ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Comparison of Nutritional and Sensory Quality of Vegetables Grown on Aquaponics Technology and Conventional Produces

BY:

Dereje Wolde Tilahun

Advisors:

Paulos Getachew (PhD)
Abebe Tadesse (PhD)

A Thesis Submitted to College of Natural and Computational Sciences of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Nutrition

June, 2019

Addis Ababa, Ethiopia

Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

Comparison of Nutritional and Sensory Quality of Vegetables Grown on Aquaponics Technology and Conventional Produces

Prepared by: Dereje Wolde Tilahun

Approved by		Signature	Date
1. ______________	(External Examiner)	____________	__________
2. Dr. Aynadis Tamene	(Internal Examiner)	____________	August 09, 2019
3. ______________	(Chairperson)	____________	__________

Advisors:-

4. Paulos Getachew (PhD) ____________

5. Abebe Tadesse (PhD) ____________

Declaration

I, the undersigned, declare that, this is original work and has never been presented in any other University as well as research institutes and all the source materials used for writing the thesis have been fully acknowledged. This paper has never been submitted to and/or presented in any other University, college or institution in candidature of any other degree, diploma, or certificate.

Name: Dereje Wolde Tilahun

Signature: ______________

Date: __________________

This thesis has been submitted for examination with my approval as University advisor.

Name: Paulos Getachew (PhD)	Name: Abebe Tadesse (PhD)
Signature: __________________	Signature: _________________

Date and place of submission: (office of Research and Graduate Programs)

Addis Ababa University

June, 2019

DEDICATION

This thesis is dedicated to my beloved father "Wolde Tilahun" who I lost him in death. It has been almost more than 21 years since I last saw you, spoke to you, touched your hand, hugged you or just sat in your presence. You left me with a lot of memories. I finally made it! I kept the promise that I made to you when I was a little boy. I went to school for 20 years with lots of ups and down in the absence of you who loves and take care of me than anyone does. I miss you! I really miss you.

ACKNOWLEDGEMENTS

It is my pleasure to express my gratitude to all those people who have supported me and had their contribution in one or other way in making this thesis possible.

First, I would like to extend my deepest gratitude to my advisor Dr. Paulos Getachew, for his excellent care and genuine guidance throughout my graduate studies and in the preparation of my thesis proposal and master's thesis. Through his guidance and encouragement, I felt confident and self-assured throughout this important endeavor.

My thanks also go to my co- advisor, Dr. Abebe Tadesse, for his guidance and sharing his knowledge on aquaponic system from the scratch to the end of this work.

Again, I would like to express my thankfulness to Dr. Kaleab Baye, for the big role he played on the wining of the small research grant from Wageningen University, Holland.

This work would not have been completed in the present form without the help and cooperation of different institution and individuals. First, we greatly appreciated Wageningen University, Holland for funding this study. Next, we would like to say thank you the department of zoological science, Addis Ababa University and the aquaponic farmers at Shewa Robit town for allowing us to use their aquaponic farm. My special thanks go to Kotebe Metropolitan University (KMU) for giving me full sponsorship to attend this MSc program.

I am also gratefully acknowledges the Center for Food Science and Nutrition laboratory staff of Addis Ababa University especially, Mr. Debebe Hailu, Yemane H/Mariam and Ms. Woinshet Abera for their generous help in my research work. I would also like to acknowledge members of Bless Agri Food Laboratory Services; especially my thanks go to Mr. Henok Ashagrie for his technical support on mineral analysis. I also wish to express my deepest gratitude to my friend Ms. Tsion Yemane for her unreserved help during beta carotene analysis.

Last but not least, I am deeply indebted to my wife, Ms.Genzeb Misganaw, for her continuous support, encouragement and enabling me such an achievement, without her it would have not been possible to complete my study. My heartfelt appreciation goes to my beloved daughter, Absalat Dereje for her patience in my absence when she needed me to take care of her.

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIXES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
DO	Dissolved Oxygen
DW	Dry Weight
DWC	Deep Water Culture
FAO	Food and Agriculture Organization of the United Nations
FW	Fresh Weight
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
LES	Laurence Experimental Station
MF	Membrane Filtration
MFC	Membrane Fecal Coliform
NFT	Nutrient Film Technique
PTFE	Pollytetrafluoroethylene membrane filter
PVC	Polyvinyl Chloride
RAS	Recirculating Aquaculture System
SPSS	Statistical Package for Social Science
TCA	Tri Chloroacetic Acid
TEA	Techno Economic Analysis
UN	United Nations

Abstract

Aquaponics is one of a sustainable and alternative food production sector that integrates aquaculture with hydroponics. It is an emerging part of aquaculture that uses the natural interaction between bacteria, fish and plants to change waste into clean water. Compared to soil-based agriculture and aquaculture production methods, aquaponics has many benefits including re-use of the wastewater from the growing fish, production of two commodities from a single system and expanded food production by urban residents. For the sustainability of aquaponics, the nutritional and sensory quality of the produces should be equivalent or better than the conventional produces. Thus, this study was aimed to compare the nutritional quality and customer preference of lettuce (*Lactuca sativa* L.) and kale (*Brassica carinata*) grown on aquaponic system with that of conventionally grown in soil-based system. Proximate composition of lettuce and kale was determined using standard methods of AOAC and mineral content was determined by Inductively Coupled Plasma Optical Emision Spectroscopy. Sensory test was also determined by using discriminatory, preference and rating acceptance test methods. Accordingly, the aquaponic lettuce from Shewa Robit site had a significant ($p<0.05$) higher amount of crude fiber, magnesium, potassium, iron, copper, and boron compared to soil-grown lettuce. Meanwhile the aquaponic lettuce from Addis Ababa University site had significantly higher amount of crude fat, crude ash, crude fiber, calcium, magnesium, phosphorus, iron, boron, copper, and manganese than soil-based lettuce. Aquaponic kale had a significant higher amount of crude protein, crude fat, crude ash, crude fiber, zinc, copper, manganese, and macro minerals than the soil-based kale. However, the aquaponic lettuce and kale at both sites had lower concentration of vitamin C and β-carotene than the soil-based lettuce. A higher concentration of nitrate (78.48μg/g) and (70.73μg/g) was obtained in aquaponic lettuce at Shewa Robit and kale at AAU site respectively, compared to the soil-based produces. The concentration of chromium, lead and nickel were lower in the aquaponic lettuce than the soil based havests at AAU site. Aquaponically grown lettuce and kale at AAU site had a lower sensory preference score compared with the soil-based produces. The microbial load of the aquaponics system water, lettuce and kale was within the framework (<235 CFU/100mL) of EPA for such products. In conclusion, in this study aquaponically grown lettuce and kale had a better nutritional quality compared with the soil-based ones. Besides, the heavy metals concentration was within the acceptable limit of WHO/FAO. Further studies to improve the sensory qualities of the aquaponic leafy vegetables are strongly recommended from this study.

Keywords: Aquaponics, nutritional quality, customer preference, microbial load, harmful metals

CHAPTER ONE

1. INTRODUCTION

1.1.Background of the study

Malnutrition is still a significant problem in the developing world. Hence, dietary quality and diversity in the food environment is required to tackle this challenge (Sibhatu *et al.*, 2015). To attain diet diversity and quality, in the food supply system agricultural production should be diversified. However, the problem associated with conventional food production on soil has been aggravated due to climate change, decline in soil productivity, arable land shrinkage, urbanization, increase in population, high demand of energy and water (FAO, 2012). These global environmental, social and economic challenges drive the need for new and improved solutions for food production and consumption (Sala *et al.*, 2017). Therefore, sustainable diversified food production system with less water and energy consumption is becoming more important (Suhl *et al.*, 2016). Addressing these issues and to diversify the agricultural subsystem, aquaponics and other systems that do not require arable land are promising. In light of nutrition, in many African countries there are major nutritional deficiencies in protein, essential minerals and vitamins. Aquaponics may provide an efficient means to provide both animal protein (fish) and mineral and vitamin sources (fresh vegetables) to populations where water/and or fertilizer resources are limited with a minimum of environmental pollution (Nichols and Savidov, 2011).

Aquaponics promises to address the mentioned problems in the framework of controlled environment agriculture (Kiss *et al.*, 2015). Aquaponics is a food production sector that combines aquaculture (fish rearing in water) with hydroponics (soilless crop production) (Rakocy *et al.*, 2012). The integrated combination is symbiotic, in which fish (daily fed a protein-rich diet) generate waste that flows into the hydroponic system. The hydroponic system has an environment suitable for bacteria to convert the waste (ammonia) into compounds required for plant growth (nitrate) (Bailey and Ferrarezi, 2017). Through aquaponics farmers can produce a great variety of leafy vegetables, herbs, fruits and fish locally (Pattillo, 2017).

The main components of aquaponic systems are: fish rearing tanks, a solid removal device (clarifier) to reduce suspended solids (fish waste, uneaten feed, biofloc), a filtering tank to minimize remaining suspended solids and the hydroponic troughs serve for the biological conversion of fish excreted ammonia to nitrate, which is less toxic to fish and to strip water nutrients (Pantanella, 2012).

For the success of aquaponics harvests in the food system, the environmental, nutritional and sensory qualities must be equivalent or better than soil-grown produces (Treftz *et al.,* 2015). From environmental perspective, aquaponics food production technology offers increased yields, higher plant survival rates, decreased land, water and pesticide uses (Pattillo, 2017; Treftz *et al.*, 2015). From the perspective of food safety in aquapònic systems, few studies addressing whether aquaponic produces are more or less susceptible to microbial contamination compared to conventional produces. Aquaponics systems are often built elevated above the ground, set-up in greenhouse and built away from soil. Hence, there seems to be much less likelihood of contamination of system water, aquaponic vegetables and fish. Another characteristic that makes aquaponics different from the conventional agricultural techniques is the organic management. So far, no chemicals are allowed to be incorporated in the aquaponic system. This is to prevent any risks of plant and fish-cross toxicity.

Although aquaponics has several environmental and economic advantages, it does not guarantee a produce with high nutritional quality and customer preference (Treftz *et al.,* 2015). As innovative technologies with aquaponics are being investigated, it is important to investigate the quality of the aquaponic produce in terms of sensory and nutritional quality, since taste and nutrition are the two drivers of consumption of the product (Treftz and Omaye, 2015). Besides, in aquaponics system some elements like iron and boron which are important for plant photosynthesis and respiration are limited (Licamele, 2009 and Tadesse, 2017). Along with other factors, this may entail a possible nutritional and sensory quality difference in the produces as compared to conventional harvests. So far, many studies around the world on aquaponics technology which mainly revolving around on fish to plant interaction, selection of plant crops and fish species which adapt the system and improvement of yield have been done.

In Ethiopia, in the past few years, aquaponics projects have been started (Love *et al.*, 2015 and Slingerland, 2017), mainly focused on production and system maintenance. However, to our knowledge, there is no study or documented data on nutritional quality of aquaponically grown vegetables in Ethiopia. Therefore, this study was carried out to determine whether aquaponically-grown leafy vegetables i.e. lettuce (*Lactuca sativa* L.) and kale (*Brassica carinata*) are as nutritionally dense as conventionally soil-grown ones focusing on their biochemical composition, sensory quality, mineral content, vitamin C, pro vitamin A and nitrate concentration and at the same time microbial load of the system water, lettuce and kale.

1.2.Statement of the problem

Aquaponics technology has several advantages compared with soil-based production systems: it produces plants with added value that can be considered "organic products", reduces the amount of hazardous nitrogen discharges to the environment, eliminates the use of pesticides and fertilizers, and reduces water and waste removal related operating costs (Pineda-Pineda *et al.*, 2015). However, these advantages do not guarantee a produce with high nutritional quality and customer preference (Treftz *et al.*, 2015). According to Licamele (2009) and Tadesse (2017), elements like boron and iron important for photosynthesis and respiration are deficient in the aquaponic system. This may indicate a possible nutritional and sensory quality differences in the produces as compared to conventional harvests. In fact, for consumers, the primary consideration for selecting and consuming a food commodity is the product's palatability (i.e. sensory quality) and nutrition (Lawless and Heymann, 2010). Therefore, nutritionally quality food products don't guarantee preference by consumers. Hence, in the food system, any new agricultural produce (i.e. aquaponic vegetables) need to incorporate both qualities to derive a quality diet to the consumer. Aquaponic vegetables being new for our society in the food system, when a consumer buys as such a produce most importantly sensory properties and consistency with previous similar products come first. Therefore, sensory evaluation is considered as an integral part in describing and controlling aquaponic produces quality in this study. In Ethiopia, aquaponic projects have been started mainly focused on ensuring food security and improvement of production of fish and vegetables (Slingerland, 2017). However, to our knowledge, there is no study on nutritional aspect as well as customer preference of aquaponic vegetables grown in Ethiopia. To address this research gap, an experiment to evaluate the nutritional quality and customer preference for products grown on aquaponic systems were performed.

1.3.Research questions

The specific questions investigated in this research are:

- Does aquaponically-grown vegetables are lower, equal or better in quality than soil-grown produces?
- Is there a difference in sensory quality between aquaponic and soil-grown vegetables?
- Does aquaponic produces are safe from harmful metals and foodborne pathogens?

1.4.Objectives

1.4.1. General Objective

- To compare the nutritional quality and sensory preference of aquaponically-grown green leafy vegetables to those grown on soil through conventional farming system

1.4.2. Specific Objectives

- To evaluate the nutritional composition (proximate, mineral, pro vitamin A, vitamin C and nitrate) of lettuce and kale grown on an aquaponic systems
- To compare the similarity and differences between the concentrations of harmful metals in aquaponically grown lettuce and kale with the conventional produces
- To evaluate the customer preference of lettuce and kale grown on aquaponic systems as compared with conventional produces
- To determine the microbiological load of water, lettuce and kale sampled from aquaponic systems

CHAPTER TWO

2. LITERATURE REVIEW

2.1.Overview of global food production systems

The significance of agriculture is strongly correlated with food security, poverty reduction, rural development as a means to achieve bigger goals including employment led economic growth. The agriculture sector, however, faces critical challenges (Sheikh, 2006) including rapid demand growth for food, increased human population, increased meat consumption and search for quality food etc. Unlike now, in early 1960s, most nations were self- sufficient in food; (Kendall & Pimentel, 1994). Due to the ever-increasing global population, equitable food provision is a great challenge (FAO, 2012). This population size is expected to reach 9.5 billion in 2050 (FAO, 2012). It is evident that as the global population increases, so does the need for sustainable growing systems. Hence, providing healthy diet with adequate amount for such population size from conventional food production systems is difficult (FAO, 2012). So, what are the solutions to feeding a growing world population in the face of climate change? Therefore, dynamic paradigm shift on food production is needed (FAO, 2012).

2.2.Soilless agricultural systems

Soilless agriculture provides plants with support and a reservoir for nutrients and water. The soil is a mediafor plant growth while controlling the quantities of water, mineral salts and most important, dissolved oxygen (Lakkireddy *et al.*, 2018). In contrast, Soilless culture can be defined as "any method of growing plants without the use of soil as a rooting medium, in which the inorganic nutrients absorbed by the roots are supplied through the irrigation water" (Savvas *et al.*, 2013). Soilless culture technique plays crucial role in 21st century in commercial food production (Lakkireddy *et al.*, 2018).

Globally, agriculture has changed unexpectedly over the last few decades, and this change continues, since the driving forces for these changes are still in place (Raviv *et al.*, 2008). Rapid scientific, economic, and technological developments throughout the world are among the major forces. The increase in world's population and the improvement in the standard of living in many countries have created a strong demand for high-value foods (Raviv *et al.*, 2008). Additionally, lack of suitable soil, disease contamination after repeated use and the desire to apply optimal conditions for plant growth are leading to the worldwide trend of growing plants in soilless media. Pantanella (2012) stated that soilless cultivation can at least double the harvest of conventional horticulture (from 1kg m^{-2} in soil to 2.3 kg m^{-2} in soilless for lettuce; from 1.2-2.4 kg m^{-2} in soil to 14-74 kg m^{-2} in soilless for tomato). Moreover, soilless growing methods decrease the excessive spread of soil borne pathogens, improve growth by a better control, save energy and increase production. Also as recent report indicated that soilless system can increase a more efficient use of water, nutrients and pesticides to decrease emission to the environment (Van Os, 1994). To summarize, the main reasons for the expansion of soil-less agricultural practices are: decreased presence of soil-borne diseases and pathogens because of sterile conditions, improved growing conditions that can be manipulated to meet optimal plant requirements leading to increased yields, increased water and fertilizer-use efficiency, and the possibility to develop agriculture where suitable land is not available (Somerville *et al.*, 2014).

In soilless system, when roots are suspended in moving water, they absorb food and oxygen rapidly. If the oxygen content is insufficient, plant growth will be slow. But if the solution is saturated with oxygen, plant growth will accelerate. Therefore, the grower's task is to balance the combination of water, nutrients, and oxygen, with the plant needs, in order to maximize yield and quality. Along with these factors, for the best results, temperature, humidity and CO_2 levels, light intensity, ventilation, pH and the plant's genetic make-up should also be considered (El-Kazzaz, 2017). Now soilless agriculture is shifted from open to close- loop system. This system is known for its efficiency in water use and maintaining the quality of the yield (Putra and Yuliando, 2015).

Growing plants in containers above ground has been at practice at various times throughout the ages. The Egyptians did it almost 4000 years ago. Wall paintings found in the temple of Deir el Bahari (Naville, 1913as cited from Hussain *et al.*, 2014) showed what appears to be the first documented case of container-grown plants. They were used to transfer mature trees from their native countries of origin to the king's palace and then to be grown this way when local soils were not suitable for the particular plant. It is not known what type of growing medium was used to fill the containers, but since they were shown as being carried by porters over large distances, it is possible that materials used were lighter than pure soil. Starting in the seventeenth century, plants were moved around, especially from the Far and Middle East to Europe to be grown in orangeries, in order to supply aesthetic value, and rare fruits and vegetables to wealthy people (Raviv *et al.*, 2008).

2.3. Types of soilless agricultural systems

2.3.1. Aeroponics

Aeroponics is the technology of growing plants in an air or mist environment without the use of soil or an aggregate media. The word aeroponic is derived from the Latin meanings of 'aero' (air) and 'ponic' (work) (Farran *et al.*, 2006). This is an alternative method of soil-less culture in growth-controlled environments. Historically, it could be traced back to more than 120 years ago (Waisel *et al.*, 2002). However, it is more advanced than hydroponics system since plant roots are submerged not in nutrient solution but suspended in the air and a nutrient solution is misted over root system to maintain a constant film of nutrients.

The growing of plants in aeroponics system is considered as safe and ecologically friendly for producing natural, healthy plants and crops. Reports show that the system is ten times more successful than conventional techniques, tissue culture and hydroponics. In addition the aeroponic system is more user-friendly as the plants are separated; they are all suspended in the air (Gopinath, Vethamoni and Gomanthi, 2017). The system has the ability to conserve water and energy. It comparatively offers lower water and energy inputs per unit growing area (Ritter *et al.*, 2001; Farran *et al.*, 2006). Although aeroponics is a unique way of growing, it is not a common means of commercial production (Sheikh, 2006).

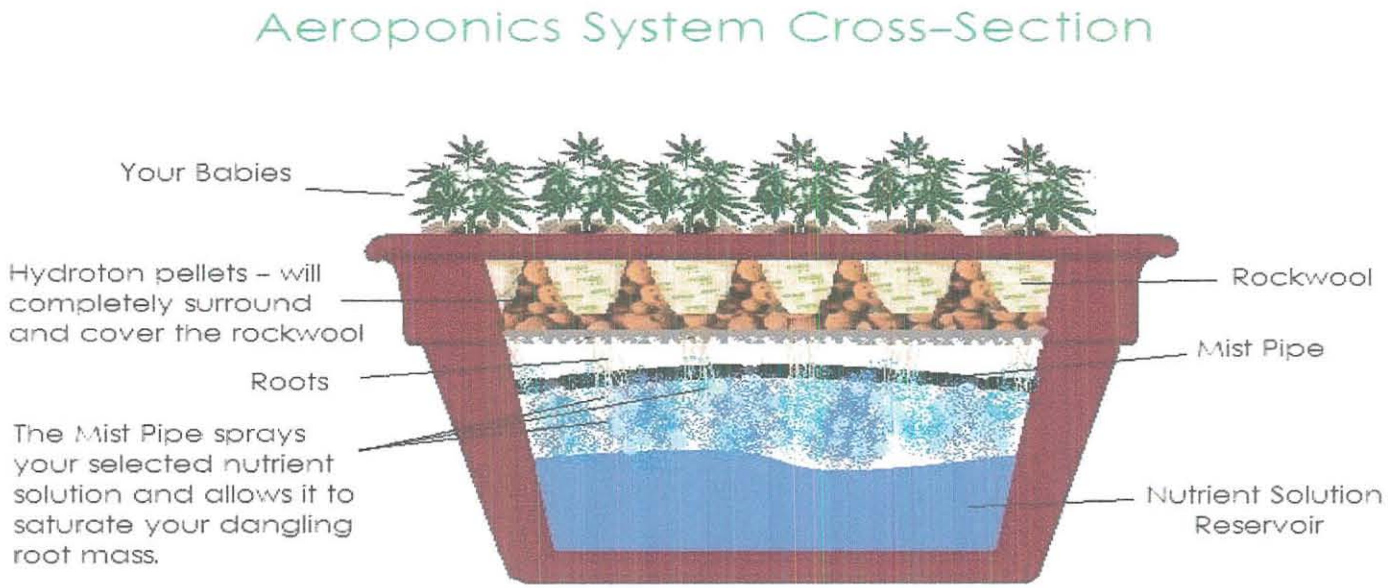


Figure 1: Aeroponic system

Source: www.piterest, accessed on 9/5/ 2018.

In general compared to hydroponic culture, aeroponics has the following advantages. (1) it is very light in weight; (2) it has no limitation of air; (3) it limits disease spread since plant-to-plant contact is reduced and thus, products of aeroponics are pesticide free); (4) it saves water, making it ideal for some countries where water resources are limiting; and (5) it is flexible and allows modification of many root-zone environmental factors such as root zone temperature (Lee SK, 1993).

2.3.2. Hydroponics

Due to huge, rapid and subsequent demand on food supply, many new trends in the farming innovative methods which include a complex agricultural production system have been evolved. Hydroponics is a technology of plant growing in a soilless medium through addition of nutrient solution required for healthy growth and development of plant (Savvas, 2003).The term hydroponics was coined by Gericke (1937 as cited from Sheikh, 2006) to mean water culture without employing any substrate. Mainly the term is used to refer to systems that do include some sort of substrate to anchor or stabilize the plant and to provide an inert matrix to hold water. However, hydroponics is the practice of growing plants in nutrient solutions (Raviv *et al.*, 2008). The determination of the essential elements required by plants was discovered using solution culture techniques. The technique has an interesting history of development and use dating back into the mid18th-century (Jones, 1982). Hydroponic production get intensified through time due to increased economic demand for producing high value crops in more stable situations (Jensen, 1997).

2.3.3. Aquaponics

Mitigating mother technologies (aquaculture and hydroponics) associated problems has cost implication and hence developing multi-trophic culture system, which is aquaponics, would be considered as most cost effective (Childress, 2002). The word aquaponics is a mixture between aquaculture and hydroponic (Martin, 2017). It's organic by definition: instead of using man made chemical fertilizers, plants are fertilized by the fish waste (without application of pesticides/herbicides (Okemwa, 2015). The main components of an aquaponic system are (Figure2): fish rearing tanks, a solid removal device (clarifier), to reduce suspended solids (fish waste, uneaten feed, biofloc), a filtering tank to minimize remaining suspended solids.

Following the filtering stage the hydroponic troughs serve for the biological conversion of fish excreted ammonia to nitrate (Pantanella, 2012). In addition to the vegetables they can grow, most aquaponics gardeners cultivate fish as well. Thus, aquaponics can be described as the farming of fish and vegetables in a symbiotic environment imitating the natural cycles of nutrients for a more sustainable agriculture. It is harmonized fish and plant production system developed based on the mutual benefits for both through microbial intervention (Tyson *et al.*, 2011).

Aquaponics provides several benefits including environmental, economical, and societal health through its potential of reducing water consumption and waste discharge to the environment, and incredibly highest productivity potential (Buzby *et al.*, 2016; Dediu *et al.*, 2012). It can be the answer to a fish farmer's problem of disposing of nutrient rich water and a hydroponic grower's need for nutrient rich water (Okemwa, 2015). In aquaponics, the fish waste provides a food source for the growing plants and the plants provide a natural filter for the fish. This creates a mini ecosystem where both plants and fish can grow.

Aquaponics first appeared at least 1,500 years ago in China (Jones, 2002). Soilless agriculture, in fact, historically dated back to several hundred years BC since the civilization of ancient Egyptians, the Chinese and other cultures (Jones, 2002). The Aztecs started a method of suspended gardens based on hydroponics at Lake Tenochtitlan during the 10th and 11th centuries. However, commercial aquaponics ideas and companies were started in Europe (Miličić, 2017). In a modern context, aquaponics emerged from the aquaculture industry as fish farmers were exploring methods of raising fish while trying to decrease their dependence on the land, water and other resources (Fernandez, 2015).

Taking apart the ancient techniques developed by different cultures at the length of the history, modern aquaponics began in 1977 with studies about the options for removing nitrates from waste aquaculture water which would be recirculated again (Bohl, 1977). Among the different options, the removal of nitrates using plants as a biofilter seemed promising which led to the birth of aquaponics (Martin, 2017). The other option was without relying on plants, mainly bacteria-adsorbent biofilters that led to the RAS, the modern recirculating aquaculture.

2.4.Principles of aquaponics and its worldwide practices

The classical working principle of aquaponics is to provide nutrient-rich aquacultural water to a hydroponic plant culture unit, which in turn removes the water that is returned to the aquaculture tanks (Figure 2). Conventional hydroponics requires mineral fertilizers in order to supply the plants with necessary nutrients (Jones, 1982). But the aquaponics systems use the available fish water that is rich in fish waste as nutrients for plant growth. Another advantage of this combination lies in the fact that excess of nutrients does not need to be removed through periodical exchange of enriched fish water with fresh water as practiced in aquaculture systems (Pade and Nelson, 2005). The system results in a symbiosis between fish, microorganisms and plants, and encourages sustainable use of water and nutrients, including their recycling. Within this synergistic interaction, the respective ecological weaknesses of aquaculture and hydroponics are converted into strengths (Lennard, 2004).

Aquaponic practices can be found throughout the world, from deserts to northern countries like United States (US) to tropical islands. In Gaza, aquaponics is saving lives. During the summer of 2014, the Israeli offensive lasted 51 days and left 2,000 Palestinians dead and tens of thousands of houses destroyed. It wasn't safe to go buy vegetables and farmers were cut off from their fields. Instead, families use their roofs to grow food. Individuals grew fish, tomatoes, eggplants, and peppers regularly and were able to feed their family (Baker, 2016).

Aquaponics is still a new and emerging technology in most African countries, including Ethiopia (SmartFish, 2013). The industry is dominated by technology and training suppliers, consultants, and community/organic/local food initiatives. There are very few well established commercial aquaponic systems and most of those that have been cross-subsidized by other economic activities, at least in the start-up phase. Many aquaponics initiatives in temperate zones are struggling with high capital, energy and labor cost demand. Constraints on pest management have been the major problems to date (Tokunaga *et al.*, 2013).

In many developing countries there are major nutritional deficiencies in protein, essential minerals and vitamins. Aquaponics may provide an efficient means to provide both animal protein (fish) and mineral and vitamin sources (fresh vegetables) to populations where water/and or fertilizer resources are limited with a minimum of environmental pollution (Nichols and Savidov, 2011).

Aquaponics has huge potential to be used by developing countries - both as commercial ventures and a way to provide food (Ter Morshuizen, 1996) owner and founder of Aquaculture Innovations. Aquaculture advocates also say it is sustainable and eco-friendly. Tony Abuta, founder of Amsha Africa Foundation, explains "Water is a precious commodity in developing nations, and because the majority of the water used is recycled through the aquaponics system, significantly less water is consumed than in traditional farming."Abuta adds: "By building Aquaponic systems in developing nations like those in Africa, there would be more food for the population, and it would be more nourishing" (Okemwa, 2015). However, there were no suitable survey tools existed to collect information on production practices and interests of individuals engaged in aquaponics. In general aquaponics increase food security, employment opportunities and economic growth. As nutrition is a key issue for developing nations, who rely mainly on staple crops such as wheat and rice, the fish farmed could also provide a valuable source of protein (Okemwa, 2015).

Figure 2: A design illustration of a simple aquaponics system

Source: http://www.shutterstock.com
Accessed on 14 August, 2018

2.5. Advantages and limitations of aquaponic technology

Waste water treatment using aquaponic plants is one of the cost-effective and beneficial phytoremediation strategies (Nuwansi *et al.*, 2016). Compared to conventional, soil-based agriculture and aquaculture production methods, aquaponics uses significantly less water and produces less waste discharge (Tyson *et al.*, 2011). It can also take place on marginal land, or indoors and in urban areas. A recent technical report by the United Nations Food and Agriculture Organization (FAO) (Somerville *et al.*, 2014) detailed some of the possible benefits of aquaponic systems compared to conventional agriculture and fisheries. The benefits of this system include: re-use of the wastewater from the growing fish, production of two commodities from a single system, reduction of pressure on land conversion, an increase in the world supply of fish without depleting wild stocks, production of food in areas with minimal water supply, and expanded food production by urban residents (Baker, 2016; Somerville *et al.*, 2014). It is estimated that aquaponics system uses 10% of the land needs and 5% of the water needs as traditional vegetable production (Baker, 2016).

From economic perspective, aquaponic system has expensive initial start-up costs compared with soil based vegetable production or hydroponics (Somerville *et al.*, 2014). Additionally, aquaponics cannot be applied in places where cultured fish and plants cannot meet their optimal temperature ranges. Currently, all aquaponics operations utilize fish diet primarily prepared by considering the physiological demand of fish and minimal possible nutrient loss to the environment despite the need to balance the plant demand to earn good profit from the whole setup (Somerville *et al.*, 2014). So far, fishmeal is considered to be ideal source of protein for aquaponic system. However, it is not cost effective for many aquaponic farmers to afford.

Studies have been conducted in search of alternative fish diets and both animal-based and plant ingredients were found to be good protein sources for fish. But, the fundamental problem to use plant ingredients as fish diet component is associated with the presence of phytic acid, which makes calcium, phosphorus and zinc unavailable (Tadesse, 2017). Hence, in many aquaponics operations nutrient imbalance for plants is observed through prolonged aquaponics operation using commercial fish diet (Ogunji *et al.*, 2008). Similarly, concentration of nutrients in aquaponics is usually lower than those found in hydroponic systems (Pantanella, 2012). According to Licamele (2009) and Tadesse (2017), in aquaponics system some elements like iron and boron which are important for plant photosynthesis and respiration are limited. Similarly, limited plant growth and development is reported in aquaponics due to the possibility of mineral imbalances in the system (Endut *et al.*, 2010; Seawright *et al.*, 1998; Rakocy, 1997).

Thus, one possible mitigation measure can be diet quality adjustment. In addition, plants have their own nutrient requirements, which vary based on plant type. Hence, depending only on conventional fish diet as nutrient source in aquaponics make the plant components suffer. Therefore, new insight should be advocated about aquaponics input (fish diet) formulation scenarios giving mutual emphasis for fish, plant, and microorganisms physiological requirements. Except multi-element ionic nutrients like nitrate and phosphate, other macro and micronutrients might be adjusted by simple addition on fish diet with critical fish and plant tolerance limit consideration (Endut, 2010).

2.6.Challenges to sustainability of aquaponics technology

The commercial development of socially, ecologically, and environmentally sustainable aquaponic systems faced several challenges that need to be addressed (palm*et al.,* 2018). According to Lehman *et al.* (1993) agriculture is a process that does not deplete any non-renewable resources that are essential to agriculture in order to sustain the agricultural practices. Both aquaponic and hydroponics can be considered a sustainable agricultural production system. Sustainable agriculture can be achieved by resembling natural ecosystems and "designing systems that close nutrient cycles, which is one of the main attributes of aquaponics (Okemwa, 2015). According to FAO, there is a potential for aquaponic systems to be part of a more sustainable future for agriculture.But the wide variety of potential system specifications and production locations necessitates that any claims to sustainability be evaluated on a case-by-case basis (Tyson *et al.,* 2011). The tangible factor of sustainability in aquaponic system could be economic feasibility. Even the most environmentally friendly systems will have a hard time making an impact if they are not able to compete profitably with conventional systems.

Balancing the aquaponic system environment for the optimum growth of three organisms will be an on-going subject of research. In aquaponic activities, one major challenging factor is the accessibility and affordability of fishmeal.

Mostly the situation is aggravated in developing countries where, the access for aquaponic components limited. Use of fishmeal as major protein source in fish diet increased the diet price and brought profit consequences (El-Saidy and Gaber, 2004). According to Tadesse (2017), there were a handful studies related to the alternative fish feed and the animal-based and plant dietary ingredients including feather meal, meat bone meal, linseed, peanut, sunflower cottonseed and poultry byproducts suggested as good sources of protein. However, the quality of such ingredients varies based on place of origin, animal condition and other environmental factors (Tadesse, 2017). Further aquaponic systems' adoption will require more public and private resources to close many knowledge gaps in properly managing these systems and successfully marketing their products to the public (Goddek *et al.,* 2015). If this technology is properly applied and monitored, it definitely has the potential to improve food security and exert the benefits outlined above.

2.7.Plants and fish species adapted to aquaponic system

Many types of vegetables have been cultivated in aquaponic systems (Adler *et al.,* 2000). However, the selection of plant species adapted to aquaponic greenhouses is related to stocking density of fish tanks and subsequent nutrient concentration of aqua cultural effluent (Diver and Rinehart, 2000). With this criterion, culinary herbs are the best choice. They grow very rapidly and command high market prices. The income from herbs such as basil, cilantro, chives, parsley, portulaca and mint is much higher than that from fruiting crops such as tomatoes, cucumbers, eggplant and okra (Rackocy *et al.,* 2006). Lettuce, herbs, and specialty greens (spinach, chives, basil, and watercress) have low to medium nutritional requirements and are well adapted to aquaponic systems.

Plants yielding fruit (tomatoes, bell peppers, and cucumbers) have a higher nutritional demand and perform better in a heavily stocked, well established aquaponic system. Greenhouse varieties of tomatoes are better adapted to low light, high humidity conditions in greenhouses than field varieties (Diver and Rinehart, 2000). Generally, not all produce can be grown in this system. Plants that thrive in an aquaponics system are those in which their nutritional requirements can be met by the fish wastewater (Baker, 2016).

Many fish species grow well in the aquaponics system too, however, most commonly used are those that are food sources such as tilapia, trout, perch and bass (Rakocy *et al.,* 1997). Several warm-water and cold-water fish species are adapted to recirculating aquaculture systems, tilapia is the fish species most commonly cultured in aquaponic systems. Tilapia is a warm-water species that grows well in a recirculating tank culture. Furthermore, tilapia is tolerant of fluctuating water conditions such as pH, temperature, oxygen, and dissolved solids. Tilapia produces a white-fleshed meat suitable to local and wholesale markets. Although some aquaponic systems have used channel catfish, largemouth bass, crappies, rainbow trout, pacu, common carp, koi carp, goldfish (*Carassius auratus*), Asian sea bass (barramundi) and Murray cod, most commercial systems are used to raise tilapia. Most freshwater species, which can tolerate crowding, will do well in aquaponic systems (including ornamental fish) (Rackocy *et al.*, 2006). Therefore, in the present study also the Nile tilapia (*Oreochromis niloticus*) which weighs an average of 70g was used for the aquaponic system.

2.8.Nutritional and sensory characteristics of aquaponic produces

2.8.1. Nutritional quality

Aquaponics provides the consumers an opportunity for eating fruits and vegetables grown closer to home. This reduces spoilage and can maximize nutritional benefits through increased diet diversity (Murphy *et al.*, 2011). Comparison of the nutritional content of aquaponically and soil-grown fruits and vegetables is challenging because of the fundamental differences between the two different methods. The difference in growing techniques may result in variations of nutritional content as well. According to the quality survey from Tronsted (1995) the highest rank in quality is obtained by the appearance and taste of a product. However, not much knowledge at present is available on the qualitative parameters of aquaponic leafy vegetables in terms of appearance, biomass, dry matter and color. A study carried out by Travieso *et al.*(2016) stated that lettuce grown under greenhouse system showed a higher value of+ fresh and dry weight, number of leaves, leaf area and chlorophyll content but lower antioxidant capacity compared with soil grown lettuce in open field.

Nutrient concentrations in aquaponics are lower than those used in hydroponic systems due to the storage of nutrients into the recirculating water and the continuous supply of minerals by fish. However, some studies have indicated that soilless growing methods have superior nutritional quality, while others indicate no significant differences (Treftz *et al.*, 2015; Gruda *et al.*, 2009). Although aquaponic systems have been studied for decades, vegetable quality and productivity of aquaponics compared with other cultivation methods still needs to be fully developed. Thus, one of the specific objectives of this study was to investigate the nutritional quality of aquaponic produces as compared with that of conventional produces. In light of nutrition, in many African countries where animal protein and fresh vegetable supplies are very low, aquaponics may provide both animal protein from fish and mineral and vitamins from fresh vegetables (Nichols and Savidov, 2012). Generally, the nutritional requirement in aquaponic plants vary with many confounding factors such as variety, life cycle stage, day length, grow methods and weather conditions which correlate with plant biochemical composition (Bittsanszky *et al.*, 2015).

2.8.2. Consumer perception towards aquaponic produces

Many key questions about the overall feasibility of aquaponic production remain unanswered. Particular concerns for start-up producers are the consumers' perception and willingness to pay for aquaponic fruits/vegetables and fish (Short *et al.*, 2017). A study in Malaysia reported that respondents had positive intensions regarding the purchase of aquaponically grown products. Consumers who use aquaponic produces believe the naturalness of the food is not affected. Yet, research on consumer acceptance of the aquaponic harvests is crucial. Nowadays, consumer awareness has increased on the health benefits of local and organic agricultural produces (Basha *et al.*, 2015; Falguera *et al.*, 2012). Moreover, consumers make more conscious decisions when buying food (Tokunaga *et al.*, 2015), especially in developed and industrial countries. Accordingly, consumer acceptance and certification criteria are of special interest for the development of commercial-scale aquaponics systems. Most available studies on aquaponics have focused mainly on production and system maintenance (Love *et al.*, 2015). Due to their healthiness, environmental friendliness, taste, freshness, and quality of the food, as well as a desire to avoid genetically modified ingredients, consumers show willingness to buy organic products. Therefore, it is possible that consumers' perceptions of aquaponic products might contain some of the same appealing attributes as organic and local production. Thus, the present study investigated the sensory quality/preference of aquaponic produces with that of conventional harvests.

2.9.Aquaponics and food safety issues

Aquaponics is gaining increased attention as a bio-integrated sustainable food production system. The waste products of one biological system serve as nutrients for a second biological system (Diver, 2000). This integration of fish and plants results in a polyculture that increases diversity and yields multiple products. Water is re-used through biological filtration and recirculation. Local food production provides access to healthy foods and enhances the local economy. However, when food plants are grown in the presence of fish culture effluent, food safety considerations become very important (Hollyer *et al.*, 2009). The coming paragraphs will detail on the microbial and chemical safety of aquaponic produces and water collected from the aquaponic system.

2.9.1. Microbial quality of aquaponic produces

One important issue in aquaponics that has not yet been studied is the assessment of microbiological quality of the water and the reduction of microbial loads to comply with food safety standards (Pantanella *et al.*, 2010). It is not uncommon to find fecal coli forms and non-pathogenic *E.coli* in aquaponics water (Baker, 2016). According to Fox *et al.* (2012) due to the constant recirculation of water coli forms are naturally present in aquaponic systems.

From the perspective of food safety in aquaponic systems, few studies addressing whether aquaponic produces are more or less susceptible to microbial contamination compared to conventional produces. Aquaponics systems are often built elevated above the ground, set-up in greenhouse and built away from soil. Hence, there seems to be much less likelihood of contamination of system water, aquaponic vegetables and fish. In addition, aquaponic systems are usually located and designed in a way that allows them to have protection from contamination unlike traditional soil farming (Baker, 2016). However, in the aquaponic system the used water from fish as nutrient for plants can pose a microbial risk for water and vegetables (Alcaraz *et al.*, 2016). Although cold blooded animals like fish have different bacteria from warm blooded animals and do not have *Escherichia coli*, the presence of coli forms may be a possible risk. Hence, aquaponics could benefit from water sterilization, however it is not clear whether drastic elimination of bacteria may harm nitrifying bacteria activity and thus be a risk factor for fish. Thus, the present also investigated the microbial load of the circulating water from the aquaponic system.

2.9.2. Chemical residues in aquaponic produces

Another characteristic that makes aquaponics different from the conventional agricultural techniques is the organic management. So far, no chemicals are allowed to be incorporated in the aquaponic system. This is to prevent any risks of plant and fish-cross toxicity. Aquaponics is mostly work with integrated pest/disease management and the use of biological treatments (Pantanella, 2012). Soil and environmental pollution is a matter of major concern and has been considered as global problem because of its adverse effect on human health, plants and animals exposed to chemicals (Abbas *et al.,* 2017). However, in aquaponics system, the used water from fish tank as nutrient for plant may cause a risk if the source is from contaminated area. There are several aspects of food safety with regard to raw vegetable produce, including chemical (i.e., pesticides, herbicides, antibiotic use, etc.), physical (i.e., foreign objects, metal or bone fragments, large pieces of soil or rocks), and biological (i.e., bacteria, parasites, viruses (chalmers, 2004).So far, no available data found regarding chemical residues in the aquaponic system and aquaponic harvests.

2.10. Economic analysis of aquaponic system

From a purely economic development perspective, Aquaponics system is still not common in rural areas because existing aquaponic systems require a large capital and operational expenditures (El Essawy, 2018). According to Somerville *et al.* (2014) aquaponics is more productive and economically feasible in certain situations, especially where land and water are limited. However, aquaponics is complicated and requires substantial start up costs.

Energy use is relatively high because of the need for both aeration and pumping in most systems (Hambrey, 2013). Most of the early research on aquaponics took place outdoors, in warm locations with limited freshwater and limited local food production such as the Virgin Islands (US). In this setting, aquaponics can be an efficient system for food production with remarkable profit (Bailey *et al.,* 1997). There were handful studies related to the cost and profit for commercial scale aquaponics (Tokunaga *et al.,* 2013; Bunyaviroch *et al.*, 2013; Bailey *et al.*, 1997). The study conducted by Tokunaga *et al.* (2015) in Hawaii concluded that the economic performance for commercial scale aquaponics had some potential, even though the potential might be not as promising as former studies suggested.

The study conducted by Bunyaviroch *et al.* (2013) investigated a commercial case in Puerto Rico and indicated that aquaponics was viable there but the profitability was limited. The study conducted by Palm *et al.* (2014), focusing on factors affecting economic sustainability of closed ebb flow aquaponics in Germany and he concluded that the availability of plant nutrients in the aquaponic system and the general performance of the combined fish and plant cultivation must be kept in mind in order to achieve economic sustainability in closed aquaponics. Based on a techno-economic study of aquaponics in South Africa, high capital and operating cost made it difficult to make profit (Lapere, 2010). Love *et al.* (2015) conducted a relatively comprehensive international survey on aquaponics production and profitability. It indicated that energy, water, and fish feed were the three major physical inputs in aquaponics. Small scale aquaponics could be operated in the backyard while commercial scale aquaponics is considered as agriculture which could make profit. It was reported that the average size of commercial aquaponics was using 10,300 L water and was occupying 0.01 ha field. Less than half operators also reported that they used supplemental light to help plant production. The survey also stated that electricity was the primary energy source for aquaponics. Aquaponics is supposed to have large potential in development and expansion (Love *et al.*, 2015). For commercial operators, 55% of them harvested less than 45 kg fish and 52% of them harvested less than 226 kg plants in the previous year. The survey also showed that more commercial aquaponics producers sold products through direct markets, such as at aquaponics facility, farm market, and restaurant, other than indirect markets, such as via grocery store and wholesale; which also indicated that aquaponics was still not a mature agriculture. From this survey, only 31% of operators made profits during the previous year, and many of them were not only selling fish and plants, but also selling aquaponics materials and services (Love *et al.*, 2015). Before committing to a large or expensive system, a full business plan considering economic, environmental, social and logistical aspects should be conducted (Somerville *et al.*, 2014). In another survey by Savidov (2004) on aquaponics system in Alberta, Canada; most consumers indicated that they would purchase aquaponic vegetables. Their study also predicted that the greatest challenge for producers would be finding the right market, meeting customer requirements in terms of quantity as well as quality and establishing proper prices (Savidov, 2004).

2.11. Concluding remarks

Aquaponics can be described as the farming of fish and vegetables in a symbiotic environment imitating the natural cycles of nutrients for a more sustainable agriculture. As nutrition is a worldwide key issue especially for developing nations, who rely mainly on staple crops such as wheat and rice, It can be the answer to provide both animal protein (fish) and mineral and vitamin sources (fresh vegetables) (Nichols and Savidov, 2011). However, limited plant growth and development is reported in aquaponics due to the possibility of mineral imbalances, continous power failure and the inaccessibility and affordability of fishmeal. In general, if this technology is properly applied and monitored, it definitely has the potential to improve food security, employment opportunities and economic growth.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in two towns of Ethiopia where aquaponic facility were available. These were Shewa Robit town and Addis Ababa (Addis Ababa University aquaponic facility). Shewa Robit is a medium size town located at 225 km northeast of Addis Ababa, in the Amhara National Regional State at elevation of about 1,280 meters above sea level (ASL) and is situated in a mountainous area. The town lies at a longitude of 10°06'N39°59'E and latitude of 10.1°N39.983°E respectively. The climate in Shewa Robit is semi-arid with mean annual rainfall of 120 mm; usually the maximum rainfall occurs in the months of July and August. The mean annual temperature is 32°C with a daily average minimum and maximum of 14°C and 36°C respectively. According to Koop (2016), the mean relative humidity in Shewa Robit is 21%. The second aquaponic site, Addis Ababa University is located in Addis Ababa. Addis Ababa is located between 8°55' and 9° 05'N Latitude and 38° 40' and 38°50' E Longitude. The city is located at the center of Ethiopia with an area of 540 km^2. Its altitude ranges from 2000m – 2800m (ASL). The temperature is mild Afro alpine temperature and warm temperate climate with annual average temperature between 10°C to 20°C and average annual rainfall is 1200mm.

3.2. Experimental setup and statistical data analysis

The experimental setup was comprised of nine fish holding tanks (circular, plastic) with 250 L water holding capacity. Each tank was attached to 80 L biofilter tank, which contains 0.25 Kg bioballs and attached to 20L clarifier (Figure 3). The plant component was nutrient film techniques (NFT) with 44 planting pots attached to each fish tank. Each system was working independently in every aspect and similar. Throughout the experiment, nutrient water flow from fish tank to clarifier then to biofilter and pumped up to hydroponics gullies and back to fish tank by gravity. All units utilized municipal tap water according to the method described by Tadesse (2017).

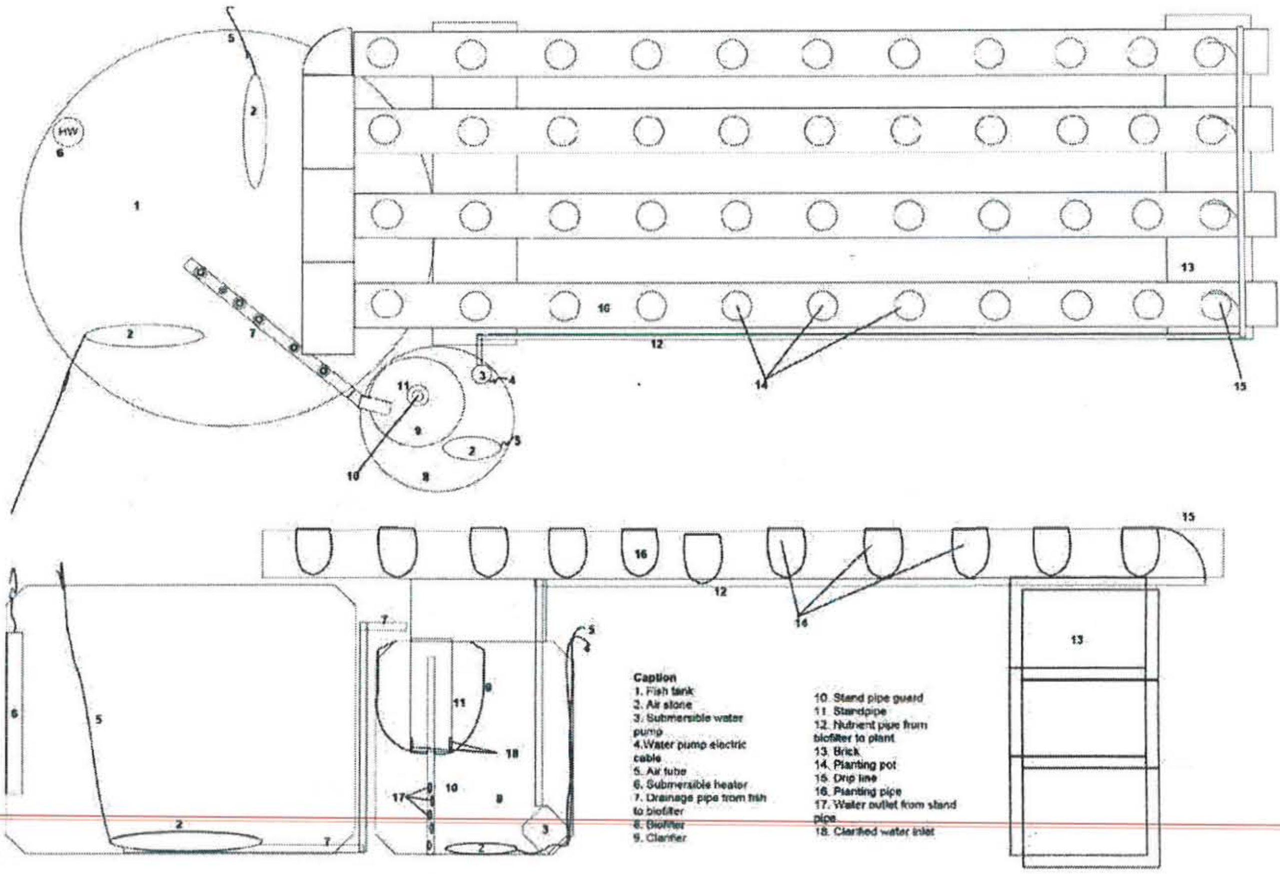


Figure 3: Schematic diagram of aquaponics unit used in the experiment.

Source: Tadesse (2017)

Statistical data analysis for the sensory evaluation

The data collected from the sensory tests were analyzed using SPSS® (version 23). Analysis of variance (ANOVA) was performed for each vegetable samples with comparison between those aquaponically and soil-grown. Significant differences were determined at $p < 0.05$. All analyses were conducted in triplicate and the results were expressed as mean ± standard error (SE)

Statistical analysis for the microbial tests

All results were recorded using MS Excel 2010 and the statistical analysis was performed with SPSS 23.0

3.3. Fish stocking

For this experiment, 54 Nile tilapia (*O. niloticus*) fingerlings were obtained from aquaponic farmers. Then stocking of fingerlings was done with the density (800g) of fingerlings in each tank. Acclimatization of fingerlings was carried out for 8 to 10 days prior to the experiment.

Figure 4: Weighing and stocking of Nile tilapia (*O. niloticus*) into fish holding tanks

3.4. Fish feeding

Fish feed in aquaponic system for fish and plant production varies widely in their mineral content as per ingredients used and species of interest. Tilapia nutritional dietary requirements for energy, protein, lipids, vitamins and minerals were established after several studies (Tadesse, 2017). Similarly, lettuce needs essential minerals in proper amount, type, and integrity for its best growth. In this study, commercial Nile tilapia feed containing protein, carbohydrates and vitamin premixes were used to feed the fish. The fish feed composition is indicated in the appendix IV. The feed was supplied twice daily (9 am and 5 pm) at the rate of 10% body weight for the first month. Then the feeding rate was adjusted to 8% for the rest of the period according to the method described by Tadesse (2017).

3.5. Planting of lettuce (*Lactuca sativa* L.) and Kale (*Brassica carinata*)

Seeds of lettuce (*L. sativa* L.) and kale (*B. carinata*) were purchased from Fan agricultural input supplier and after preparing the grow beds, the seeds were sown on hydroponic grow beds and in open field without chemical treatments or fertilizers on the same day. Thenafter 36 days of planting, roots of seedlings were transplanted to aquaponics facility for the experiment. Then, seedlings with 4-5 branching young leaves were placed in a small media filled plastic mesh pots and placed in a plastic line pipe which wholes are cut and attached to the system in the late afternoon on 1st November, 2017. The upper part of the roots remains in the air while the lower parts grow vigorously in the well aerated water. Finally, after 65 days of planting kale and lettuce samples were harvested from aquaponic system and field on the same day.

3.6. Sample collection and preparation

3.6.1. Sample collection and preparation for compositional analyses

Samples of both lettuce and kale were collected from field and aquaponic systems after 65 planting days by taking same size and equal number of leaves. Homogenized sample was prepared for each vegetable by mixing each leaf together and bagged with clean plastic bags in ice box. Then, samples were brought to Center for Food Science and Nutrition laboratory of Addis Ababa University for compositional analyses. Then, the vegetables were cleaned with tap water and rinsed with deionzed water to remove dust and extraneous matter. The cleaned vegetable samples were freeze dried using (MINI-LYODEL 1683-11, Delvac pumps-India). Finally, the dried vegetable samples were ground using high speed lab mill (FW100-China), homogenized and stored in a tightly closed clean bottle until further analyses as indicated in Figure 5.

Figure 5: Lyophilized, ground and homogenized lettuce and kale samples

3.7. Methods of analyses

Proximate composition determination

Moisture content determination

Moisture content of lettuce and kale was determined by drying the samples in an air drying oven (Gallenkamp, model OV 880, England) by using (AOAC, 2000); the Official Method 925.09. Five gram of composite dried and ground vegetable sample was accurately weighed and placed into a previously cleaned, dried and weighed metal crucible with fresh sample (W_2). The crucible with its content was put into a drying oven at 105°C for 5hr and cooled in desiccators at room temperature for 30 minutes. Then, the crucible with residue was weighed until a constant weight was obtained (W_3).The moisture content was determined using the following equation.

$$\text{Moisture (\%)} = \frac{(W2-W3)}{W1} * 100 \qquad \text{Equation (1)}$$

Where,

W_1 = weight of sample

W_2 = final weight of crucible + fresh sample

W_3 =weight of crucible + weight of sample after oven dried

Determination of crude ash content

Ash content was determined by high temperature incineration in an electric muffle furnace according to the AOAC (2000), using official method 925.09. First, clean porcelain crucible, dried at 105^0C in an air drying oven (Gallenkamp, model OV 880, England) for 30 minutes was cooled in desiccators and weighed (W_1). Then 2.5 g of powder vegetable sample was weighed into previously dried and weighed porcelain crucible (W_2). The sample was charred by hot plate at 120^0C for 1 hr until the contents were carbonized and turn black. The crucible with its content was placed in a Muffle furnace (CARBOLITE-Hope Sheffield, 5302RR-England) set at 550 ^{0}C for 5 hr to ignite until ashing was completed. The crucible with its residue was cooled in desiccators at room temperature for 30min and weighed (W_3). The total ash was expressed as percentages on dry matter basis as follows:-

Crude ash content (%) $=\frac{(W3-W2)}{W1}*100$ Equation (2)

Where,

W_1= weight of sample

W_2= weight of empty crucible

W_3= weight of crucible and sample after ashing

Determination of crude protein

Crude protein was determined by the method of AOAC (2000) using Kjeldahl method. Homogenous dried and ground vegetable sample (0.5g) was weighed into a digestion tube. Then, mixture of catalysts (5 g K_2SO_4, 1 g $CuSO_4$) and 6 mL of concentrated H_2SO_4 were added and the tube was gently shaken uniformly to wet and mix the sample with the acid. The digestion tube containing rack with exhaust system was loaded on a pre-heated digestion block for an hr and heated to 370°C for 3 hr allowing digestion. All samples were digested until the solutions turned green and clear. 50mL of distilled water and 25mL 40% sodium hydroxide solution were added to the digested sample solution. The distillation process was automatically started by using Kjeltec analyzer after sample was cooled. The digestion tube was placed in the distillation unit and safety door was closed. Distilled water (80 mL), 30mL of receiver solution (2% boric acid) and 50 mL of 40 % NaOH were added to digestion tubes, respectively. Then, the steam valve of Kjeltec was opened automatically and distilled approximately for 4-7 minutes. The distillate was titrated with standardized 0.1 N HCl using methyl red indicators until pink reddish end point was achieved. The percentage total nitrogen and crude protein contents were calculated using equation 3.

$$\% \text{ of nitrogen} = \frac{(V2 - V1)*N*14.01*100}{\text{Sample wt (mg)}}$$

Equation (3)

Where,

N = Normality of titrant (standard hydrochloric acid) (0.1N)

V1 = Volume in mL of standard hydrochloric acid solution used in the titration of the blank sample

V2 = Volume in mL of standard hydrochloric acid solution used in the titration for the sample

14.01 = the molecular weight of nitrogen

The % of nitrogen is converted to % of protein by using a conversion factor of 6.25.

Crude protein content (%, w/w) = %Nitrogen* conversion factor (i.e. %N*6.25) Equation (3)

Determination of crude fat

Crude fat content of both lettuce and kale samples was determined by AOAC (2000) method using Sohxlet extractor (insert company name, FP-SCZ-D, Hanan, China (Mainland). Two gram of each vegetable sample was weighed (W_1). A clean and dried muslin thimble containing the sample and covered with fat free cotton at the bottom and top was placed in the extraction chamber. 50mL of the extracting solvent (petroleum ether, boiling point 40-60^{0}C) was poured into the metal cap and fitted into the extraction unit. The extraction process was carried out for 2 hr for soaking and 2 hr for extraction. Then the thimbles with their residue were removed from the Sohxlet system and placed in drying oven at 105°C for 30 minutes. Then, the thimbles were cooled in desiccators and weighed (W_3). The fat content obtained was expressed as a percentage of the initial weight of the sample using the following formula.

Crude fat content (%) $= \frac{W_3-W_2}{W_1}*100$ Equation (4)

Where,

W_1= weight of sample (g)

W_2= weight of extraction thimble (g)

W_3= weight of extraction thimble with the dried crude fat (g)

Determination of crude fiber

Crude fiber was determined by the method of AOAC, (2000) using the official method 962.09. Two gram vegetable samples were transferred into a 750 mL Erlenmeyer flask and 200ml of boiling 1.25% H_2SO_4 was added and the flask was immediately set on a hot plate (IKA3581400, 230 VAC, Cole-Parmer-Canada) at 130 ^{0}C and condenser connected to it. The content was brought to boil within 1 minute and the sample was digested for 30 minutes. At the end of the 30 minutes, the flask was removed and the content was filtered through a linen cloth in a funnel and subsequently washed with boiling water until the washings were no longer acidic. The samples were washed back into the flask with 200mL boiling 1.25% NaOH solution. The condenser was again connected to the flask and the content of the flask was boiled for 30 minutes. It was then filtered through the linen cloth and thoroughly washed with boiling water until the washings were no longer alkaline. The residue was transferred to a clean crucible with a spatula and the remaining particles washed off with 15mL ethanol into the crucible. The crucible with its content was then dried in drying oven at 105^0C for 2hrs and cooled in desiccators and weighed (M_1). The crucible with its content was then ignited in a furnace at 550^0C for 2hrs, cooled and re-weighed (M_2). The loss in weight gave the crude fiber content and was expressed as a percentage of the initial weight of the sample. The total crude fiber was expressed in percentage as follows:-

Crude fiber content (%)= $\frac{(M2-M3)}{M1}*100$ Equation (5)

Where,

M_1 = weight of sample

M_2 = weight of crucible and sample after drying

M_3 = weight of crucible and sample after ashing

Determination of total carbohydrate

The percentage carbohydrate content was determined by subtracting the sum of the percentages of moisture, ash, protein and fat content from 100%.

Total carbohydrate content (%) =100–(%Moisture+%Ash+%Crude %protein+%Crude fat) ... Equation (6)

Utilizable carbohydrate content (%) = total carbohydrate – crude fiber

Calorific value (kcal/100g)

Energy value is quantified using an indirect calculation method. The three groups of nutrients, which provide the body with energy, are carbohydrates, fats and proteins (James, 1995). One gram of carbohydrate (C) was assumed to give 4Kcal energy; one gram of fat (F) 9Kcal energy and one gram of protein (P) 4Kcal. Therefore, determination of calorific value (Kcal/100g) of the vegetable samples was determined according to James (1995) as shown in equation 7 below.

Energy value = (P*4) + (F*9) + (C*4) in Kcal/100g of the sample Equation (7)

Where,

P = Protein content (%)

F = Fat content (%)

C = Available total carbohydrate (%)

Mineral analysis

Digestion of vegetable samples for mineral determination

The concentration of minerals in the lettuce and kale samples was performed by ICP-OES (Spectro ARCOS FHS12, Germany, 2010) after microwave digestion (AOAC, 2000, method 999.10).

About 0.5 g of freeze dried, ground and homogenized vegetable samples were weighed and placed in a digestion vessel. 3mL of 30% hydrogen peroxide (H_2O_2) and 5mL of concentrated nitric acid (HNO_3) was added in each digestion vessel and closed with the lid respectively. This was followed by adjusting the instrument to the appropriate temperature, pressure and time for digestion. Then, digestion vessels were placed in the microwave digestion machine (Model-MW-DE-01, U-Thermo International H.K) (Figure 6). After 52 minutes, digestion was completed which is signaled by the alarm sound from the instrument. Digested samples were cooled in a plastic container with cold water. Finally, all digested samples were makeup with distilled water to the final volume of 25 mL and transported to Debrezeit HORTICOOP Ethiopia P.L.C for analysis. The mineral content of lettuce and kale was determined after the standard solutions were prepared.

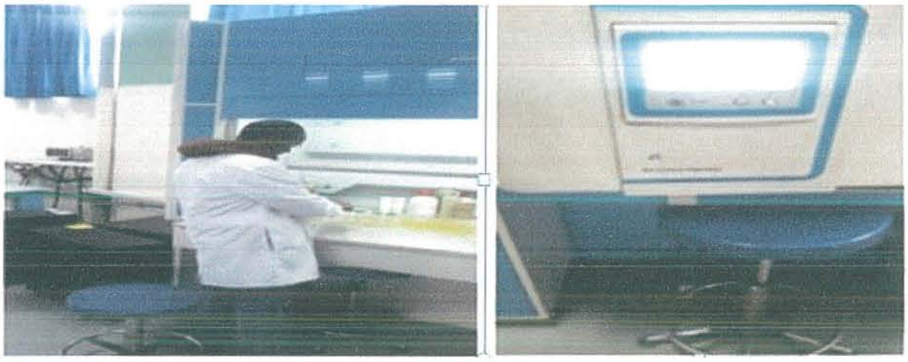


Figure 6: Microwave digestion of vegetable samples for mineral analysis

Elemental analysis of vegetable samples

Analysis of the mineral contents of lettuce and kale samples was determined by ICP-OES (Spectro ARCOS FHS12, Germany, 2010) and the concentration of all elements was determined using the following formula.

$$\text{Metal concentration (ppm)} = \frac{N*TV}{Wt} *DF \qquad \text{Equation (8)}$$

Where,

N = elemental concentrations of the sample solution read from the instrument

TV = total volume of the sample extract

DF = dilution factor

Wt = weight of sample

3.7.1. Beta carotene analysis

The beta-carotene content of lettuce and kale samples was determined according to the method by Hart and Scott (1995) with some modifications.

Samples preparation

About 3g vegetable samples were placed in a mortar, homogenized and extracted with 50mL cold acetone applied in small portions. The acetone extracts were collected and placed in a 125mL Erlenmeyer flask, the residue added and the mixture stirred manually for 1minute. The extract was removed, filtered through a sintered glass and the residue stirred manually with a mixture of 15mL acetone and 25mL hexane for 5 minute. All extracts were combined and placed in a separatory funnel.

The upper phase was removed and the remaining portion was evaporated to dryness in a rotary vacuum evaporator (RE-2000A, China) at about 35°C. Then, the concentrated extract was dried under liquid nitrogen gas and weighed.

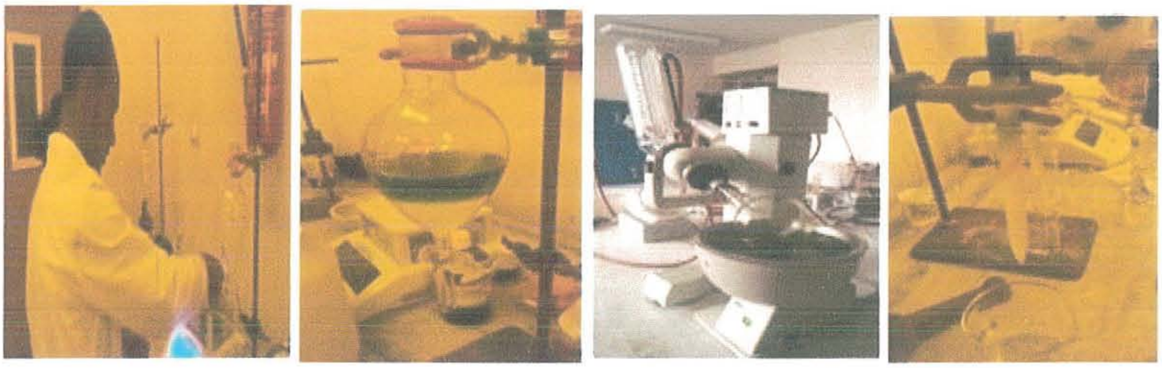


Figure 7: Preparation of vegetable samples for beta carotene analysis

Clean-up procedure

To remove color interference during HPLC analyses, the crude extract was cleaned up with open- column silica gel. Briefly, open glass column of 1.2mm diameter was cleaned, rinsed with n-hexane and dried. Then, small piece of defatted cotton was put at the bottom to prevent silica gel loss. 15g of dried silica was dissolved in n-hexane. Then, the silica was packed into the open-column with continuous solvent addition to avoid bubble formation and cracking. The dried crude extract was reconstituted with small amount of n-hexane and loaded to the column. Then, first 5mL of n-hexane was added into the column gradually. This resulted in the broader elution of orange pigment from the green extract. Then, to narrow the brown beta carotene pigment, a 4:1 combination of n-hexane to ethyl acetate was added gradually. The clear yellowish fraction was collected and dried under nitrogen. The fraction was kept at -20°C until the HPLC analyses (Rodriguez-Amaya, 2001). Then, prior to injection into HPLC the extract was dissolved in cold acetone, filtered through a 0.22mm PTFE syringe filter (Millipore) and about 10mL of sample extract was placed into sample vials.

HPLC instrument

HPLC (Shimadzu- CTO-20AC S. No L20214605018AE 220-240V~ 50-600VA, Japan) which consists of binary pump, auto sampler, column, and thermostat and chemistation software was used.

Column

A stainless spherisorb C18 (4.6 x150 mm) separating column with 3μm Particle size and column temperature of 30°C was used.

Mobile phases

The mobile phases consisted of an isocratic solvent system of acetonitrile: triethylamine: methanol in the ratio of 80:10:10 with the flow rate of 0.7mL/min. The mobile phase was degassed using an ultra-sonic bath and filtered through 0.22mm Millipore filter paper before use.

Standardization, quantification and identification

Stock standard beta carotene (Sigma Chemical Co., USA) was prepared by dissolving approximately 10mg of beta carotene in 100 mL of n-hexane in a 100mL volumetric flask. This solution was stored in polypropylene, autoclavable plastic vials at -20°C. A working standard was prepared by dilution of the stock standard solution with n-hexane to 100mL, where after the concentration of beta carotene was determined by measuring the absorbance of the diluted solution at 450 nm on HPLC. Standardization was done using 5, 10, 20, 40, 50, 80 and 100 ppm of beta carotene standard solutions. A calibration plot of peak area against concentration (μg/mL) was prepared for the standard solution. Peak identification for samples was based on the retention time and comparison with standards.

Method validation

In this study, a simple, efficient and accurate improved HPLC method mainly derived from the procedures of Hart & Scott (1995) with some modification was used for the determination of beta-carotene in the fresh kale and lettuce cultivated through aquaponic and soil-based system. Chromatographic method validation was done by checking different parameters such as identification, accuracy, recovery, linearity, LOD and LOQ

Identification

Identification of beta-carotene from the test sample was done according to retention time on the HPLC chromatogram which was obtained after running standard beta-carotene with different concentrations (5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm and 100ppm). Accordingly, the beta-carotene retention time was 9.0969; precision of the retention time measured by using percent relative standard deviation was 0.198%, percent relative standard below 2% is acceptable (FDA, 2002).

Table 1: HPLC based Beta carotene identification

Standard β-carotene (5,10,20,40,50,80,and 100)ppm	**Mean (min)**	**N**	**Standard Deviation**	**% RSD**
	9.10	8	0.018	0.198

N= number of replications, RSD= Relative Standard Deviation

Well resolved chromatograms were obtained from running the different concentrations of beta-carotene standards in HPLC (Appendix VI). Besides, there was no mobile phase interfrance (Appendix VII).

Precision

The repeatability of the analytical method was tested by injecting 7 replicates of 5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm and 100ppm of beta-carotene standard under the same analytical conditions within the same day. According to FDA standard the acceptable level of percent relative standard deviation for precision is ≤ 2%. For the peak area % RSD is less than 2%, which is acceptable.

Table 2: Repeated injection of different standard beta-carotene concentrations to check precision of the method

	β-carotene concentration						
	5ppm	**10ppm**	**20ppm**	**40ppm**	**50ppm**	**80ppm**	**100ppm**
Peak Area (nm)	444848	1026147	1821854	3003999	4526516	6852071	7889030
	444062	1025374	1823599	2999233	4546285	6850527	7882525
	447008	1027291	1824384	2997094	4563495	6855641	7890406
	448243	1029663	1826946	2995487	4577869	6861285	7876587
	447564	1031168	1827463	2994531	4594261	6867161	7869765
	448746	1035209	1831823	2993971	4610502	6873452	7860232
	448341	1038440	1835939	2993182	4629732	6879870	7862357
Mean	446973	1030470	1827429	2996785	4578380	6862858	7875843
SD	1824.27	4862.80	4949.71	3781.43	36200.47	11129.01	12204.40
% RSD	0.408	0.472	0.271	0.126	0.791	0.162	0.155

SD= Standard Deviation, RSD= Relative Standard Deviation

Limit of detection and limit of quantification

Among concentrations of 0.08, 0.05, 0.025 and 0.0125 ppm of beta carotene standard the instrument detected the smallest analyte concentration of 0.0125 ppm (LOD) (Appendix VIII) with signal to noise ratio of 8.375 which is greater than 3, thus acceptable. Below this concneteration (0.0125ppm) beta carotene peak was not detected (Appendix X). The limit of quantification of the instrument was 0.025 with signal to noise ratio 17.6 which is greater than 10 and acceptable (Table 3).

Table 3: Limit of detection and Limit of quantification of beta carotene

Beta-carotene	LOD (ppm)	Signal to noise ratio (S/N)	LOQ (ppm)	Signal to noise ratio(S/N)
	0.0125	8.38	0.025	17.60

LOD= Limit of Detection LOQ= Limit of Quantification S/N= Signal to Noise ratio

0.0125ppm

Linearity

Coefficient of determination (R^2) is the main criteria that FDA uses to check the acceptability of linearity of data, which was obtained from y-intercept of the linear regression line for the peak area versus concentration plot. As reported in Table 4, the coefficient of determination lied between 0.9945-0.9999 indicating the presence of strong relationship between the concentration of the analyte and peak area. The correlation coefficient was almost equivalent with 0.998, which showed a reliable linearity (FDA, 2002). Linearity of the analysis was evaluated by injecting five series of beta-carotene standards (5, 10, 20, 50, and 80 ppm) to indicate the presence of direct linear relationship between the concentration of the analyte and the peak area on the chromatogram with 7 runs (Appendix XI).

Table 4: Linearity check

Beta-carotene	Number of runs (N)	Calibration curve equation	R^2
	7	Y=85531X+126693	0.9981

Accuracy and recovery

According to FDA (2002) mean percent recovery should be within the range of 70-120 to be acceptable method. As shown in Table 5, the method used was accurate within the desired recovery range set by FDA and the percent relative standard deviation were less than 1% (FDA, 2002).

Table 5: Accuracy and recovery of different concentrations of beta-carotene injected into HPLC

	β-carotene Spiking Concentration					**β-carotene %Recovery**				
	5ppm	**10ppm**	**20ppm**	**50ppm**	**80ppm**	**5ppm**	**10ppm**	**20ppm**	**50ppm**	**80ppm**
	3.74	10.51	19.81	51.44	78.63	74.80	105.10	99.05	102.80	98.29
	3.71	10.50	19.83	51.67	78.61	74.20	105.00	99.15	103.30	98.26
	3.75	10.52	19.84	51.87	78.67	74.90	105.20	99.20	103.74	98.34
	3.76	10.55	19.87	52.04	78.73	75.10	105.50	99.35	104.08	98.41
	3.75	10.57	19.88	52.23	78.80	75.00	105.70	99.40	104.46	98.50
	3.77	10.62	19.93	52.42	78.88	75.30	106.20	99.65	104.84	98.60
	3.76	10.65	19.98	52.64	78.95	75.20	106.50	99.90	105.28	98.69
Mean ± SD	3.74 ± 0.01	10.57 ± 0.06	19.87 ± 0.06	52.04 ± 0.42	78.75 ± 0.13	74.92 ± 0.36	105.60 ± 0.57	99.38 ± 0.30	104.00 ± 0.86	98.44 ± 0.16
%RSD	0.26	0.53	0.29	0.80	0.16	0.48	0.54	0.30	0.83	0.16

After the method validation, beta-carotene content of the soil-based and aquaponically grown lettuce and kale was determined.

Sample analysis

HPLC analysis of beta carotene from the sample was carried out by injecting 20µL of the extract in to the HPLC column. The column was eluted isocratically with the mobile phase at a flow rate of 0.7mL/min. The effluent was monitored with UV detector responses set at a wave length of 450 nm. Duration of the run time was 15min/sample. The concentration of beta carotene in the sample was quantitated in the HPLC using the peak area under the curve with working standard solution as reference. All results were expressed as µg/100g fresh weight of the vegetables (lettuce and kale) and β-carotene concentration of samples was calculated as follows:

$$\beta\text{-carotene } \mu g/100g \text{ FW } (Cx) = \frac{A_x * C_s (\mu g/mL) * \text{total volume of (mL)}}{A_s * \text{ sample weight}}$$

Where,

Cx= concentration of carotenoid of sample

Ax= peak area of sample under curve

Cs= concentration of standard curve

As= peak area of the standard

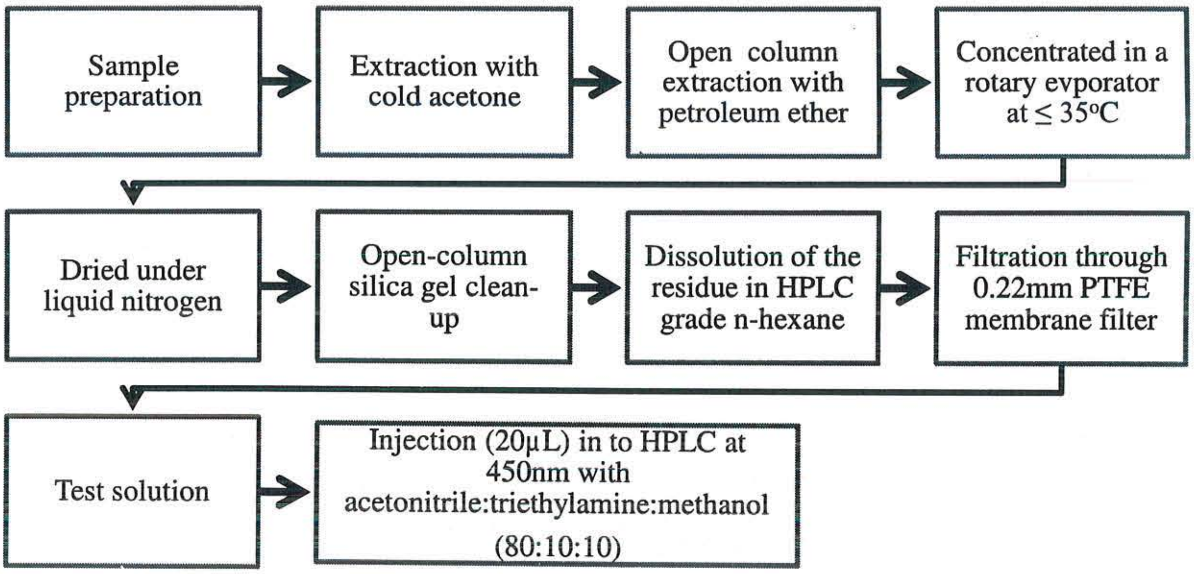


Figure 8: Schematic representation of beta carotene analysis

3.7.2. Vitamin C content

The vitamin C content of vegetables was determined according to AOAC (2016) using the official method 962.09.

Standard vitamin C (ascorbic acid (AA) solution)

About 100mg of AA was weighed out in beaker and dissolved with 5% metaphosphoric acid. Then, the solution was making up to 100mL with 5% metaphosphoric acid. Each mL of the solution contains 1mg AA. To prepare 10,20,30,40 & 50 µg, 0.5, 1, 1.5,2 & 2.5mL of standard solution was taken in test tubes respectively and diluted to 4mL with 5% metaphosphoric acid.

Control preparation

From 4ml AA, 1mL of solution was taken and transferred to 50mL volumetric flask and 1mL saturated Bromine water solution was added and diluted to 50mL with 5% metaphosphoric acid. Then, the solution was aerated in a conical flask and 0.5g thiourea was added to the solution to expel excess bromine. From the clear solution, 0.5mL was taken in test tube and diluted to 4mL with 5% metaphsphoric acid.

Sample preparation

About 5g of vegetable sample was extracted with 100ml of 6% TCA by mortar & pestle for 2-5 minutes. Then, the suspended solid was removed by centrifuging. In a conical flask containing sample solution 1-2 drops of saturated Bromine solution was added & aerated. To 10mL aliquot, 10mL of 2% thiourea was added and from this solution 4ml was pipette into each of 3 test tubes. Also one test tube was set aside to serve as blank and to each of the remaining tubes 1mL of 2, 4-di nitro phenyl hydrazine (DNPH) was added. Then all test tubes were placed in water bath at 37^{0}C For 3 hr and cooled in an ice bath for approximately 5 minutes. 5mL of 85% H_2SO_4 was added slowly while the tubes were in an ice bath in the meantime, 1mL of 2% DNPH was added to the blank and all tubes were mixed very well. Then, all tubes were let to stand at room temperature for 30 minutes. Finally the absorbance of the standards, blank and test samples was read at 515nm using spectrophotometer (THERMO SCIENTIFIC-evolution 220 UV- Vis spectrophotometer-US). Vitamin C concentration was calculated as follows:

$$\text{Vitamin C (mg/100g)} = \frac{[(As - Ab)]*10}{[(As_{td} - Abs_{td})]}$$

Where

As = absorbance of sample

Ab = absorbance of blank

A_{std}= absorbance of standard concentration (mL)

A_{bstd}= absorbance of blank for standard and

10 = dilution factor

3.7.3. Nitrate concentration

Nitrate concentration in composite dried, ground and homogenized vegetable leaves was determined according to the method described by (Lastra, 2003) using salicylic acid with some modifications.

Reagent preparation

All reagents were of analytical grade

a) Salicylic acid solution: In a 100mL volumetric flask, about 5g of salicylic acid was weighed and dissolved in concentrated sulfuric acid and diluted with the same acid. Then, the solution was stored in an amber bottle at 4°C until analyses was carried out.

b) Nitrate- N standard solution: about 500 mg of KNO_3 was weighed and placed in 500mL volumetric flask. Then, dissolved in deionized water and make up with distilled water to the final volume of 500 mL. Working standard solutions of 10, 20, 30, 40, 50, 60, 70, 80, and 100mg/L were prepared by diluting the standard solution with deionized water.

c) 2N Sodium hydroxide solution: 40g of sodium hydroxide pellets was weighed and dissolved in deionized water and fill up to 500mL with deionized water.

Extraction procedure

About 0.1g of freeze-dried, ground and homogenized powder of lettuce and kale leave samples were extracted with 10mL hot deionized water and kept in water bath (YCW-0125, GFL- Germany) at 80 °C for 30 minutes with continuous shaking using water bath with shaker. After 30 minutes, the solution was removed from the water bath (YCW-0125, GFL- Germany) and cooled at room temperature and then filtered through whatman No. 40 filter paper. The supernatants were decanted and kept for analyses.

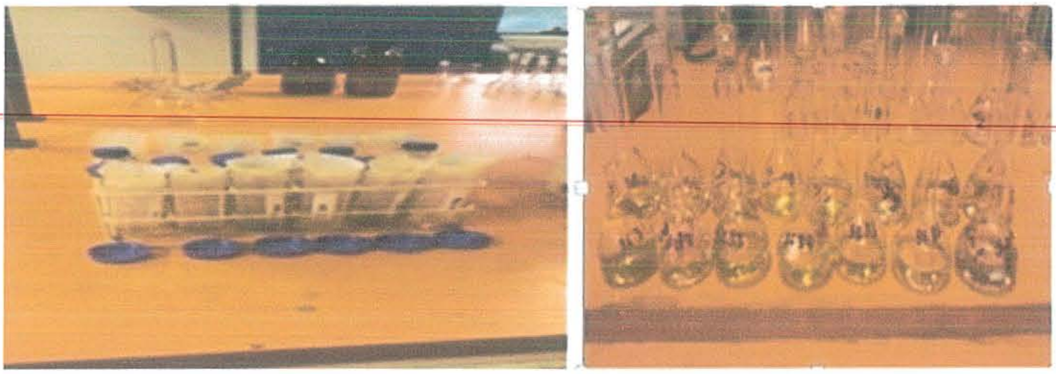


Figure 9: Extracted kale and lettuce samples for nitrate content determination

Determination of calibration curve

0.1 mL aliquots of working standard solutions (i.e. 10-100mg/L) $KNO_3$was measured and placed in 50 mL plastic screw caped test tubes and mixed with 0.4mL salicylic acid. Then, each test tube was left aside for 20 minutes until the formation of nitro salicylic acid is completed. After 20 minutes at room temperature, 9.5 mL of 2N NaOH solution was slowly added and mixed well to obtain 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0mg/L NO_3-N solution. Upon the addition of 2N NaOH solution, yellow color solution was formed. Then, the solution was left aside to cool down at room temperature and finally absorbance was determined at 410 nm using spectrophotometer (Uv-Vis, Liantrisant, and Model-CF728YW-UK).

Nitrate determination

0.1mL of the extracts waspipetteinto 15mL screw caped plastic test tubes, and mixed thoroughly with 0.4 mL of 5% (w/v) salicylic acid in concentrated H_2SO_4. After 20 minutes at room temperature, 9.5 mL of 2N NaOH was added slowly with a micropipette to raise the pH. Samples were cooled to room temperature and absorbance at 410 nm was determined in a Liantrisant, and Model-CF728YW-UK) spectrophotometer. For extracts from dried and ground vegetables, a blank of 0.1 mL H_2O and the normal reagents were also prepared. Nitrate concentrations were expressed as µg NO_3 per g dry weight (ppm) and nitrate concentration in vegetables was calculatedas follows:

$$Cx = \frac{C*V}{W}$$

Where,

C = the concentration of sample as derived from the standard curve (ppm)

V = the final volume of sample after filtration (ml) and

W = sample weight (g)

Statistical data analysis for compositional analyses

Statistical analysis was performed using SPSS® (version 23). Student's t-test was carried out to determine level of significance between means. All analyses were conducted in triplicate and results were expressed as mean ± standard error.

3.8. Sensory evaluation of lettuce sample from Shewa Robit and AAU sites

3.8.1. Lettuce harvesting from Shewa Robit and AAU site

The vegetables from both sites as well as from field and aquaponic systems were harvested between 7AM and 8AM in the morning for consistency by hand when they reached maturity stage. Then, lettuce samples were placed in a clean plastic bag then in ice box and immediately transported to the Shewa Robit Higher Education Preparatory and General Secondary School and Center for Food Science and Nutrition laboratory of AAU respectively, where sensory evaluation was done using score sheets.

3.8.2. Lettuce sample preparation and sensory evaluation

Freshly harvested lettuce samples were cleaned with tap water to remove dust particles and immersed in diluted lemon juice for a few minutes to avoid microbial contamination. Then, the lettuce samples were washed and rinsed with tap water again to washout the lemon juice to avoidits masking effect on taste. Prior to the experiment, bite-sized samples, about 15g was placed on identical clear plastic plates coded with three random numbers representing the sources of vegetable samples. Finally, samples were served for sensory evaluation at room temperature.

Figure 10: Lettuce sample preparation for sensory evaluation

3.8.3. Orientation about sensory evaluation

The sensory evaluation study was designed to determine preference and overall acceptability of aquaponically grown vegetables as compared with field-grown vegetables on the basis of visual appearance, color, taste, texture and overall acceptability. Difference test, preference test, and rating acceptance test methods were applied. Before each test session, participants were given orientation about the aquaponics technology and overall sensory evaluation procedure as shown in Figure 11.

First participants were provided with two samples on plastic plates (one aquaponically grown lettuce and the other soil-grown lettuce) and they were asked to taste and evaluate the difference between the two samples on the basis of visual appearance, color, taste, texture and overall acceptability. Preference tests were conducted after the difference test was completed. Participants were given two coded samples (one aquaponically grown lettuce and the other soil-grown lettuce) and were asked to mark the sample they preferred. Rating acceptance test was conducted using a 9-point hedonic scale rated from (1=dislike extremely, 2 = dislike very much, 3= dislike slightly 4 = dislike moderately, 5= neither like nor dislike, 6 = slightly like, 7= like moderately, 8 = like very much and 9= Like extremely). Finally, the participants rated the samples by putting number scores under the degree of their satisfaction for each sensory attributes (Appendix III and IV).

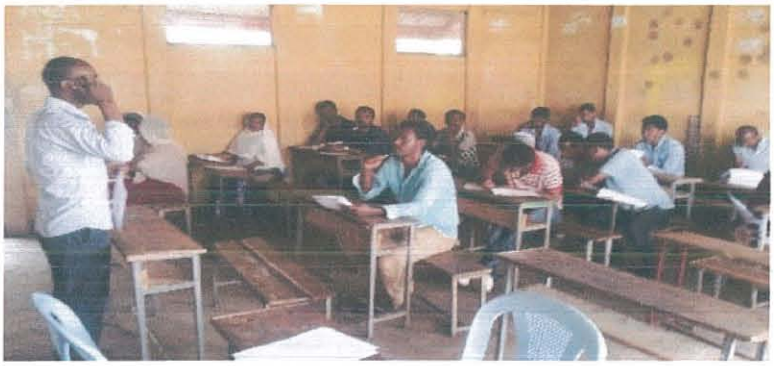


Figure 11: Orientation about sensory evaluation procedure

3.8.4. Kale sample preparation for sensory evaluation at AAU site

Freshly cut composite samples of kale were collected from field and aquaponic systems from AAU site and brought to laboratory in clean plastic bag in ice box for sensory evaluation. Then, the fresh harvests were cleaned with tap water to remove dust particles. Then, the kale leaves were sliced to smaller pieces with a kitchen knife, immersed in 1L of boiling water at 96°C, cooked for 70 minutes and drained using a sieve. The drained samples were immediately cooled at room temperature and finally served to participants for sensory evaluation (Figure 12).

Figure 12: Kale sample preparation for sensory evaluation at AAU site

3.8.5. Procedure for sensory evaluation of kale harvested from AAU site

The sensory evaluation study was designed to determine preference and overall acceptability of aquaponically grown vegetables over field-grown ones. Difference test, preference test, and rating acceptance tests using 5 sensory attributes were used to evaluate the lettuce samples.

First participants were provided with two samples on plastic plates (one aquaponically grown kale and the other soil-grown kale) and were asked to taste and evaluate the difference between aquaponic and soil-grown kale samples on the basis of visual appearance, color, taste, texture and overall acceptability (Appendix III). Preference tests were conducted after the difference test was completed. Participants were given two coded samples (one aquaponically grown kale and the other soil-grown kale) and were asked to mark the sample they preferred (Appendix IV). Rating acceptance test was conducted using a 9-point hedonic scale rated from (1=dislike extremely, 2 = dislike very much, 3= dislike slightly 4 = dislike moderately, 5= neither like nor dislike, 6 = slightly like, 7= like moderately, 8 = like very much and 9= Like extremely). The participants rated the samples by putting number scores under the degree of their satisfaction for each sensory attributes.

3.8.6. Participant selection

First, we recieved the approval letter from the ethical committee (CNS-IBR) of college of Natural & Computational Sciences Institutional Review Board after the submission of proposal and fulfilling all the criteria required. Participants were untrained panelists and were member of the community at Shewa Robit town; 19 male and 3 female aged from 26-42. Similarly, the participants from AAU site included 10 female and 23 male members, ages 22 to 38 with a mean age of 30 years who were graduate students at AAU. Panelists were selected according to the following criteria: people without food allergies specifically for vegetables (i.e. lettuce and kale), non-smokers, people who consume vegetable products at least once a week, people available for all sensory tests, people interested in participating, and people able to express their feeling verbally regarding the product (Zhao *et al.*, 2007).

3.9.Microbiological test

Microbial analysis of water from fish tank and vegetables was performed by using the membrane filtration technique (MF) according to the International Standards Organization (ISO) protocols, for the detection of total coliforms (TC), (ISO 9308- 1:2000, 2000); and fecal coliform (EC), (ISO 9308- 1:2000; 2000).

Sample preparation

Six samples (including three vegetable samples and three water samples from the aquaponic system) were collected from both sites (i.e. Shewa robit and Addis Ababa University aquaponic facilities). Briefly, water and vegetable samples were collected aseptically following standard procedures. Water samples from fish tanks were collected by submerging the pre-sterilized glass container into the fish tank and immediately recapping the glass container. For vegetable samples, composite and representative freshly harvested lettuce and kale leaves from aquaponic system were placed into glass containers that contain a saline solution. Within 12 hours after collection, samples were immediately submitted to an accredited private testing laboratory (Bless Agri Food Laboratory Service) for analyses.

Analysis for total coliform and fecal coliform

Total coliform and fecal coliform bacteria were analyzed from system water and vegetables grown in aquaponic system. The membrane filtration (MF) method ISO 9308-1:2000, 2000 with LES-ENDO and mFC agar was taken as the reference method for total coliform and fecal coliform detection respectively.

Filtration and incubation of samples

For MF (Membrane Filtration) technique, a-100mL water sample was filtered through hydrophilic mixed nitrocellulose esters membranes of 0.45-µm pore size and 47mm of diameter for all organisms. The filter was then placed on the selective medium which is incubated at 37°C and at 44°C for 24 ± 2 hr for coliform and *E.coli* respectively, with subsequent further biochemical characterization of the typical lactose-positive colonies, leading to the detection and enumeration of coli form bacteria and *E.coli* within 2 to 3 days.

Evaluation and confirmation, standard test

The characteristic colonies on the membrane are counted as lactose-positive bacteria. For coliform bacteria and *E.coli*, subculture was carried out of randomly selected characteristic colonies for confirmatory tests: oxidase and indole production. The number of lactose-positive coliforms bacteria and *E.coli* likely to be present in 100mL of the sample are counted.The oxidase-negative and lactose positive colonies described by the MF method were counted as total coliforms and the indole- positive at 44°C for 24 ± 2 h gas from lactose-forming coliform colonies were confirmed as *E.coli.* The numbers of bacterial colonies were calculated using the following formula.

$$Cs = \frac{Z^{*}\ Vs}{V_{tot}}$$

Where

Cs = the estimated number of cfu in the reference volume (Vs);

Z = the sum of colonies counted on plates or on membranes derived from dilutions $d_1, d_2 \ldots d_i$ or derived from separate volumes of the test portion (sample or dilution);

V_s= the reference volume chosen to express the concentration of the micro-organisms in the sample;

V_{tot}= the calculated total volume of original sample included in the plates enumerated.

V_{tot}= either the sum of the separate volumes of the test portion (sample or dilution) or calculated by equation (2):

$$Vtot = (n_1 v_1 d_1) + (n_2 v_2 d_2) + \ldots\ldots\ldots + (n_i v_i d_i)$$

Where

Vtot is the calculated total volume of original sample included in the plates enumerated;

$N_1, n_2 \ldots n_i$, is the number of plates counted for dilution $d_1, d_2 \ldots d_i$;

$V_1, v_2 \ldots v_i$, is the test volume used with dilution $d_1, d_2 \ldots d_i$;

$d_1, d_2 \ldots d_i$, is the dilution used for test volume $v_1, v_2 \ldots v_i$ (d= 1 for undiluted sample, d = 0.1 for a ten-fold dilution

3.10. Frame work of the research experiments

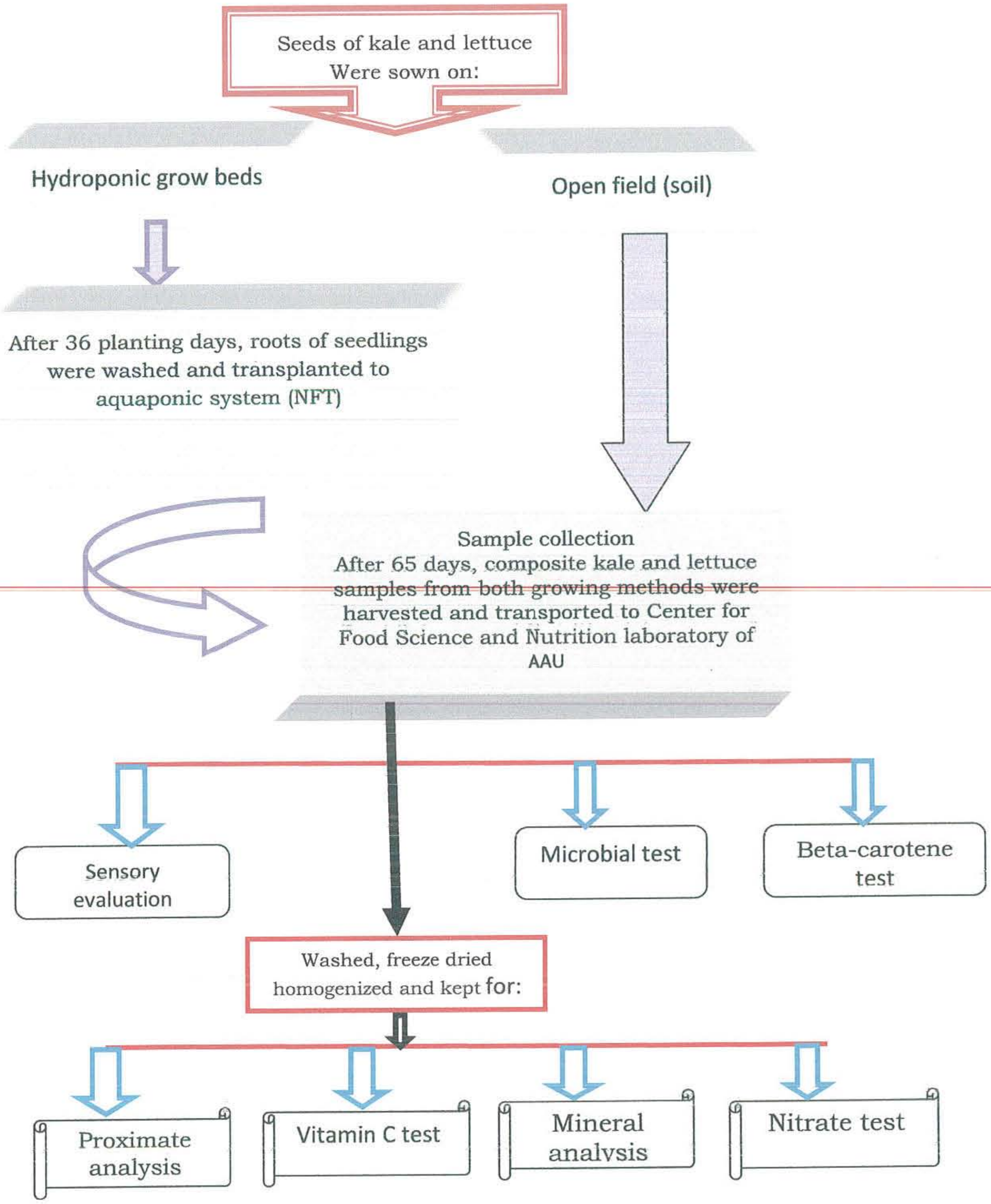


Figure 13: Experimental frame of the whole study

CHAPTER FOUR

4. RESULTS AND DISCUSSION

Comparing biochemical composition of aquaponic produces reported in different studies might not be accurate. This is because of many confounding factors such as seed variety, environmental conditions, aquaponic facility etc. Hence, in this document results are compared with range values of previous studies as a contribution to the research pool. The detailed results are reported and discussed as follows.

4.1. Proximate composition of lettuce and kale at AAU site

The results of proximate composition of aquaponically and soil-grown lettuce (*L. sativa* L.) and kale (*Brassica carinata*) harvested from Addis Ababa University site are presented in Table 6.

Table 6: Proximate composition of lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
Nutrient (g/100g)	Soil-based lettuce	Aquaponic lettuce
Moisture	5.20 ± 0.20	4.70 ± 0.10
Crude protein	14.65 ± 0.00	16.23 ± 0.00
Crude fat	9.75 ± 0.05	9.95 ± 0.15
Crude ash	14.00 ± 1.60	$21.80 \pm 0.60^{*}$
Crude fiber	18.38 ± 0.18	$26.78 \pm 1.75^{*}$
Utilizable carbohydrate	68.20 ± 0.05	$47.32 \pm 0.85^{**}$
Gross energy (kcal/100g)	419.15 ± 0.25	$343.75 \pm 2.05^{**}$
	Kale	
Nutrient (g/100g)	Soil-based kale	Aquaponic kale
Moisture	7.30 ± 0.10	$5.60 \pm 0.20^{*}$
Crude protein	9.23 ± 0.00	12.60 ± 0.00
Crude fat	3.25 ± 0.25	$5.75 \pm 0.25^{*}$
Crude ash	9.55 ± 0.05	$4.20 \pm 0.20^{**}$
Crude fiber	21.44 ± 0.79	$27.22 \pm 0.27^{*}$
Utilizable carbohydrate	70.67 ± 0.10	$73.85 \pm 0.65^{*}$
Gross energy (kcal/100g)	348.85 ± 1.85	$397.55 \pm 0.35^{**}$

All values are expressed as mean ± SE. (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test $^{*}p<0.05$; $^{**}p<0.01$.

Through conventional soil-based system, soil provides all the necessary nutrients required for normal plant growth. However, in soilless systems like aquaponics plants obtain their nutrient from fish waste. Studies on chemical concentrations of fish effluents showed that certain elements were deficient in aquaponic system which in turn could result in difference in plant biochemical composition. Based on this anticipated variation in nutrients, in this study the proximate composition of vegetables grown on aquaponic system was compared with the soil-based harvests. Accordingly, there was no significant difference ($p<0.05$) in crude protein and crude fat contents between aquaponically and soil-grown lettuce. Meanwhile, there was a significant difference in carbohydrate and caloric value ($p<0.01$), crude ash and crude fiber content ($p<0.05$) between vegetables cultivated in the two systems (Table 6). Accordingly, aquaponically grown lettuce had higher ash content as compared to soil-grown lettuce. This value was relatively higher than the value reported by Gladys and Aba (2015). Ash content is generally a measure of the mineral content of food samples (Onwuka, 2005). Thus, higher ash content from aquaponically grown lettuce might indicate the presence/abundance of important minerals. Higher protein content was obtained in aquaponically grown lettuce. However, statistically there was no significant difference. In fact; this value was even higher than previous report by Ranawade *et al.* (2017). Lettuce is a good source of dietary fiber (Dahl and Stewart, 2015). In this study, the crude fiber content of aquaponically grown lettuce was higher than soil-grown harvests. This difference could be due to variation in environment in which the produce cultivated. In an animal study, the inclusion of red oak leaf lettuce in the diet lowered cholesterol (Nicolle *et al.*, 2004). Thus, growing lettuce aquaponically might benefit consumers with improved nutrients such as fiber. The soil grown lettuce had higher carbohydrate and gross energy contents than the aquaponic lettuce (Table 6). Kale is one of the green leafy vegetables that belong to the Brassicaceae or Cruciferеae family. It is a species of plant that includes many common foods like cabbage, broccoli, cauliflower kale, Brussels sprouts, collard greens, Savoy, kohlrabi and Chinese kale (Agarwal *et al.*, 2017). It is easy to cultivate and can grow in colder temperatures. The proximate composition of kale (*Brassica carinata*) cultivated through soil-based and aquaponic system is shown in Table 6.

As reported in Table 6, the aquaponic kale had significantly higher contents of crude fat, crude fiber, carbohydrate and caloric value ($p<0.05$) than the soil-based harvest. The average fat content obtained in this study was higher than those reported by Afrin *et al.* (2018) and Gladys and Aba (2015) on similar harvests. Similarly, the carbohydrate content of both aquaponic and soil-based kale in this study was higher than the contents in other vegetables such as lettuce.

Kale is an excellent source of fiber, which is an important nutrient to reduce diet related diseases (Emebu *et al.*, 2011). In this study, the highest fiber content was obtained from aquaponically grown kale. In contrast, the crude ash content was significantly lower in the aquaponic kale ($p<0.01$). However, the ash content of the aquaponic kale in this study was higher than the value in other vegetables such as spinach (*Spinacia oleraceae*) 1.1g/100g, lettuce (*L. sativa*) 0.8g/100g and cauliflower (*Brassica oleraceae*) 0.6g/100g (Hanif *et al.*, 2006). Numerically, the aquaponic kale had higher crude protein content than the soil-based harvest. In fact this value was higher than the report by Ranawade *et al.* (2017) for hydroponic and aquaponically grown spinach (2.9 and 2.7) g/100g respectively. Afrin *et al.* (2018) also reported a lower protein content of 1.89 and 2.29% for aquaponic cauliflower than the value in the present study. The higher protein content might be linked with nitrogen accumulation in the form of NH_3 in the system (Acikgoz, 2011). This accumulation of nitrogen in the young leaves will be converted to nitrogenous biomolecules like protein. The accumulation of N-nitrate could be a good indicator of the efficiency of the aquaponic system.

4.2. Mineral composition of aquaponically and soil-grown lettuce and kale

Macro minerals

Vegetables are the major sources of vitamins A and C, minerals, dietary fiber and polyphenols/flavonoids. They also add flavor to diets (Asfaw, 1997). Aquaponics provides an opportunity for growing and consuming fresh fruits and vegetables cultivated closer to home (i.e. implying important role in inmproving diet diversity). But it is challenging to compare the nutritional content of aquaponically grown vegetables with soil-grow ones because of the fundamental differences between the two different methods (Murphy *et al.*, 2011). Soil is usually the most available growing medium for plants, proving essential nutrients for proper growth. In contrast, in aquaponic system, plants obtain nutrients from effluents of fish which is deficient in certain nutrients (Munguia-Frago *et al.*, 2015). Hence, it is important to know if this variation influenced the mineral composition of the harvests. Therefore, in this study mineral contents of aquaponic and soil- grown lettuce were compared. The aquaponically grown lettuce had a significantly higher composition of all macro minerals except potassium and sulfur ($p<0.001$) (Table 7). The calcium concentration was higher than the values reported by Pantanella *et al.* (2010). One of the critical elements in aquaponics is potassium, which is an important component in fruiting and ripening (Pantanella, 2012). In this study, potassium concentration was lower in the aquaponic lettuce. However, this value is higher than the reports by Saha *et al.* (2016) (1.71%). In contrast, the sodium concentration was higher in the aquaponic lettuce in the present study. According to Vicente *et al.* (2009) vegetables are low in sodium and high in potassium content which agrees with the reports in this study for aquaponic lettuce. Potassium is not needed by the fish and it was not added to the fish feed in the aquaponic system. Hence, it is reported that aquaponic systems that rely solely on fish waste to supply nutrients for the plants have low levels of K, P, Fe, Mn, and sulfur (Roosta and Hamidpour 2011). However, the reverse is true for reports in this study. Minerals are basically classified as macro and micros based on the relative requirement of each mineral by human beings. The macro mineral composition of aquaponic and soil-based kale at AAU site is given in Table 7.

As reported in Table 7, the concentrations of major minerals including calcium, magnesium, sodium, potassium, phosphorus and sulfur were significantly higher in aquaponic kale than soil-based harvests ($p<0.001$). As per previous reports, concentrations of nutrients in aquaponics are usually lower than other growing methods like hydroponic system. Howver, the higher concentration of macro-minerals in the aquaponically grown kale in this study can be due to the varriation in nutritional requirements of aquaponic plants with day length, variety, and weather conditions. Besides, variation in pH, solubility, plant absorption and mineralization of the fish food can also influence the biochemical content in aquaponic crops (Pineda-Pineda *et al.*, 2017). Moreover, appropriate fish stocking rate and fish feed composition have direct influence on the biochemical composition of aquaponic crops and fish as well. A study carried out by Pineda-Pineda *et al.* (2017) indicated that with different fish stocking density there was a differentiation in the concentration of nutrients in plant tissues. Thus, in this study high macro mineral content in aquaponic kale might suggest that there was sufficient amount of NH_4^+ inthe aquaponic solution and easily absorbed by the plant.Therefore, with controlled system aquaponics can provide vegetables with high major mineral compositions.

Table 7: Macro-mineral content of lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
Minerals (mg kg^{-1})	Soil-based lettuce	Aquaponic lettuce
Calcium	18033.50 ± 41.5	24547.54 ± 153.02***
Magnesium	2744.64 ± 7.43	4590.47 ± 29.31***
Sodium	427.32 ± 1.39	774.22 ± 5.42***
Potassium	74507.93 ± 454.42	62338.59 ± 188.39***
Phosphorus	5589.27 ± 38.82	7324.73 ± 21.44***
Sulfur	4054.38 ± 7.26	3286.79 ± 16.19***
	Kale	
Minerals (mg kg^{-1})	Soil-based Kale	Aquaponic Kale
Calcium	18675.45 ± 26.43	36094.18 ± 58.31***
Magnesium	1691.59 ± 8.16	3441.78 ± 23.48***
Sodium	132.83 ± 0.27	463.34 ± 1.86***
Potassium	33347.21 ± 71.66	41482.24 ± 228.46***
Phosphorus	3977.55 ± 22.06	4679.69 ± 18.83***
Sulfur	9015.84 ± 39.86	10600.72 ± 61.70***

All values are expressed as mean ± SE. (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test; $^{***}p<0.001$.

Micro minerals

The mean concentrations of essential micro minerals in the soil-based and aquaponic lettuce and kale from Addis Ababa University site are summarized in Table 8.

Table 8: Micro mineral contents of lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
Micro-minerals (mg L^{-1})	Soil- based lettuce	Aquaponic lettuce
Iron	1709.09 ± 4.25	2165.66 ± 2.78***
Zinc	81.44 ± 1.58	63.85 ± 1.21**
Boron	21.68 ± 0.38	30.56 ± 0.47*
Copper	62.54 ± 1.31	130.85 ± 2.26***
Manganese	36.09 ± 0.26	76.35 ± 1.20***
	Kale	
Micro-minerals (mg L^{-1})	Soil- based Kale	Aquaponic Kale
Iron	503.19 ± 1.01	177.18 ± 1.92***
Zinc	40.35 ± 0.08	62.78 ± 0.27***
Boron	22.49 ± 0.49	20.06 ± 0.31*
Copper	5.30 ± 0.11	8.64 ± 0.31**
Manganese	32.46 ± 0.78	76.48 ± 1.08***

All values are expressed as mean ± SE (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

As reported in Table 8, aquaponically-grown lettuce had significantly higher concentration of iron, copper, manganese ($p<0.001$) and boron ($p<0.05$). In contrast, soil-based lettuce had increased zinc content than the aquaponic harvest ($p<0.01$). The iron content in this study was higher than the range value reported by Pineda-Pineda *et al.* (2017) on aquaponic lettuce grown with 10 kg m^{-3} fish density. Among the essential elements required for plant growth, iron plays a major role in respiratory and photosynthetic reactions. Thus, iron deficiency reduces chlorophyll production and characterized by intervienal chlorosis in young leaves (McCauley *et al.*, 2009). Similarly, boron concentration in aquaponically-grown lettuce was higher than the value in the soil-based harvest. Pineda-Pineda *et al.* (2015) also reported a similar trend. Boron is an essential micronutrient which is required for successful plant growth. Plants suffering from boron deficiency exhibit chlorotic in young leaves and death of the main growing point (terminal bud). Also manganese concentration was significantly higher in the aquaponically-grown lettuce compared to the soil-based harvest. These results agreed with Pineda-Pineda *et al.* (2015) who found 72.3mg/kg of manganese in lettuce leaves cultivated in an aquaponic system which was stocked with a fish density of 20kg m^{-3}.

Copper, a redox active metal, plays an important role in the oxidative defense system. In fact, oxidative stress is one of the characteristics of copper deficiency (Vicente *et al.,* 2009). Hence, the higher copper concenteration in the aquaponic lettuce might help in preventing the plant from possible oxidative stress. In contrast, the zinc content in the aquaponic lettuce was significantly lower than the soil-based harvest. Trace minerals like iron, copper, zinc and manganese are considered as essential elements for normal life processes (Fraga *et al.,* 2005). In most developing countries micronutrient deficiencies are still standing problems. One of the major factors related with these deficiencies is low diet diversity score. Thus, new food production systems like aquaponics with proper management will increase the diet diversity in rural and arid areas where production of fish, fruits and vegetables are low. Yet, the essential mineral concentrations need to be evaluated from aquaponic harvests. In this study, the trace minerals concentration was compared between aquaponic and soil-based kale.

As reported in Table 8, the concentrations of zinc ($p<0.001$), copper ($p<0.01$) and manganese ($p<0.001$) in the aquaponic kale were significantly higher than the values in soil grown kale. The zinc concentration in the aquaponic kale in this study waslowerthan the value reported by Pineda-Pineda *et al.* (2017). Meanwhile, the values were higher than the report by Baloch *et al.* (2015) (1.86 to 17.88) g/100g for cauliflower. But the iron ($p<0.001$) and boron ($p<0.05$) concentrations were significantly lower in the aquaponic kale. This might be due to the variability of cultivars and availability of iron and other nutrients like boron in the aquaponic system. Similarly, the higher NH_4^+ concentration in the aquaponic solution due to high fish density might have an effect on absorption of nutrients. Compared to other studies, lower fish density (800g/cm^3) was used in this study. According to Pineda-Pineda *et al.* (2017) iron and boron contents in aquaponic vegetables were decreased with a fish density of 15-20 kg m^{-3}.

Iron plays a major role in plant growth, plant respiratory and photosynthetic reactions. Iron deficiency reduces chlorophyll production and is characterized by intervienal chlorosis with a sharp distinction between veins and chlorotic areas in young leaves (McCauley *et al.,* 2009). In this study, iron deficiency was observed in kale leaves which grew through aquaponic system. This was proved by the appearance of interveinal chlorosis on young kale leaves. Similalry, boron is an essential micronutrient required for plant growth, in which the deficiency exhibit chlorotic young leaves. Boron concentration was lower in the aquaponic kale compared with soil grown kale.

4.3. Harmful metal concentration in lettuce and kale sample from AAU site

Soil and environmental pollution are a major concern in agricultural practices and has been accepted as a global problem because of adverse effect on human health, plants and animals (Abas *et al.,* 2010). Similarly in aquaponics system, the used water from fish tank as nutrient source for plants may cause toxicity if the source is contaminated. Thus, in this study, the harmful metal concentration in aquaponically grown lettuce was compared with the soil-grown harvest.Harmful metal toxicity has received special attention globally due to neurotoxin, carcinogenic and several other impacts arising from their consumption even at lower concentration (Abas *et al.,* 2010).

The mean concentrations of harmful metals found in the soil-based and aquaponically grown lettuce from Addis Ababa University site are summarized in Table 9.

In recent years, reports indicated the contamination of leafy vegetables with harmful metals as a result of environmental pollution. Anthropogenic activities, such as urban life, industry and agriculture increase the lead, chromium, cadmium, and nickel concentration in soils and water. Thus, the pollution led to accumulation of harmful metals in vegetables (Naser *et al.,* 2009). One of the literally assumed advantages of aquaponics is the reduced levels of harmful metals in the harvests. With this regard, the concentration of harmful metals in aquaponic and soil-grown kale was evaluated in this study.

Table 9: Harmful metal concentration in lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce		
Harmful metals (mg L^{-1})	Soil-based lettuce	Aquaponic lettuce	Recom. Max. L. for vegetables (mg kg $^{-1}$) FAO/WHO
Chromium	3.03 ± 0.69	2.58 ± 0.62	2.30
Cadmium	0.09 ± 0.00	$0.18 \pm 0.01^{**}$	0.20
Lead	0.36 ± 0.11	0.08 ± 0.15	0.30
Nickel	1.66 ± 0.38	$0.26 \pm 0.04^{*}$	-
Mercury	0.13 ± 0.01	0.09 ± 0.00	0.03 µgg-1
Arsenic	0.67 ± 0.21	1.59 ± 0.37	0.43
	Kale		
Chromium	3.54 ± 0.59	1.96 ± 0.23	
Cadmium	0.18 ± 0.01	0.18 ± 0.01	
Lead	1.48 ± 0.11	1.79 ± 0.39	
Nickel	0.84 ± 0.02	0.74 ± 0.05	
Mercury	0.53 ± 0.07	0.81± 0.10	
Arsenic	0.19 ± 0.17	$1.21 \pm 0.28^{*}$	

All values areexpressed as mean ± SE (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test; $^{*}p<0.05$; $^{**}p<0.01$.

There is a rising concern on toxic metal contamination of vegetables grown on soil-based system. This is due to the contamination of rivers, lakes and other water bodies with industrial effluents. Thus, it is out of necessity in this study that the concentrations of toxic metals in soil-based and aquaponic harvests were compared. Accordingly, the concentrations of cadmium and nickel were significantly higher and lower in the aquaponic lettuce than in soil-based harvest at $p<0.01$ and $p<0.05$ respectively. The concentrations of cadmium were lower than the levels reported by Itanna (2002) from soil-based lettuce collected in Addis Ababa. Among the listed harmful metals, cadmium is of particular concern because the high mobility in the plant-soil system. Emission of cadmium into the environment can result from incineration of metal scrap, use of phosphate fertilizers, metal plating activities and abrasion from automobile tyres and so on. It is well known that vegetables absorb these metals from the soil and atmosphere through dusts deposited on their surfaces (Rahlenbeck *et al.,* 1999). The higher cadmium level in the aquponic harvests might be due the proximity of the cultivation areas to the main traffic road (i.e atmospheric pollution). There is a positive relationship between atmospheric metal deposition and higher concentrations of harmful metals in plants (Bai*et al.,* 2011). In contrast, nickel concentration was lower in the aquaponic lettuce significantly. In fact, the nickel concentration in both soil-based and aquaponic lettuce was lower than the results obtained by Itanna (2002) (i.e. 1.86 mg kg^{-1} in lettuce harvested from Kera, Addis Ababa). Nickel has potential toxicity to both plants and animals. Nickel phytotoxicity results in chlorosis, weak plant growth, yield depression and reduced nutrient uptake and disorders in plant metabolism (Poulik, 1999). From the present study, one can hypothesize the possibility of reducing some harmful metals contamination through aquaponic system, as the system is well protected in a green house. With this regard, numerically in the aquaponic lettuce the levels of chromium, lead and mercury were lower than the values in soil-based harvest. The lower concentrations of mercury in aquaponically grown lettuce might also be linked with the exclusion of herbicides/pesticides from the aquaponic system. As reported in Table 9, numerically the concentration of chromium and nickel were lower in aquaponic kale. In fact, the chromium concentration in the soil-grown kale exceeded the FAO/ WHO maximum limit of 2.3 mg L^{-1}for leafy vegetables (Itanna, 2002). But lead, mercury and arsenic concentration were higher (Table 9). In fact, the lead concentration in both harvests exceeded the FAO/ WHO maximum limit of 0.3 mg L^{-1}for leafy vegetables (Itanna, 2002). This might be attributed from factors including gas emissions from vehicles (Banerjee *et al.,* 2010). The toxicity of mercury is more severe than the other metals, causing serious loss of vision, hearing and mental retardation and death (Abbas *et al.,* 2010). In this study the aquaponic kale had an increased mercury level by 0.28ppm than the soil grown kale. But the mercury concentration in both harvests is within the standard limit set by FAO/WHO. Similarly, arsenic concentration in the aquaponic kale was significantly higher ($p<0.05$). There was no significant change in cadmium concentration between the two harvests. Cadmium concentration in kale in this study was lower than the value reported by Rahlenbeck *et al.* (1999) from kale leaves collected from Addis Ababa city.

4.4. Beta carotene content

Beta-carotene belongs to a group of more than 600 compounds, together called carotenoids (Bogacz-Radomska and Harasym, 2018). It is generally regarded as the most important and widely used carotenoid. B-carotene is a secondary metabolite synthesized by plants and belongs to compound group of carotenoids (Bogacz-Radomska and Harasym, 2018). It is used as a food coloring agent, an antioxidant, and an important and pro-vitamin A source (Schierle *et al.*, 2004). Moreover, carotenoids also protect the cells by directly quenching the excess energy of excited chlorophyll or quenching the highly reactive singlet oxygen as antioxidants (Mou, 2009). As it has a major role in photosynthesis, it is important to evaluate the effect of different production systems on beta carotene concentration of the harvests. Accordingly, in this study the beta carotene concentration in kale and lettuce cultivated through aquaponics and soil-based system was compared. As reported in Table 10, soil-based lettuce had significantly higher beta-carotene concentration than aquponic lettuce ($p<0.001$). This might be attributed from the less exposure of aquaponic plants to sunlight and temperature which had significant influence on carotenogenesis. The two processes in photosynthetic tissues are biosynthesis and photo degradation. These processes are affected by environmental factors, particularly exposure to temperature and sunlight (Kimura and Rodriguez-Amaya, 2003). As light regulates many developmental and morphogenetic processes in plants, the variation may be due to the light conditions used in the aquaponic cultivation of vegetables. The aquaponic farm was covered by polyethylene roof during the experiment period. In addition to this variation in cultivation methods is considered to be a factor for differentiation in beta-carotene concentrations of harvests. Plants cultivated in an open field have the access to absorb sufficient amount of light energy from the sun in order to make their own food through the process of photosynthesis.

Table 10: β-carotene content of lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
	Soil-based lettuce	Aquaponic lettuce
β-carotene (μg/100g)	958.88 ±1.23	$758.78 \pm 0.29^{***}$
	Kale	
	1009.11±1.35	$796.66 \pm 0.66^{***}$

All values are expressed as mean ± SE. n=3, on fresh wet basis. *indicates significant difference between mean values across the same row using student's t-test; $^{***}p<0.001$.

As reported in table 10, the soil-based kale at AAU site had a significantly higher amount of beta-carotene than the aquaponic harvest ($p<0.001$). This might be due to less exposure of aquaponics system to sunlight and temperature which had significant decreasing influence on carotenogenesis (Kimura and Rodriguez-Amaya, 2003). The aquaponic system in this study was covered by polyethylene roof. This controls the amount of sunlight and temperature to which the vegetables are exposed. Similarly, in this study lower beta-carotene concentration was found in aquaponic lettuce.

4.5. Vitamin C content

Vitamin C (ascorbic acid) is a water-soluble vitamin and is found in variable quantities in fruits, vegetables and organ meats (e.g. liver and kidney) (Padayatty *et al.*, 2003). It is a reducing agent and synthesized from glucose in the liver of most mammalian species, but not humans. One of the important properties of vitamin C is its antioxidant activity. Antioxidant activity of vitamin C helps to prevent certain diseases such as cancer, cardiovascular diseases, common cold, age-related muscular degeneration and cataract (Devaki and Raveendran, 2017). It also enhances the bioavailability of iron in the diet during absorption. In this study, vitamin C concentration in kale and lettuce harvested through different production systems was evaluated. Accordingly, the soil-based lettuce had significantly higher vitamin C concentration than the aquaponic harvest ($p<0.001$) (Table 11). Mou (2012) discussed that ascorbic acid content of lettuce leaves increased under strong light conditions and decreased under weak light or shaded conditions. The lower vitamin C concentration in the aquaponic lettuce might then be related with the lower light intensity in the system (shaded by polyethylene plastic). Kale is also rich in vitamins particularly in vitamin C (17.7 mg kg^{-1}) and rated as the second highest among 22 vegetables tested (Dias, 2012). As reported in Table 6,the vitamin C concentration in aquaponic kale was significantly lower than the amount in soil grown kale ($p<0.001$). In fact, the vitamin C content in the aquaponic kale in this study was lower also from previous similar harvests (Agarwal *et al.*, 2017). Meanwhile, the Vitamin C content of the soil-grown kale was higher compared with previous reports on *Brassica oleraceae* var. capitata (56.37 mg/100g) and cauliflower (61.5mg/100g) (Ogbede *et al.*, 2015). Different factors can be associated to this lower content of vitamin C in aquaponic kale. One possible reason could be exposure to light because ascorbic acid is formed during photosynthesis. Apparently, the two production systems had different exposure to light, the aquaponics system being roofed with polyethylene plastic. Hence, lower vitamin C content might be expected from the later system. In this study, similarly vitamin C content in aquaponically grown kale was significantly lower (Table 11) than the soil based harvest. Mou (2009) discussed that ascorbic acid content of lettuce leaves increased under strong light conditions and decreased under weak light or shaded conditions.

Table 11: Vitamin C content of lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
	Soil-based lettuce	Aquaponic lettuce
Vitamin- C (mg/100g)	51.30±0.01***	26.41±0.01
	Kale	
	69.42 ± 0.01	5.08± 0.01***

All values are expressed as mean ± SE. (n=3), on dry basis *indicates significant difference between mean valuesacross the same row using student's t-test: $^{***}p<0.001$.

4.6. Nitrate concentration

Surpass concentration of nitrate in vegetables is becoming a global food safety issue. Excess nitrate consumption may harm the health of the consumer as it can be nitrite causing methaemoglobinaemia or carcinogenic nitrosamines (Afali and Elahi, 2014; Blom-Zandstra *et al.*, 1989). The role of nitrogen fertilizers on growth, performance and quality of products in soil-based agriculture is increased to achieve more productivity and this leads to overuse of these fertilizers, which in turn leads to nitrate aggregation in plants. The accumulation of nitrate in plants depends upon many environmental factors such as amount and form of N application, light intensity, temperature, water supply and photoperiod (Blom-Zandstra, 1989). Compared to the conventional soil-based system, nitrate produced in aquaponics systems comes from protein content in the fish feed. Hence, with appropriate fish density, the levels of nitrate are sufficient for normal plant growth (Bittsanszky *et al.*, 2015).

In aquaponic system there is no nutrient solution added. The whole system relies on fish feed and waste from the fish. The primary waste from the fish is full of nitrite (toxic) need to be converted into nitrate using denitrifying bacteria. Thus, the regulation of nitrate and nitrite in the aquaponic system is important. Therefore, one of the specific objectives of the present study was to investigate the nitrate concentration of the aquaponic harvests as compared with the soil-based harvests. The nitrate concentration of lettuce and kale cultivated through aquaponic and soil-based system is presented in Table 12.

Table 12: Nitrate concentration in lettuce and kale cultivated through soil-based and aquaponic system at Addis Ababa University site

	Lettuce	
	Soil-based lettuce	Aquaponic lettuce
Nitrate ($\mu g\ g^{-1}$)	44.25 ± 0.09	40.27 ± 0.01**
	Kale	
	23.18 ± 0.03	70.73 ± 0.01***

All values are expressed as mean ± SE. (n=3), on dry basis.*indicates significant difference between mean values across the same row using student's t-test: $^{**}p<0.01$.

The soil-grown lettuce had significantly a higher amount of nitrate than the aquponic harvest at $p<0.01$ (Table 12). Variablity in cultivars, cultivation methods, and availability of nitrate in vegetables could be the possible reasons for the difference in nitrate concentration between soil-grown and aquaponics lettuce. The nitrate values obtained in this study were lower than the values (545.3 mg kg^{-1} fresh weight) reported by Pantanella (2012) for lettuce samples. Major factors affecting the nitrate concentration include plant species, plant variety, plant part and stage of maturity, drought, high temperature, shading, deficiencies in certain nutrients (Keeney, 1970). In aquaponic systems, plant and fish density also influences nitrate content of the vegetables (Petrea *et al.,* 2013). The comparable levels of nitrates found in aquaponically-grown lettuce with the soil-based harvest, suggested aquaponics to be a relevant food production system for healthy vegetables. The lettuce harvested from both systems had a nitrate level within the Commision Regulation (EC, 2011) food safety limits ranging from 125 to 2500 mg NO$_3^-$/kg fresh for lettuce grown under cover and 2000 mg·kg$_{-1}$ for that grown in the open air.

The nitrate concentration in the aquaponic kale was significantly higher than the value in the soil based harvest ($p<0.001$) (Table 12). In aquaponics system, ammonia which is a byproduct of fish metabolism is converted into nitrite and nitrate through nitrification process. Then, plants will uptake the water (containing nitrate and other nutrients) through their roots while it travels through plant growing beds. Thus, the higher nitrate concentration found in aquaponically grown kale in the present study could be due to the variability in cultivars. Excess amount of nitrate (NO_3^-) in the aquaponic system can promote a rapid plant growth and increase the accumulation of nitrate in leaves (Alcaraz *et al.*, 2016). The nitrate concentration in both harvests was below the set upper limits of nitrates (<2000 mg kg^{-1} fresh weight) of vegetable products (EC, 2011). Thus, with proper management aquaponic systems can maintain a normal level of nitrate so as the plant growth can be improved maintaining nutritional quality.

4.7. Sensory evaluation of lettuce and kale harvested from AAU site

In addition to yield and nutritional quality, sensory qualities of vegetables are very important aspects of quality. The high initial capital of aquaponic systems will be worthless if the final produces are not preferred by the consumer. In addition, little is known about the nutritional quality and consumer preference of products grown in aquaponic systems. Because of variation in production practices, there might be also a difference in organoleptic characteristics of products (Selma *et al.,* 2012). Hence, as innovative method aquaponic harvests need to pass sensory evaluation for future expansion and recommendations. Accordingly, the types of sensory evaluation of the vegetable samples in this study included difference, preference and rating acceptance tests on the basis of sensory attributes like appearance, color, taste, texture and overall acceptability. Both lettuce and kale samples were presented at room temperature to the participants. Each panelist evaluated the intensity of the attribute, in a score sheet based on 9 point hedonic scales. With this regard, the sensory attributes of lettuce and kale harvested from aquaponic and soil-based systems were compared.

Discriminatory test

Discriminatory sensory test determined if panelists could detect an overall difference between aquaponically and soil-based vegetables. As reported in Table 13, most of the participants were able to differentiate between aquaponically and soil grown lettuce based on the different sensory attributes. Among the 17 participants, 88%, 47%, 82%, and 59% of the participants were able to identify the difference between aquaponically and soil-grown lettuce on the basis of overall appearance, color, taste and texture respectively. Similarly, most of the participants were able to differentiate between aquaponically and soil grown kale based on different sensory attributes. Among the 16 participants, 63%, 75%, 63%, 63% and 50% of them were able to identify the difference between aquaponically and soil-grown kale on the basis of appearance, color, aroma, taste and texture respectively.

Table 13: Discriminatory sensory test on lettuce and kale cultivated through aquaponics and soil-based system at Addis Ababa University site

	Lettuce	
Sensory attributes	Frequency (Yes, %)	Frequency (No, %)
Appearance	15, 88	2, 11
Color	8, 47	9, 52
Taste	14, 82	3, 17
Texture	10, 58	7, 41
	Kale	
Sensory attributes	Frequency (Yes, %)	Frequency (No, %)
Appearance	10, 62	6, 38
Color	12, 75	4, 25
Aroma	10, 62	6, 38
Taste	10, 62	6, 38
Texture	8, 50	8, 50

Values are expressed as number of panelists responding yes or no upon detecting differences

Preference test

Since the panelists were able to detect difference in the sensory attributes except color between the two harvests, the next step was identifying the type of difference detected. Many producers and researchers throughout the world are attracted to aquaponic system for its potential of providing more yields within short period of time and year-round. However, little is known about consumer preferences for produces from aquaponic systems. In food product evaluation, there are two major measurements of consumer testing; measurement of preference and measurement of acceptance (Lawless and Heymann, 2010). In this study, the results of the consumer preference tests are listed in Table 14.

Accordingly as reported in Table 14, out of 17 participants, 59% of participants indicated that they preferred soil-grown lettuce whereas 41% of participants preferred aquaponically grown lettuce. Regarding to kale sample among the 16 participants, 75% show their preference for aquaponically grown kale over the soil-grown ones.

Table 14: Consumer preference test between lettuce and kale cultivated through aquponics and soil-based at Addis Ababa University site

Vegetables	Number of panalists preferred the sample	Percent (%)
Aquaponic lettuce	7	41
Soil-grown lettuce	10	59
Aquaponic kale	4	25%
Soil-grown kale	12	75%

Rating acceptance test

Descriptive statistics was used to compute the mean values from the panelist's evaluation of the attribute ratings for appearance, color, taste, texture, and overall acceptability of aquaponically and soil-grown lettuce. Panelists were asked to rate the attributes for both the conventional, soil-based and aquaponic produces by a 9-point hedonic scale with 1 being extremely dislike and 9 being extremely like. As shown in Table 15, there was no significant difference between the two harvests in rating the appearance, color and taste of the lettuce. In contrast, there was a significant difference ($p<0.05$) in texture and overall acceptability, in both attributes the soil-based lettuce having the higher acceptance rate. Similarly, Short *et al.* (2018) reported that lettuce samples grown in aquaponic system were preferred less than the soil-grown lettuce samples. Texture is a complicated term that relies on the mouth feel of a food quality as perceived in the mouth. Textural attributes that need to be considered during rating acceptance test include dryness, tenderness or wetness (Treftz, 2015). In this study, the values for texture were also scored high in the soil-grown lettuce compared to the aquaponic produce.

Table 15: Sensory scores for rating acceptance test on lettuce and kale grown on aquaponic and soil- based system at Addis Ababa University site

	Sensory attributes					
Vegetables	Appearance	Color	Aroma	Taste	Texture	Acceptability
Aquaponic lettuce	7.35 ± 0.24	7.82 ± 0.25	-	6.82 ± 0.46	6.71 ± 0.34*	7.00 ± 0.27*
Soil-grown lettuce	7.94 ± 0.20	7.94 ± 0.20	-	7.47 ± 0.27	7.71 ± 0.31	7.76 ± 0.22
Aquaponic kale	6.63 ± 0.35*	6.75 ± 0.48	6.69 ± 0.46	6.50 ± 0.48*	6.75 ± 0.49	6.56 ± 0.47
Soil-grown kale	7.56 ± 0.27	7.50 ± 0.30	7.25 ± 0.28	7.69 ± 0.29	7.69 ± 0.27	7.67 ± 0.25

All values are expressed as Mean ± SE. *indicates significant difference in mean values within the same column using student t-test at $^*p<0.05$

As shown in Table 15, the soil-grown kale had shown a higher appearance and taste acceptability rating score than the aquaponic harvest ($p<0.05$). Consumers may try a new product like aquaponic produces if attracted by its appearance (Barrett*et al.,* 2010). Similarly, the soil-based kale had higher color, aroma, texture, and overall acceptability rating score. Among which, the higher score for aroma might be due to its higher sulfur content compared to the aquaponically grown kale. The sulfur content in such plants is directly correlated with glucosinolate concentration and strongly influences the flavor of vegetables (Schnug, 1990). Taste also influences the purchasing behavior of consumers (Pollard *et al.,* 2002), aquaponic vegetables are only competitive when they have a good taste. In this study, the aquaponic kale had a competitive acceptancerating score in all the tested attributes with the soil grown harvest. With proper management of the aquaponic system, in the future a more preferred aquaponic produce with high nutritional quality can be harvested.

4.8. Proximate composition of lettuce at Shewa Robit site

Proximate composition

In this study, the other aquaponic site used for the nutritional and sensory evaluation of aquaponic harvests was at Shewa Robit town, Ethiopia. This replication of the evaluation at AAU' site supported to check the consistency of nutritional and sensory quality of aquaponic produces inorder to draw tangable conclusions and recommendations on the potential and sustainability of this innovative technology.

As shown in Table 16, there was no significant difference in proximate composition between the two harvests except the crude fiber content. The crude fiber in the soil based lettuce was significantly higher than the value in aquaponic harvest. The fiber content of the lettuce samples from Shewa Robit site was higher than the value reported by Gladys *et al.* (2015) (1.25-1.94g/100g dry weight). Similarly, there was no significant difference in crude protein, crude ash and fat contents between the aquaponic and soil grown lettuce from Shewa Robit site. Meanwhile, there was a significant difference in crude fiber content ($p<0.05$) (Table 16).

Table 16: Proximate composition of lettuce (*L. sativa* L) leaves cultivated through soil-based and aquaponic system from Shewa Robit site

	Lettuce	
Nutrient (g/100g)	Soil-based lettuce	Aquaponic lettuce
Moisture	6.40 ± 0.40	8.00 ± 0.00*
Crude protein	17.83 ± 0.00	16.56 ± 0.00
Crude fat	5.50 ± 0.00	6.00 ± 0.00
Crude ash	20.20 ± 0.20	19.25 ± 0.25
Crude fiber	25.73 ± 0.18	24.33 ± 0.18*
Utilizable carbohydrate	50.06 ± 0.20	50.19 ± 0.25
Gross energy (kcal)	321.06 ± 0.80	321.00 ± 1.00

All values are expressed as mean ± SE. (n=3) on dry basis. *indicates significantdifference between mean values across the same row using student's t-test; *$p<0.05$.

4.9. Mineral content of lettuce at Shewa Robit site

Macrominerals

The aquaponically grown lettuce at Shewa Robit site had a significantly higher concentration of magnesium ($p<0.05$) and potassium ($p<0.001$) than the soil-based harvest. In contrast, the calcium ($p<0.01$), sodium ($p<0.001$), phosphorus ($p<0.01$) and sulfur ($p<0.001$) concentrations were lower in the aquaponic lettuce (Table 17).

Table 17: Macro minerals content of lettuce (*L. sativa* L) cultivated through soil-based and aquaponic system at Shewa Robit site

	Lettuce	
Macro minerals (mg kg^{-1})	Soil- based lettuce	Aquaponic lettuce
Calcium	17275.59 ± 120.73	16281.09 ± 171.14**
Magnesium	3638.78 ± 26.61	3809.16 ± 28.66*
Sodium	4363.40 ± 18.07	3672.23 ± 26.27***
Potassium	60695.05 ± 185.17	77318.49 ± 493.49***
Phosphorus	5107.39 ± 16.59	4879.50 ± 27.17**
Sulfur	3199.58 ± 19.12	2811.33 ± 9.03***

All values are expressed as mean ± SE on dry basis. *indicates significant difference between mean values across the same row using student's t-test; *$p<0.05$, **$p<0.01$, **$p<0.001$.

Micro-minerals

The concentration of the micro-minerals iron, zinc, copper, manganese and boron of soil-based and aquaponically grown lettuce at Shewa Robit site was reported in Table 18. Accordingly, the concentrations of iron ($p<0.001$), copper ($p<0.01$) and boron ($p<0.05$) were significantly higher in the aquaponic lettuce.Pineda-Pineda *et al.* (2017) also reported higher iron content in aquaponic lettuce which wasgrown in asystem with 10 kg m^{-3} fish density. Iron plays a major role in plant growth specifically in respiratory and photosynthetic reactions. Hence, iron deficiency reduces chlorophyll production and is characterized by intervienal chlorosis (McCauley *et al.,* 2009). Boron is also an important nutrient for the plant metabolism, in which plants suffering from boron deficiency exhibit chlorosis in young leaves. Therefore, the higher contents of iron and boron (which are basically found in soil) in aquaponic lettuce indicated that aquaponics can replicate the soil system in food production. Yet, the emphasis on the monitoring of aquaponic system should get emphasis. Except with manganese, the same trend was found from AAU site.

Table 18: Concentration of micro-minerals in lettuce (*L. sativa* L.) cultivated through soil-based and aquaponic system at Shewa Robit site

	Lettuce	
Micro-minerals (mg L^{-1})	Soil-based lettuce	Aquaponic lettuce
Iron	1409.26 ±16.02	5102.30 ± 32.15***
Zinc	62.47 ± 1.51	44.74 ± 0.92**
Copper	16.48 ± 0.52	21.40 ± 0.47**
Manganese	69.95 ± 1.89	61.67 ± 1.27*
Boron	24.32 ± 0.71	29.55 ± 0.87*

All values are expressed as mean ±SE. (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

4.10. Harmful metals concentration in lettuce sample from Shewa Robit aquaponic site

The mean concentrations of harmful metals found in the soil-based and aquaponically grown lettuce sampled from Shewa Robit site are summarized in Table 19. Due to the contamination of rivers, lakes and other water bodies with industrial effluents. There is a rising concern on toxic metal contamination of vegetables grown on soil-based system. Thus, the impact of new food production systems on the concentration of harmful metals should be evaluated. In this study,the concentrations of cadmium, arsenic and lead were significantly higher in the aquaponic lettuce than in soil-based harvest at $p<0.01$ and $p<0.05$ respectively. The concentration of cadmium was lower than the levels reported by Itanna (2002) from soil-based lettuce collected in Addis Ababa. Among the listed harmful metals, cadmium is of particular concern because of high mobility in the plant-soil system. Emission of cadmium into the environment can result from incineration of metal scrap, use of phosphate fertilizers, metal plating activities and abrasion from automobile tyres and so on. It is well known that vegetables absorb theses metals from the soil and atmosphere by dusts deposited on their surfaces (Rahlenbeck *et al.*, 1999). The higher cadmium level in the aquponic harvests might be due to the location of the system nearer to the main road in which the plant can absorb it easily from the environment. In contrast, nickel concentration was lower in the aquaponic lettuce significantly. This might be due to the fact that the experiment was carried out in a greenhouse which reduces largely the contamination of the leaves by air pollution. The nickel concentration in both soil-based and aquaponic lettuce was lower than the results obtained by Itanna (2002) (i.e. 1.86 mg kg^{-1} in lettuce harvested from Kera, Addis Ababa). Nickel has potential toxicity to both plants and animals. Nickel phytotoxicity results in chlorosis, weak plant growth, yield depression and reduced nutrient uptake and disorders in plant metabolism (Poulik, 1999).

Also, numerically in the aquaponic lettuce the levels of chromium, lead and mercury were lower than the values in soil-based harvest. Environmental lead pollution occurs through traffic emissions. Rahlenbeck *et al.* (1999) reported lead concentration of 0.25 mg kg^{-1} in lettuce grown in soil-based system in Addis Ababa. Higher lead concentration in aquaponic lettuce at AAU site might be due to either environmental pollution or from fish feed. Chromium is a human carcinogen with primary route of exposure through inhalation (Costa and Klein, 2006). The estimated safe daily dietary intake of chromium is 50 to 200 μg (Anderson, 1997). The concentration of chromium in both aquaponic and soil-grown lettuce was found to be lower than the report by Itanna (2002) (i.e. 9.47 mg kg^{-1} in lettuce collected from kera, Addis Ababa Ethiopia).

Table 19: Harmful metal concentration in lettuce (*L. sativa* L.) cultivated through soil-based and aquaponic system at Shewa Robit site

	Lettuce	
Harmful metals (mg L^{-1})	Soil-based lettuce	Aquaponic lettuce
Chromium	2.22 ± 0.83	1.87 ± 0.31
Cadmium	0.08 ± 0.015	0.09 ± 0.00
Lead	0.08 ± 0.04	0.21 ± 0.04
Nickel	1.49 ± 0.50	0.38 ± 0.03
Mercury	0.52 + 0.18	0.16 + 003

All values are expressed as mean ±SE (n=3)on dry basis.*indicates significant difference between mean values across the same row using student's t-test;$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

4.11. Vitamin C content

The vitamin C content of lettuce cultivated in aquaponic and soil-based systems at Shewa robit site is outlined in Table 20.

Lettuce is a good source of vitamin C. Vitamin C is one of the most important antioxidants (Travieso *et al.,* 2016). In this study, the soil-grown lettuce had significantly higher vitamin C content (23.32 mg/100g) compared to the aquaponic harvest (15.16 mg/100g) (Table 20). Among the environmental factors influencing plant growth, light is a major onewhich can affect photosynthesis. Generally, lower light intensity during growth will result in less ascorbic acid content in plant tissues (Travieso *et al.,* 2016). As LI and Kubota (2009) described, high intensity of light increased the levels of ascorbic acid in lettuce. Vegetables cultivated under greenhouse (like in present study in an aquaponic system) were less exposed to sun light hence it results in low plant photosynthesis. Thus, the lower vitamin C concentration in the aquaponic lettuce both in Addis Ababa and Shewa robit site might be linked with reduced light intensity in the system.

Table 20: Vitamin C content of lettuce (*L. sativa* L.) cultivated through soil-based and aquaponic system at Shewa Robit aquaponic site

	Lettuce	
	Soil-based lettuce	Aquaponic lettuce
Vitamin C (mg $100g^{-1}$)	23.32 ± 0.01	15.16 ± 0.08^{***}

All values are expressed as mean ± SE (n=3) on dry basis. *indicates significant difference between mean values across the same row using student's t-test; $^{***}p<0.001$).

4.12. Nitrate concentration

Excess nitrate accumulation in edible vegetables is a problem, causing diseases like methemoglobinemia in children and gastrointestinal cancer in adults (Afali and Elahi, 2014). In this study the level of nitrate in lettuce leaves cultivated through aquaponic and soil-based system from Shewa Robit site is presented in Table 21.

As reported in Table 21, the nitrate concentration in the aquaponic lettuce was significantly higher than the value in the soil-grown harvest ($p<0.001$). Nitrate produced from fish waste contains high levels of nitrogen. The nitrate concentration in both harvests in this study was lower than the values reported by Pantanella (2012). However, the concentration of nitrates found in aquaponically-grown lettuce do not exceed the maximum allowed limit (<2000 mg NO_3^-/kg). Both aquaponically and soil-grown lettuce from Shewa robit site had a significant higher nitrate concentration than the AAU site harvests at $p<0.01$ and 0.001 respectively. This variation could be due to the difference in fish density from both sites (i.e. the fish density stock at AAU aquaponic site was comparatively lower (< 1 kg/fish tank than the Shewa robit site). According to Bittsanszky *et al.* (2015) appropriate fish stoking rates monitors the levels of nitrate in the aquaponic solution.Moreover, there are other confounding factors which cause the accumulation of nitrate in plants.

Table 21: Nitrate concentration in soil-based and aquaponically grown lettuce (*L. sativa* L.) leaves harvested from Shewa Robit aquaponic site

	Lettuce	
	Soil-based lettuce	Aquaponic lettuce
Nitrate ($\mu g\ g^{-1}$)	28.26 ± 0.06	$78.48 \pm 0.05^{***}$

All values are expressed as mean ± SE. n=3 onn dry basis. *indicates significant difference between mean values across the same row using student's t-test: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

4.13. Sensory evaluation of lettuce sample from Shewa Robit site

For the future success of aquaponics, the sensory evaluation of the harvests is necessary. However, so far little is known about the nutritional quality and consumer preference of harvests grown in aquaponic systems. Accordingly, the sensory attributes of lettuce harvested from aquaponic and soil-based systems from Shewarobit site was compared. The sensory tests applied were discriminatory test, preference test, and rating acceptance test on the basis of appearance, color, taste, texture and overall acceptability.

Discriminatory test

As reported in Table 22, all of the 23 panelists were able to distinguish the sensory difference between aquaponically and soil grown lettuce based on appearance, colour, taste, texture and overall acceptability (Table 22).

Table 22: Discriminatory sensory test on aquaponically and soil-grown lettuce (*L. sativa* L.) at Shewa Robit site

Sensory attributes	Frequency (Yes, %)
Appearance	23, 100
Color	23, 100
Taste	23, 100
Texture	23, 100

Values are expressed as number of panelists responding yes or no upon detecting differences

Preference test

Soilless culture systems like aquaponics, the most intensive food production method in today's agriculture industry, are based on environmentally friendly technology which can result in higher yields, safe and quality products. Similarly, sensory, nutritional and microbiological qualities of produces from soilless agriculture have been considered as important factors to determine the effectiveness of the system compared to traditional methods (Selma *et al.*, 2012). However, very few studies are available on consumer preferences for products grown in soilless culture systems (Short *et al.*, 2018). As aquaponics is a new food production technology, the consumer acceptability and preference towards aquaponically-grown vegetables will only be realized if consumers perceive these emerging technologies to be accepted in terms of sensory evaluation.

In this study, the result for preference test between aquaponic and soil-grown lettuce from Shewa robit site is presented in Table 23. Based on the study, 13 out of 23 (57%) of the participants preferred the aquaponically grown lettuce to the soil grown lettuce (Table 33). Similarly, a study carried out by Khandaker and Kotzen (2018) showed that aquaponically grown bitter gourd was preferred to that of market-bought bitter gourd.

Table 23: Sensory preference test between aquaponically and soil-grown lettuce harvested from Shewa Robit aquaponics site

Vegetable	Number of panalists preferred the sample	Percent (%)
Aquaponic lettuce	13	57
Soil-based lettuce	10	43

Rating acceptance test

Acceptance rating test was used to evaluate the attribute ratings for appearance, color, taste, texture and overall acceptability of aquaponically grown vegetables. Panelists were asked to rate the attributes for both the soil-based and aquaponic produces by 9-point hedonic scale with 1 being extremely dislike and 9 being extremely like. Aquaponically grown lettuce had a higher acceptance rating score in all the attributes tested (Table 24). Specifically, the score for texture and taste was significantly higher at $p<0.05$. The results in this study agree with reports by

Khandaker and Kotzen (2018).Consumers may try a new product like aquaponic produces if attracted by its appearance (Barret *et al.,* 2010). Taste also influences the purchasing behavior of consumers (Pollard *et al.,* 2002); aquaponic vegetables are only competitive when they have a good taste. Thus, the higher score in all the sensory attributes of the aquaponic lettuce is an important indicator of future success of aquaponics.

Table 24: Sensory scores for descriptive analyses on lettuce (*L. sativa* L.) cultivated through aquaponic and soil-based system at Shewa Robit aquaponics site

	Sensory attributes				
Vegetables	Appearance	Color	Texture	Taste	Acceptability
Aquaponic lettuce	7.39 ± 0.17	7.43 ± 0.18	7.26 ± 0.43*	7.39 ± 0.32*	7.00 ± 0.39
Soil-based lettuce	6.78 ± 0.47	6.61 ± 0.40	5.96 ± 0.49	5.96 ± 0.55	6.39 ± 0.47

All values are expressed as mean ± SE. *indicates significant difference between mean values within the same column using student's t-test; *$p<0.05$.

4.14. Results of microbial test

The microbiological test was performed to determine the microbial load of water samples from the fish tanks and vegetables harvested from aquaponic system by membrane filtration techniques using standard methods of ISO 9308-1:2000. Microbial investigation of water in aquaponics and on the harvests is not studied well so far. It is not uncommon to find fecal coli forms and non-pathogenic *E.coli* in aquaponics water (Baker, 2016). Due to the constant recirculation of water coli forms are naturally present in aquaponic systems (Fox *et al.*, 2012). However, so far only few studies were conducted to address the susceptibility of aquaponic produces to microbial contamination. Aquaponics systems are located above the ground, set-up in greenhouse or building away from soil. Hence, there seems to be much less likelihood of environmental contamination of the system. Moreover, aquaponic systems are usually designed in a way that allows protection from contamination of outside wildlife unlike traditional soil farming (Baker, 2016). However, in the aquaponic system the use of fish waste as nutrient source for plants can represent a microbial risk for the water and vegetables (Alcaraz *et al.,* 2016). Therefore, in this study the microbial safety of the circulating water in aquaponics and the vegetables was investigated.

Table 25: Total coliform and fecal coliform bacteria estimated from system water and vegetables from Shewa Robit and Addis Ababa University aquaponic site

Source	Sampling site	Sample	Total coliform cfu/100ml	Fecal coliform cfu/100ml
Fish tank 1	AAU	Water	$1.5*10^3$	<10
Fish tank 2	AAU	Water	$2.2*10^2$	<10
Vegetable	AAU	Kale	$8.3*10^2$	<10
Vegetable	AAU	Lettuce	$3.0*10^2$	<10
Fish tank 1	Shewa Robit	Water	$4.7*10^1$	<10
Vegetable	Shewa Robit	Lettuce	$2.8*10^1$	<10

*<10 CFU/100mL implies fecal coliform strain is below the detection limit in samples, AAU- Addis Ababa University (sampling site)

The number of total and fecal coliform microbes (indicator *E.coli* (CFU/100ml)) was measured using standard procedures (ISO). Indicator microbes were estimated from vegetables and water samples and the data were analyzed in reference to the EPA-recommended recreational water-quality standards for *E. coli* (EPA 1986).

Microbial load in the aquaponic system water

As reported in Table 25, the water sample collected from AAU aquaponic site (fish tank 1 and 2) had higher total coliform bacteria count than the water sampled from the Shewa Robit aquaponic site. But, fecal coliform microbes (*E.coli*) were below the detection limit (<10 CFU/100mL) in system water sampled from both sites confirming no potential pathogens. The system water from AAU aquaponic farm contained approximately $3.17\log_{10}$ CFU/100mL of total coliform and <10 CFU/100mL fecal coliform respectively. Whereas $1.67\log_{10}$ CFU/100mL of total coliform and <10 CFU/100mL fecal coli form bacteria was detected in system water collected from Shewa Robit site. The fish tanks at AAU aquaponic greenhouse were open and easily accessible for being contaminated by rodents. In fact, dead rodents were also found in one of the fish tanks. This might be one of the factors for the microbial load difference between the two aquaponic sites. One of the reasons for the development of aquaponics is the proliferation of soil borne pathogens into soil-based harvests. Yet again there is a possibility that aquaponic system also can be microbial contaminated arising from the feces of warm-blooded animals like rodents. This underlines the importance of sanitary and hygienic confined environment for the safety of aquaponic produces.

In fact, in both aquaponic sites in this study, poor sanitary (poor good agricultural/aquaponic practices) were observed. These included rough floor, poor ventilation, poor layout, poor waste management system etc.

Microbial load of lettuce and kale

The microbial load of vegetables (lettuce and kale) grown in an aquaponic system was determined following standard procedures of ISO-2000. The vegetables were collected aseptically, and placed into a sample bottle with 250 mL of sterile water. This was followed by vigorous shaking of the contents to wash the vegetable surface. Membrane filtration technique was applied. The water sample collected after washing the surface of vegetables from AAU site contained approximately 2.47log10 cfu/100ml and 2.92log10 cfu/100ml total coliform bacteria for lettuce and kale respectively (Table 25). These values were lower than the reports by Sirsal and Neal (2013) who found 3.2log cfu g^{-1} in romaine lettuce cultivated throgh aquaponics under greenhouse. Similarly, Scudery *et al.* (2011) reported count of 6.0 log cfu g^{-1} for lettuce grown in a hydroponic floating system. These values are comparable the standard limit <235 CFU/100mL set by EPA (1986), for such produces. The presence of coliforms may be a possible risk, especially for high quality ready-to-eat vegetables like lettuce. Thus, aquaponics could benefit from water sterilization, however it is not clear whether drastic elimination of bacteria may harm nitrifying bacteria activity and thus be a risk factor for the fish stock (Pantanella, 2012). Based on previous studies and the findings of the present study, soilless system like aquaponics were demonstrated to be effective in controlling microbial contamination as the produces from the soilless system had lower microbial count and slower microbial growth (Selma *et al.*, 2012).

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Addressing the challenges that soil-based agriculture is facing, systems like aquaponics that do not require arable land are becoming promising. In aquaponics the fish generated waste (ammonia) will be converted into nitrate (utilizable by plants) by bacteria to produce vegetables, herbs, fruits and fish. However, for the sustainability of aquaponics the environmental, nutritional and sensory attributes of the produces must be equivalent or better than soil-grown harvests. Therefore, this study was carried out to determine whether aquaponically-grown lettuce (*L. sativa*L.) and kale (*B. carinata*) are as nutritionally dense as soil-grown harvests focusing on their biochemical composition, sensory quality and the microbial load of the system water and vegetables.

Accordingly, there was no significant difference in moisture, crude protein and crude fat contents between aquaponically and soil-grown lettuce harvested from AAU site. In contrast, the aquaponic lettuce had significant higher contents of crude ash and crude fiber. Unlike the soil-grown harvests, there was no significant difference in macronutrient composition between aquaponic lettuce harvested from AAU and Shewa robit sites except the crude fat content. Therefore, in terms of macronutrients composition, the aquaponic system provides a more consistent harvest than soil-based system. In this study kale was the other vegetable grown through both systems for the evaluation. Kale grown through aquaponics at AAU site had significantly higher contents of crude fat, crude fiber, carbohydrate and caloric value than the soil-based harvest. In contrast, the crude ash content was significantly lower. The higher crude protein content linked with NH_3 accumulation in the aquaponic kale was a good indicator of the efficiency of the aquaponic system.

The aquaponic lettuce from AAU site had a significantly higher composition of all macro minerals except potassium and sulfur. Meanwhile, the aquaponic lettuce from Shewa Robit site had significantly higher concentrations of magnesium and potassium than the soil-based harvest. In contrast, the calcium, sodium, phosphorus and sulfur concentrations were lower. The lower concentration of minerals like calcium in aquaponic lettuce might be correlated with high accumulation of NH_4^+ in the aquaponic solution, which might interfere the absorption of cations by the plant. Therefore, maintaining and monitoring aquaponic system should get more emphasis than the initial investment.

Aquaponic lettuce from both AAU and Shewa Robit sites had higher concentrations of iron, copper and boron. Higher iron and boron levels indicated that aquaponics can replicate the soil system in food production. In contrast, iron and boron concentrations were significantly lower in aquaponic kale, in which the deficiencies were observed by leaf chlorosis. Therefore, this study underlines the importance of formulating nutrient dense fish feed, so that the waste would provide important minerals like iron and boron. Considering other antioxidant compounds, this study investigated the concentration of beta-carotene and vitamin C in the aquaponic harvests. Accordingly, the soil-based lettuce and kale had significantly higher beta-carotene concentration than the aquaponic harvests from AAU site. Similarly, vitamin C concentration in the aquaponic lettuce and kale was found lower. This might be attributed from the less exposure of aquaponic plants to sunlight and temperature which had significant influence on carotenogenesis. Hence, future considerations on the type of polyethylene sheet to regulate the light and temperature of the system as per the requirements of the plants is important. There was a comparable and higher nitrate concentration in the aquaponic lettuce and kale compared with the soil-based harvests.

Due to the rising concern of harmful metals concentration in soil-grown harvests, in this study the possibility of reducing toxic metals through aquaponics system was evaluated. The nickel concentration was significantly lower in the aquaponic lettuce harvested from AAU site than the soil-grown harvest. In fact, numerically the levels of chromium, lead and mercury were lower in the aquaponic lettuce. Similar result was found in the aquaponic lettuce from Shewa Robit site. Similarly, the concentrations of chromium and nickel were lower in aquaponic kale. In fact, the chromium concentration in the soil-grown kale exceeded the FAO/ WHO maximum limit for leafy vegetables.

The high initial capital of aquaponic systems will be worthless if the final produces are not preferred by the consumer. In this study, majority of the sensory panelists were able to differentiate between aquaponically and soil grown lettuce/kale from AAU and Shewa Robit sites based on overall appearance, color, taste and texture. Also most of the panelists who detected difference preferred the aquaponic lettuce from both sites to the soil grown harvest. Similarly, the aquaponic lettuce/kale had a comparable acceptance rating using 9-point hedonic scale with the soil-grown harvests. This marked the potential of aquaponics to supply the consumer with quality vegetables in the future.

Irrespective of microbial of aquaponics system, no fecal coliform microbes (*E.coli*) were detected in system water sampled from both AAU and Shewa Robit sites, confirming no potential pathogens. Similalry, the water collected after washing the surface of lettuce and kale from both sites contained CFU count comparable with the set limit of EPA (1986) for such harvests.

5.2. Recommendations

For the sustainability of aquaponics technology further research should be carried involving the following issues:

- The experiments need to be done for other potential fruits and vegetables
- The experiment should be done in highly controlled/monitored system to avoid confounding variables.

In this study the major challenge was power failure, which resulted in plant wilting quickly. As such research should be done with backup alternate energy sources like solar, wind, or hydroelectric.

REFERENCES

Abbas, M., Parveen, Z., Iqbal, M., Riazuddin, M., Iqbal, S., Ahmed, M., & Bhutto, R. (2010). Monitoring of toxic metals (cadmium, lead, arsenic and mercury) in vegetables of Sindh, Pakistan. *Kathmandu University Journal of Science, Engineering and Technology*, *6*(2): 60-65.

Abbas, T., Rizwan, M., Ali, S., Zia-ur-Rehman, M., Qayyum, M. F., Abbas, F., & Ok, Y. S. (2017). Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. *Ecotoxicology and Environmental Safety*, *140*, 37-47.

Acikgoz, F. E. (2011). Mineral, vitamin C and crude protein contents in kale (*Brassica oleraceae* var. acephala) at different harvesting stages. *African Journal of Biotechnology*, *10*(75): 17170-17174.

Adler, P.R., Harper,J.K. Wade,E.W. Takeda, F. &Summerfelt, S.T.(2000). Economic analysis of an aquaponic system for the integrated production of rainbow trout and plants. *International Journal of Recirculating Aquaculture*,*1*(1): 15-34.

Afali, S. F., & Elahi, R. E. Z. V. A. N. (2014). Measuring nitrate and nitrite concentrations in vegetables, fruits in Shiraz. *Journal of Applied Sciences and Environmental Management*, *18*(3): 451-457.

Afrin, R., Rana, K. S., Islam, M. K., Zahan, S., & Salam, M. A. (2018). Cauliflower (*Brassica oleracea*) production incorporated with sea salt in aquaponic system. *European Journal of Biotechnology and Bioscience*, **6**(2): 05-11.

Agarwal, A., Raj, N., & Chaturvedi, N. (2017). A Comparative study on proximate and antioxidant activity of *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) leaves. *International Journal Advanced Research Biological Sciences*, *4*(4): 22-29.

Agriculture Organization of the United Nations. Fisheries Department. (2000). *The State of World Fisheries and Aquaculture, 2000* (Vol. 3). Food & Agriculture Organization.

Alcarraz, E., Flores, M., Tapia, M. L., Bustamante, A., Wacyk, J., & Escalona, V. (2016,). Quality of lettuce (*Lactuca sativa* L.) grown in aquaponic and hydroponic systems. In *VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits-Ethical and Technological Issues 1194* (pp. 31-38).

AlShrouf, A. (2017). Hydroponics, aeroponic and aquaponic as compared with conventional farming. *American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)*, *27*(1): 247-255.

Anderson, R. A. (1997). Chromium as an essential nutrient for humans. *Regulatory Toxicology and Pharmacology*, *26*(1): S35-S41.

AOAC (2000). Association of Official Analytical Chemists. Official methods of analysis of AOAC international, 17thEdn., Washington DC, USA.

AOAC (2005) Official methods of analysis. 18th edition. Association of Official Analytical Chemists, Washington, D. C., Official methods 923.03, 954.02, 979.09.

AOAC (2016). Official methods of analysis of AOAC international (17th ed.). Methods 962.09. Arlington, VA, USA: AOAC International.

AOAC(2000). Official Methods of Analysis, 17thEdn. Association of Official Analytical Chemists, Arlington, VA, USA.

Asfaw, Z. (1997). Conservation and use of traditional vegetables in Ethiopia. In *Traditional African Vegetables: Proceedings of the IPGRI International Workshop on Genetic Resources of Traditional Vegetables in Africa. Conservation and Use. ICRAF-HQ, Nairobi. Institute of Plant Genetic and Crop Plant Research, Rome* (pp. 57-65).

Bai, J., Cui, B., Chen, B., Zhang, K., Deng, W., Gao, H., & Xiao, R. (2011). Spatial distribution and ecological risk assessment of heavy metals in surface sediments from a typical plateau lake wetland, China. *Ecological Modelling*, *222*(2), 301-306.

Bailey, D. S., Ferrarezi, R. S. (2017) Valuation of vegetable crops produced in the UVI commercial aquaponic system. *Aquaculture Reports7*: 77-82

Bailey, D. S., Rakocy, J. E., Cole, W. M., Shultz, K. A., & St Croix, U. S. (1997). Economic analysis of a commercial-scale aquaponic system for the production of tilapia and lettuce. In *Tilapia Aquaculture: Proceedings of the Fourth International Symposium on Tilapia in Aquaculture, Orlando, Florida* (pp. 603-612).

Baker, K. A. (2016). Microbiological and quality characteristics of Alfalfa (*Medicago sativa*) and Mung Bean (*Vigna radiata*) sprouts gown using different water sources and treated post-harvest. (88):1455-1463.

Baloch, A. B., Xia, X., & Sheikh, S. A. (2015). Proximate and mineral compositions of dried cauliflower (*Brassica oleracea* L.) grown In Sindh, Pakistan. *Journal of Food and Nutrition Research, 3*(3): 213-219.

Banerjee, D., Bairagi, H., Mukhopadhyay, S., Pal, A., Bera, D., & Ray, L. (2010). Heavy metal contamination in fruits and vegetables in two districts of West Bengal, India. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 9*(9): 1423-1432.

Barnhart, C., Hayes, L., &Ringle, D. (2016). Food safety hazards associated with smooth textured leafy greens produced in aquaponic, hydroponic, and soil-based systems with and without roots in retail. *The University of Minnesota Aquaponics*.

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition, 50*(5): 369-389.

Basha, B. M., Mahapatra, S., & Manna, B. (2015). Optimum dimensions of reinforced soil berm for vertical expansion of municipal solid waste (MSW) landfills.

Bittsánszky, A., Pilinszky, K., Gyulai, G., & Komives, T. (2015). Overcoming ammonium toxicity. *Plant Science, 231*, 184-190.

Blidariu, F., Alexandru, D., Adrian, G., Isidora, R., & Dacian, L. (2013). Evolution of nitrate level in green lettuce conventional grown under natural conditions and aquaponic system. *Scientific Papers Animal Science and Biotechnologies, 46*(1): 244-250.

Blom-Zandstra M. (1989). Nitrate accumulation in vegetables and its relationship to quality. *Annals of Applied Biology, 115*(3): 553-561.

Bogacz-Radomska, L., & Harasym, J. (2018). Β-Carotene properties and production methods. *Food Quality and Safety, 2*(2): 69-74.

Bohl, M. (1977). Some initial aquaculture experiments in recirculating water systems. *Aquaculture, 11*(4): 323-328.

Bongaarts, J. (2006). United Nations department of economic and social affairs, population division world mortality report 2005. *Population and Development Review, 32*(3): 594-596.

Breimer, T. (1982). Environmental factors and cultural measures affecting the nitrate content in spinach. *Fertilizer Research, 3*(3): 191-292.

Buchanan, D. N., & Omaye, S. T. (2013). Comparative study of ascorbic acid and tocopherol concentrations in hydroponic and soil-grown lettuces. *Food and Nutrition Sciences*, *4*(10): 1047.

Bunyaviroch, C., Ding, X., Mamayek, S., & Manning, B. (2013). *Aquaponic Systems in Puerto Rico: Assessing Their Economic Viability* (Doctoral dissertation, Ph. D. Thesis, Worcester Polytechnic Institute, Worcester, MA, USA.

Buzby, K. M., Waterland, N. L., Semmens, K. J., & Lin, L. S. (2016). Evaluating aquaponic crops in a fresh water flow through fish culture system. *Aquaculture*, *460*: 15-24.

Central Statistical Agency (CSA) (October, 2016) Demographic and Health Survey of Ethiopia key indicators report, Addis Ababa, Ethiopia.

Chalmers, G. A. (2004). Aquaponics and food safety. *Lethbridge, Alberta April.*

Chen, X. G., Gastaldi, C., Siddiqi, M. Y., & Glass, A. D. M. (1997). Growth of a lettuce crop at low ambient nutrient concentrations: a strategy designed to limit the potential for eutrophication. *Journal of Plant Nutrition*, *20*(10): 1403-1417.

Childress, V. W. (2002). Promising alternatives in agri-technology: aquaponics.(Resources in Technology). *The Technology Teacher*, *62*(4): 17.

Commission Regulation (EC) No. 1258/2011 of 2 December 2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for nitrates in foodstuffs. Off J Eur Union L 320/15, 3.12.2011. Available from: http:// www.fsai.ie/uploadedFiles/Reg1258_2011.pdf (28. 05. 2013).

Conversa, G., Santamaria, P., & Gonnella, M. (2004). Growth, yield, and mineral content of butterhead lettuce (*Lactuca sativa* var. capitata) grown in NFT. *Acta Horticulture*, *659*: 621-628.

Costa, M., & Klein, C. B. (2006). Toxicity and carcinogenicity of chromium compounds in humans. *Critical Reviews in Toxicology*, *36*(2): 155-163.

Dahl, W. J., & Stewart, M. L. (2015). Position of thecademy of nutrition and dietetics: health implications of dietary fiber. *Journal of the Academy of Nutrition and Dietetics*, *115*(11): 1861-1870.

Dediu, L., Cristea, V., & Xiaoshuan, Z. (2012). Waste production and valorization in an integrated aquaponic system with bester and lettuce. *African Journal of Biotechnology*, *11*(9): 2349-2358.

DESA, U. (2015). *United Nations Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2015 revision, key findings and advance tables*. Working Paper No. ESA/P/WP. 241.

Devaki, S. J., & Raveendran, R. L. (2017). Vitamin C: sources, functions, sensing and analysis. In *Vitamin C*. IntechOpen.

Dias, J. S. (2012). Nutritional quality and health benefits of vegetables: a review. *Food and Nutrition Sciences*, *3*(10): 1354.

Diver, S., & Rinehart, L. (2000). *Aquaponics-Integration of hydroponics with aquaculture*. Attra.

Dogheim, S. M., Ashraf, E. M., Alla, S. G., Khorshid, M. A., & Fahmy, S. M. (2004). Pesticides and heavy metals levels in Egyptian leafy vegetables and some aromatic medicinal plants. *Food Additives and Contaminants*, *21*(4), 323-330.

El Essawy, H. A. S. (2018). Aquaponics as a sustainable alternative to new land reclamation and conventional agriculture in Egypt.

El-Kazzaz, K. A., & El-Kazzaz, A. A. (2017). Soilless agriculture a new and advanced method for agriculture development: an introduction. *Agricultural Research & Technology*, *3*(2): 1-10.

El-Saidy, D. M., & Gaber, M. M. (2004). Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia, Oreochromis niloticus (L.) diets. *Aquaculture Research*, *35*(9), 859-865.

Emebu, P. K., & Anyika, J. U. (2011). Proximate and mineral composition of kale (*Brassica oleracea*) grown in Delta State, Nigeria. *Pakistan Journal of Nutrition*, *10*(2): 190-194.

Endut, A., Jusoh, A., Ali, N., Nik, W. W., & Hassan, A. (2010). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource Technology*, *101*(5): 1511-1517.

EPA. (1986). Ambient Water Quality Criteria for Bacteria-1986. EPA/440/5-84-002. Office of Water Regulations and Standards. USEPA, Washington, DC.

Ethiopian Public Health Institute (EPHI) (2013) Ethiopia national food consumption survey, Addis Ababa, Ethiopia.

European Commission (EU). (2011).No1258/2011 of 2 December 2011amending Regulation (EC) No1881/2006 as regards maximum limits for nitrates in foodstuffs. *Official Journal of European Union*. L320: 15- 17.

Falguera, V., Aliguer, N., &Falguera, M. (2012). An integrated approach to current trends in food consumption: Moving toward functional and organic products?. *Food Control, 26*(2): 274-281.

FAO (2012) the state of world fisheries and aquaculture. Rome, Italy: Food and Agriculture Organization of the United Nations

FAO/WHO 1999. Expert Committee on Food Additives, Summary and Conclusions, 53rd Meeting, Rome.

Farran, I., & Mingo-Castel, A. M. (2006). Potato minituber production using aeroponics: effect of plant density and harvesting intervals. *American Journal of Potato Research, 83*(1): 47-53.

FernándezCañero, R., Pérez Urrestarazu, L., & EgeaCegarra, G. (2015). Design and preliminary assessment of a vertical aquaponics system for ornamental purposes. In *International Conference on Living Walls and Ecosystems Services, p 1-41.*

Fox, B. K., Tamaru, C. S., Hollyer, J., Castro, L. F., Fonseca, J. M., Jay-Russell, M., & Low, T. (2012). A preliminary study of microbial water quality related to food safety in recirculating aquaponic fish and vegetable production systems.

Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine*, *26*(4-5): 235-244.

Fytianos, K., & Zarogiannis, P. (1999). Nitrate and nitrite accumulation in fresh vegetables from Greece. *Bulletin of Environmental Contamination and Toxicology*, *62*(2): 187-192.

Gericke, W. F. (1937). Hydroponics crop production in liquid culture media. *Science*, *85*(2198): 177-178.

Gladys, N. N., & Aba, I. P. (2015). Effects of maturity on the proximate composition of Lettuce (*Lactuca sativa*). *Academic Research International*, *6*(1): 55.

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K. V., Jijakli, H., &Thorarinsdottir, R. (2015). Challenges of sustainable and commercial aquaponics. *Sustainability*, *7*(4): 4199-4224.

Gopinath, P., Vethamoni, P. I., & Gomathi, M. (2017). Aeroponics soilless cultivation system for vegetable crops. *Chemical. Science. Revolution. Letter.6*: 838-849.

Graber, A., Antenen, N., & Junge, R. (2014). The multifunctional aquaponic system at ZHAW used as research and training lab. In *3. konferenca VIVUS, s področjakmetijstva,* *naravovarstva, hortikulture in floristiketerživilstva in prehrane= 3rd Conference VIVUS, Conference on Agriculture, Environmentalism, Horticulture, Floristics, Food Production and Processing, Strahinj, Slovenia, 14–15 November 2014* (pp. 245-255).

Gruda, N. (2009). Do soilless culture systems have an influence on product quality of vegetables? Plant Sciences, 147: 141-147.

Hambrey, J. (2013). Aquaponics Research Project: The relevance of aquaponics to the New Zealand aid programme, particularly in the Pacific. *New Zealand Aid Programme Ministry of Foreign Affairs and Trade*: 1-96.

Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S., & Rasheed, M. (2006). Use of vegetables as nutritional food: role in human health. *Journal of Agriculture Biological Science*, *1*(1): 18-22.

Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry*, *54*(1), 101-111.

Hollyer, J., Tamaru, C., Riggs, A., Klinger-Bowen, R., Howerton, R., Okimoto, D., & Martinez, G. (2009). On-farm food safety: Aquaponics. *Food Safety Technology 38*(7).

Hussain, A., Iqbal, K., Aziem, S., Mahato, P., & Negi, A. K. (2014). A review on the science of growing crops without soil (soilless culture)-a novel alternative for growing crops. *International Journal of Agriculture and Crop Sciences*, *7*(11), 833.

International Organization for Standardization (ISO) 9308-1:2000 (2000). Water quality – detection and enumeration of Escherichia coli and coli form bacteria – Part 1: Membrane filtration method, Geneva: International Organization for Standardization.

Itanna, F. (2002). Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiopian Journal of Health Development*, *16*(3): 295-302.

J. R. Miller, K. A. Hudson-Edwards, Lechler P J et d. (2004).Heavy metal contamination of water, soil and produce within riverine communities of the Rlo Pilcomayo basin, Bolivia. *Scientific Total Environment*, 320, 189-209.

James, C. S. (1995). Analytical chemistry of food. Chapman and Hall, London. pp. 64-65.

Jensen, M. H. (1997). Hydroponics. *HortScience*, *32*(6), 1018-1021.

Jones Jr, J. B. (1982). Hydroponics: its history and use in plant nutrition studies. *Journal of Plant Nutrition*, *5*(8): 1003-1030.

Jones, S. (2002). Evolution of aquaponics. *Aquaponics Journal*, *6*(1).

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, *88*(11): 1863-1868.

Keeney, D. R. (1970). Nitrates in plants and waters. *Journal of Milk and Food Technology*, *33*(10): 425-432.

Kendall, H. W., & Pimentel, D. (1994). Constraints on the expansion of the global food supply. *Ambio*: 198-205.

Khandaker, M., & Kotzen, B. (2018). The potential for combining living wall and vertical farming systems with aquaponics with special emphasis on substrates. *Aquaculture Research*, *49*(4), 1454-1468.

Kimura, M., & Rodriguez-Amaya, D. B. (2003). Carotenoid composition of hydroponic leafy vegetables. *Journal of Agricultural and Food Chemistry*, *51*(9), 2603-2607.

Kiss, G., Jansen, H., Castaldo, V. L., & Orsi, L. (2015). The 2050 City. *Procedia Engineering*, *118*: 326-355.

Koop, M. (2016). "Aquaponics and the potential of BSFL farming in Ethiopia."*Forestry and Natural resource management.*

Kosma, C., Triantafyllidis, V., Papasavvas, A., Salahas, G., & Patakas, A. (2013). Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. *Emirates Journal of Food and Agriculture:25(12)*974-979.

Lakkireddy, K. K. R., Kasturi, & K. (2018). Role of hydroponics and aeroponics in soilless culture in commercial food production. *Research & Reviews: Journal of Agricultural Science and Technology*, *1*(3): 1-8.

Lapere, P. (2010). *A techno-economic feasibility study into aquaponics in South Africa* (Doctoral dissertation, Stellenbosch: University of Stellenbosch).

Lastra, O. C. (2003). Derivative spectrophotometric determination of nitrate in plant tissue. *Journal of AOAC International*, *86*(6): 1101-1105.

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices*. Springer Science & Business Media.

Lee, S. K. (1993). Aeroponic system as a possible alternative for crop production in Singapore. *Commonwealth Agricultural Digest*, *3*(1): 1-14.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3): 207-220.

Lehman, H., Clark, E. A., & Weise, S. F. (1993). Clarifying the definition of sustainable agriculture. *Journal of Agricultural and Environmental Ethics*, *6*(2): 127-143.

Lennard, W. A. (2004). Aquaponics research at RMIT University, Melbourne Australia. *Aquaponics Journal*, *35*:18-24.

Li, Q., & Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*, *67*(1): 59-64.

Licamele, J. D. (2009). Biomass production and nutrient dynamics in an aquaponics system (Doctoral dissertation. University of Arizona, *Department Of Agriculture AND Biosystems Engineering).*

Lorenz, O. A. (1978). Potential nitrate levels in edible plant parts. In *Soil–Plant–Nitrogen Relationships*: 201-219.

Love, D. C., Fry, J. P., Genello, L., Hill, E. S., Frederick, J. A., Li, X., &Semmens, K. (2014). An international survey of aquaponics practitioners. *PloS One*, *9*(7): e102662.

Love, D. C., Fry, J. P., Li, X., Hill, E. S., Genello, L., Semmens, K., & Thompson, R. E. (2015). Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture*, *435*: 67-74.

Lyons, D. J., Rayment, G. E., Nobbs, P. E., & McCallum, L. E. (1994). Nitrate and nitrite in fresh vegetables from Queensland. *Journal of the Science of Food and Agriculture*, *64*(3): 279-281.

Martin, A. D. (2017). *Technical and economical study of Aquaponics feasibility in northern Finland* (Doctoral dissertation, Master's thesis. University of Oulu Faculty of Technology. 62p, 2 App).

Martínez-Ballesta, M. C., Dominguez-Perles, R., Moreno, D. A., Muries, B., Alcaraz-López, C., Bastías, E.,& Carvajal, M. (2010). Minerals in plant food: effect of agricultural practices and role in human health. A review. *Agronomy for Sustainable Development*, *30*(2): 295-309.

Mattheis, J. P., & Fellman, J. K. (1999). Preharvest factors influencing flavor of fresh fruit and vegetables. *Postharvest Biology and Technology*, *15*(3): 227-232.

Maynard, D. N., Barker, A. V., Minotti, P. L., & Peck, N. H. (1976). Nitrate accumulation in vegetables. In *Advances in Agronomy*, 28: 71-118.

McCauley, A., Jones, C., & Jacobsen, J. (2009). Plant nutrient functions and deficiency and toxicity symptoms. *Nutrient Management Module*, *9*: 1-16.

Miličić, V., Thorarinsdottir, R., Santos, M. D., & Hančič, M. T. (2017). Commercial aquaponics approaching the European market: To consumers' perceptions of aquaponics products in Europe. *Water*, *9*(2): 80.

Mou, B. (2005). Genetic variation of beta-carotene and lutein contents in lettuce. *Journal of the American Society for Horticultural Science*, *130*(6): 870-876.

Mou, B. (2009). Nutrient content of lettuce and its improvement. *Current Nutrition & Food Science*, *5*(4): 242-248.

Mou, B. (2012). Nutritional quality of lettuce. *Current Nutrition & Food Science*, *8*(3): 177-187.

Munguia-Fragozo, P., Alatorre-Jacome, O., Rico-Garcia, E., Torres-Pacheco, I., Cruz-Hernandez, A., Ocampo-Velazquez, R. V., & Guevara-Gonzalez, R. G. (2015). Perspective for aquaponic systems:"omic" technologies for microbial community analysis. *BioMedical research international* 480386 (1-10).

Muramoto, J. (1999). Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. *Center for Agroecology and Sustainable Food Systems, University of California, Santa Cruz.*

Murphy, M. T., Zhang, F., Nakamura, Y. K., & Omaye, S. T. (2011). Comparison between hydroponically and conventionally and organically grown lettuces for taste, odor, visual quality and texture: A pilot study. *Food and Nutrition Sciences*, *2*(02): 124.

Naser, H. M., Shil, N. C., Mahmud, N. U., Rashid, M. H., & Hossain, K. M. (2009). Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. *Bangladesh Journal of Agricultural Research*, *34*(4): 545-554.

Nichols, M. A., & Savidov, N. A. (2011, March). Aquaponics: Protein and vegetables for developing countries. In *I International Symposium on Sustainable Vegetable Production in Southeast Asia 958* (pp. 189-193).

Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., Rock, E., Mazur, A.,& Rémésy, C. (2004). Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. *Clinical Nutrition*, *23*(4): 605-614.

Nuwansi, K. K. T., Verma, A. K., Prakash, C., Tiwari, V. K., Chandrakant, M. H., Shete, A. P., & Prabhath, G. P. W. A. (2016). Effect of water flow rate on polyculture of koi carp (Cyprinus carpio var. koi) and goldfish (Carassius auratus) with water spinach (Ipomoea aquatica) in recirculating aquaponic system. *Aquaculture International, 24*(1), 385-393.

Ogbede, S. C., Saidu, A. N., Kabiru, A. Y., & Busari, M. B. (2015). Nutrient and anti-nutrient compositions of *Brassica oleracae* Var. Capitata L. *IOSR Journal of Pharmacy, 3*: 19-25.

Ogunji, J., Toor, R. U. A. S., Schulz, C., & Kloas, W. (2008). Growth performance, nutrient utilization of Nile tilapia *Oreochromisniloticus* fed housefly maggot meal (magmeal) diets. *Turkish Journal of Fisheries and Aquatic Sciences*, *8*(1): 141-147.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., & Kurata, K. (2007). Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology*, *45*(3): 189-198.

Okemwa, E. (2015). Challenges and opportunities to sustainability in aquaponic and hydroponics systems. *International Journal of Scientific Research and Innovative Technology*, *2*: 54-76.

Onwuka, G. I. (2005). Food analysis and instrumentation: theory and practice. *Naphthalic prints, Surulere, Lagos, Nigeria*, 219-230.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H.,& Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition*, *22*(1): 18-35.

Pade, J. S., & Nelson, R. L. (2005). Village aquaponics. In *International Conference and Exhibition on Soilless Culture: ICESC 2005 742* (pp. 197-199).

Palm, H. W., Bissa, K., & Knaus, U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part II: fish and plant growth. *Aquaculture, Aquarium, Conservation & Legislation*, *7*(3): 162-175.

Pantanella, E. (2012). Nutrition and quality of aquaponic systems.

Pantanella, E., Cardarelli, M., Colla, G., Rea, E., & Marcucci, A. (2010). Aquaponics vs. hydroponics: production and quality of lettuce crop. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 927*: 887-893.

Pantanella, E., Cardarelli, M., Di Mattia, E., & Colla, G. (2010). Aquaponics and food safety: Effects of UV sterilization on total coliforms and lettuce production. In *International Conference and Exhibition on Soilless Culture 1062* (pp. 71-76).

Pattillo DA (2017) An overview of aquaponic systems: hydroponic components. North Central Regional Aquaculture Center.

Pattillo, A. (2016). Food safety, economics and environmental impacts of aquaponics in Iowa.

Petrea, Ş. M., Cristea, V., Dediu, L., Contoman, M., Lupoae, P., & MOCANU, M. (2013). Vegetable Production in an Integrated Aquaponic System with Rainbow Trout and Spinach. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Animal Science & Biotechnologies*, *70*(1).

Pineda-Pineda, J., Miranda-Velázquez, I., Rodríguez-Pérez, J. E., Ramírez-Arias, J. A., Pérez-Gómez, E. A., García-Antonio, I. N., & Morales-Parada, J. J. (2015). Nutrimental balance in aquaponic lettuce production. In *International Symposium on New Technologies and Management for Greenhouses- 1170*: 1093-1100.

Pineda-Pineda, J., Valdez-Zamora, A., Miranda-Velázquez, I., Rodríguez-Pérez, J. E., Ramírez-Arias, J. A., & Lozano-Toledano, A. (2017). Yield of two cultivars of lettuce (*Lactuca sativa* L.) in hydroponic and aquaponic systems. In *International Symposium on New Technologies for Environment Control, Energy-Saving and Crop Production in Greenhouse and Plant 1227* (pp. 347-354).

Pollard, W. E. (2002). Use of consumer panel survey data for public health communication planning: an evaluation of survey results. In *Proceedings of the section on health policy statistics* (pp. 2720-4).

Poulik, Z. (1999). Influence of nickel contaminated soils on lettuce and tomatoes. *Scientia Horticulturae*, *81*(3): 243-250.

Proca, C., Micu, D., Danielescu, C., & Manea, F. (2009). Case study of the risk assessment of nitrates on human health in the west side of Romania. In *Exposure and Risk Assessment of Chemical Pollution—Contemporary Methodology* (pp. 509-516).

Putra, P. A., & Yuliando, H. (2015). Soilless culture system to support water use efficiency and product quality: a review. *Agriculture and Agricultural Science Procedia*, *3*: 283-288.

Rahlenbeck, S. I., Burberg, A., & Zimmermann, R. D. (1999). Lead and cadmium in Ethiopian vegetables. *Bulletin of Environmental Contamination and Toxicology*, *62*(1): 30-33.

Rakocy, J. E., Bailey, D. S., Shultz, K. A., & Cole, W. M. (1997, November). Evaluation of a commercial-scale aquaponic unit for the production of tilapia and lettuce. In *Fourth International Symposium on Tilapia in Aquaculture* (pp. 357-372).

Rakocy, J. E., Bailey, D. S., Shultz, R. C., & Thoman, E. S. (2004, September). Update on tilapia and vegetable production in the UVI aquaponic system. In *New Dimensions on Farmed Tilapia: Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, Held September* (pp. 12-16).

Rakocy, J. E., Masser, M. P., &Losordo, T. M. (2006). Recirculating aquaculture tank production systems: aquaponics—integrating fish and plant culture. *SRAC publication*, *454*: 1-16.

Ranawade, P. S., Tidke, S. D., & Kate, A. K. (2017). Comparative cultivation and biochemical analysis of *Spinaciaoleraceae* grown in aquaponics, hydroponics and field conditions. *International. Journal. Curricula. Microbiology. Applied. Science*, *6*(4): 1007-1013.

Raviv, M., Lieth, J. H., & Raviv, M. (2008). Significance of soilless culture in agriculture. *Soilless Culture Theory and Practice*: 1-11.

Reid, M. S. (1992). Maturation and maturity indices.

Resh, H. M. (2016). *Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower*.

Ritter, E., Angulo, B., Riga, P., Herran, C., Relloso, J., & San Jose, M. (2001). Comparison of hydroponic and aeroponic cultivation systems for the production of potato minitubers. *Potato Research*, *44*(2): 127-135.

Rodriguez-Amaya, D. B. (2001). A guide to carotenoid analysis in foods. International Life Sciences Institute Press, Washington D. C., USA

Roosta, H. R., & Hamidpour, M. (2011). Effects of foliar application of some macro-and micro-nutrients on tomato plants in aquaponic and hydroponic systems. *Scientia Horticulturae*, *129*(3), 396-402.

Saha, S., Monroe, A., & Day, M. R. (2016). Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. *Annals of Agricultural Sciences*, *61*(2): 181-186.

Sala, S., McLaren, S. J., Notarnicola, B., Saouter, E., & Sonesson, U. (2017). In quest of reducing the environmental impacts of food production and consumption. *Journal of Cleaner Production*, *140*: 387-398.

Savidov, N., & Brooks, A. B. (2004). *Evaluation and development of aquaponics production and product market capabilities in Alberta*. Crop diversification Centre South, Alberta Agriculture, Food and Rural Development.

Savvas, D. (2003). Hydroponics: A modern technology supporting the application of integrated crop management in greenhouse. *Journal of Food Agriculture and Environment*, *1*, 80-86.

Savvas, D., Gianquinto, G., Tuzel, Y., Gruda, N. (2013). Soilless culture. FAO plant production and protection paper 217, pp: 303-354.

Schierle, J., Pietsch, B., Ceresa, A., Fizet, C., & Waysek, E. H. (2004). Method for the determination of β-carotene in supplements and raw materials by reversed-phase liquid chromatography: Single laboratory validation. *Journal of AOAC International*, *87*(5): 1070-1082.

Schnug, E. (1990). Sulphur nutrition and quality of vegetables. *Sulphur Agriculture*, *14*: 3-7.

Scuderi, D., Restuccia, C., Chisari, M., Barbagallo, R. N., Caggia, C., & Giuffrida, F. (2011). Salinity of nutrient solution influences the shelf-life of fresh-cut lettuce grown in floating system. *Postharvest Biology and Technology*, *59*(2), 132-137.

Selma, M. V., Luna, M. C., Martínez-Sánchez, A., Tudela, J. A., Beltrán, D., Baixauli, C., & Gil, M. I. (2012). Sensory quality, bioactive constituents and microbiological quality of green and red fresh-cut lettuces (*Lactuca sativa* L.) are influenced by soil and soilless agricultural production systems. *Postharvest Biology and Technology*, *63*(1): 16-24.

Sheikh, B. A. (2006). Hydroponics: key to sustain agriculture in water stressed and urban environment. *Pakistan. Journal of Agriculture., Agriculturall. Engineering., Veterinary. Science*, *22*: 53-57.

Short, G., Yue, C., Abbey, M., Anderson, N., Phelps, N., Venturelli, P., & Vickers, Z. (2018). Consumer preferences for aquaponic produce: Implications from an experimental auction.

Short, G., Yue, C., Anderson, N., Russell, C., & Phelps, N. (2017). Consumer perceptions of aquaponic systems. *HortTechnology*, *27*(3): 358-366.

Sibhatu, K. T., Krishna, V. V., Qaim, M. (2015) Production diversity and dietary diversity in smallholder farm households. *Proceedings of the National Academy of Sciences*, *112* (34): 10657-10662

Sirsat, S. A., & Neal, J. A. (2013). Microbial profile of soil-free versus in-soil grown lettuce and intervention methodologies to combat pathogen surrogates and spoilage microorganisms on lettuce. *Foods*, *2*(4): 488-498.

Slingerland, D. I. (June, 2017) Aquaponics Ethiopia: Developing a business model for sustainable implementation of small scale aquaponics systems improving food and nutrition security of rural and peri-urban households in Ethiopia.

SmartFish, E. F. F. (2013). Small-scale aquaponics launched in Ethiopia by FAO under the SmartFish project. *FAO Aquaculture Newsletter*, *51*, 53.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., & Lovatelli, A. (2014). Small-scale aquaponic food production: integrated fish and plant farming. *FAO Fisheries and Aquaculture Technical Paper.*

Suhl, J., Dannehl, D., Kloas, W., Baganza, D., Jobs, S., Scheibe, G., Schmidt, U. (2016) Advanced aquaponics: evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agricultural Water Management*,*178*: 335-344.

Tadesse, A. (2017). *Aquaponics productivity response for Niger seed cake (Guizotiaabyssinica) inclusion and increased level of mineral supplementation in fish diet* (Doctoral dissertation, Addis Ababa University Addis Ababa, Ethiopia).

Tamin, M., Harun, A., Estim, A., Saufie, S., & Obong, S. (2015). Consumer acceptance towards aquaponic products. *Journal of Busines Management*, *17*, 49-64.

Tamme, T., Reinik, M., Roasto, M., Meremäe, K., & Kiis, A. (2010). Nitrate in leafy vegetables, culinary herbs, and cucumber grown under cover in Estonia: content and intake. *Food Additives and Contaminants*, *3*(2), 108-113.

TerMorshuizen, L. D. (1996). *Distribution patterns of fishes in the head region of a turbid Eastern Cape estuary.* .

Tokunaga, K., Tamaru, C., Ako, H., & Leung, P. (2013). Economics of commercial aquaponics in Hawaii. In *Aquaponics in Hawaii Conference.*

Tokunaga, K., Tamaru, C., Ako, H., & Leung, P. (2015). Economics of Small-scale Commercial Aquaponics in Hawaii. *Journal of the World Aquaculture Society*, *46*(1): 20-32.

Travieso, L. L., Leon, A. P., Logegaray, V. R., Frezza, D., & Chiesa, A. (2016). Loose leaf lettuce quality grown in two production systems. *European Scientific Journal, ESJ*, *12*(30).

Treftz, C., Zhang, F., Omaye, S. T. (2015) Comparison between hydroponic and soil-grown strawberries: sensory attributes and correlations with nutrient content. *Food and Nutrition Sciences*, 6:1371-1380

Tronstad, R. (1995). Product position. *Direct farm marketing and tourism handbook. University of Arizona, États-Unis, Cooperative Extension Service.*

Tyson, R. V., Treadwell, D. D., & Simonne, E. H. (2011). Opportunities and challenges to sustainability in aquaponic systems. *HortTechnology*, *21*(1): 6-13.

UNICEF. (2017). Global Nutrition Report 2016: from promise to impact: ending malnutrition by 2030.

Van Os, E. A. (1994). Engineering and environmental aspects of soilless growing systems. *Hydroponics and Transplant Production 396*: 25-32.

Vicente, A. R., Manganaris, G. A., Sozzi, G. O., & Crisosto, C. H. (2009). Nutritional quality of fruits and vegetables. *Postharvest handling: A systems approach*: 57-106.

Waisel, Y., & Eshel, A. (2002). Functional diversity of various constituents of a single root system. *Plant roots: the hidden half*, *3*: 157-174.

Yu, L Yan-bin, W Xin, Gou, S. Yi-bing, W. Gang. (2006). Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, *China. Journal of Environmental Science*,*18*(6), 1124 – 1134.

Zhao, X., Chambers IV, E., Matta, Z., Loughin, T. M., & Carey, E. E. (2007). Consumer sensory analysis of organically and conventionally grown vegetables. *Journal of food science*, *72*(2), S87-S91.

Appendixes

Appendix I: Certificate of ethical clearance for sensory evaluation

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

OFFICE OF THE DEAN

Ref. No. CNSDO/245/10/2018
Date January 25, 2018

To Whom It May Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 11/01/2018 Minute No. IRB/031/2018 has examined the project proposal entitled "**Nutritional quality and consumer preference of vegetables grown in aquaponic System in Ethiopia**" By Dereje Wolde.

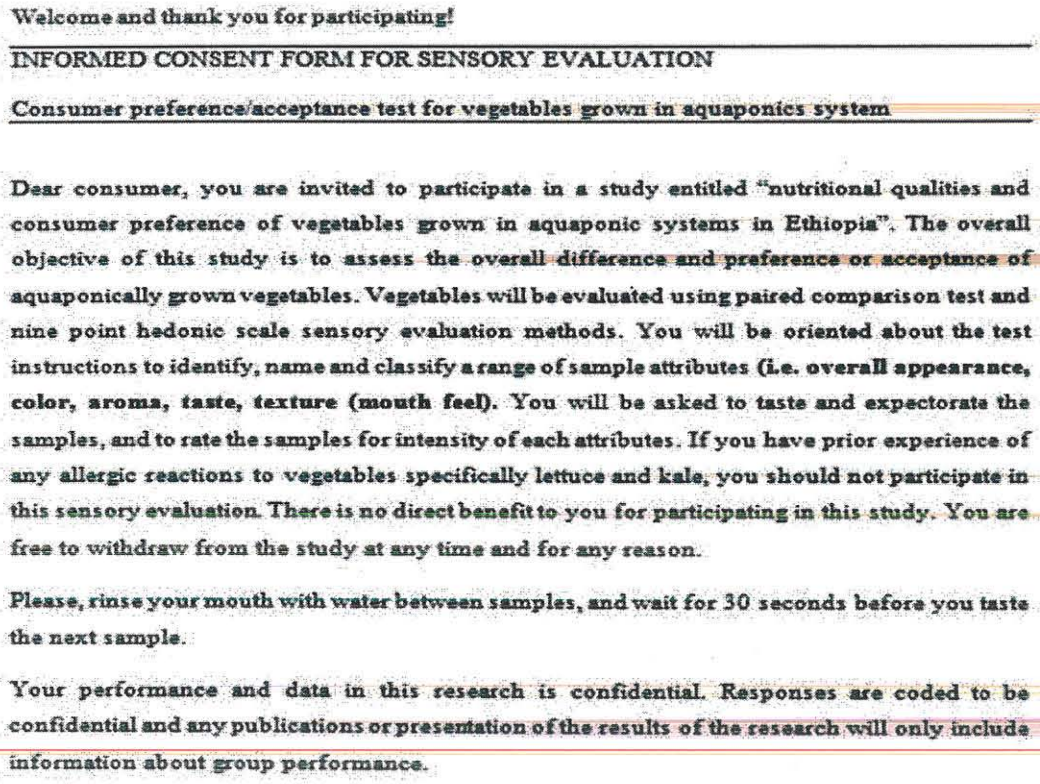
The proposal is approved for implementation.

With regards,

Shib[illegible] Temesgen [illegible]./
Dean, College of Natural & Computational Science

Tel.+251-11-123-94-72
Fax: +251-11-123-94-69
POBox 1176 Addis Ababa, Ethiopia
E-mail: dean_cns@aau.edu.et
Please Quote our reference number in you correspondence
"Examine all things; hold fast that which is good"

Appendix II: informed consent form for sensory evaluation.



Welcome and thank you for participating!

INFORMED CONSENT FORM FOR SENSORY EVALUATION

Consumer preference/acceptance test for vegetables grown in aquaponics system

Dear consumer, you are invited to participate in a study entitled "nutritional qualities and consumer preference of vegetables grown in aquaponic systems in Ethiopia". The overall objective of this study is to assess the overall difference and preference or acceptance of aquaponically grown vegetables. Vegetables will be evaluated using paired comparison test and nine point hedonic scale sensory evaluation methods. You will be oriented about the test instructions to identify, name and classify a range of sample attributes (i.e. overall appearance, color, aroma, taste, texture (mouth feel). You will be asked to taste and expectorata the samples, and to rate the samples for intensity of each attributes. If you have prior experience of any allergic reactions to vegetables specifically lettuce and kale, you should not participate in this sensory evaluation. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason.

Please, rinse your mouth with water between samples, and wait for 30 seconds before you taste the next sample.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance.

Appendix III: Questions asked to sensory panelists on discriminatory test

Difference test

DATE ___/___/___ TIME _______ PRODUCT ________________

Dear participant, here are two samples for evaluation. Is there a difference between the two samples in the following sensory attributes listed in the following table?

Attributes	Is there a difference: yes (1), no (2)
Overall Appearance	
Color	
Taste	
Texture (mouth feel)	

Appendix IV: Questions asked to sensory panelists on preference test

Preference test

Product ____________________ **Date** __/___/____ **Time** _________

Dear participant, here are two samples for evaluation. (Put '√' sign on the sample you prefer).

Which sample do you prefer?

Please examine code 263 first.

Code	221	263
Place tick		

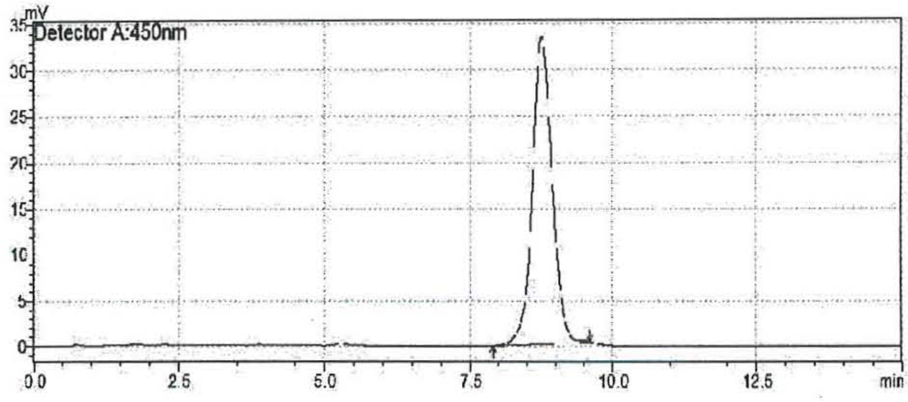
You must make a choice

Thank you!

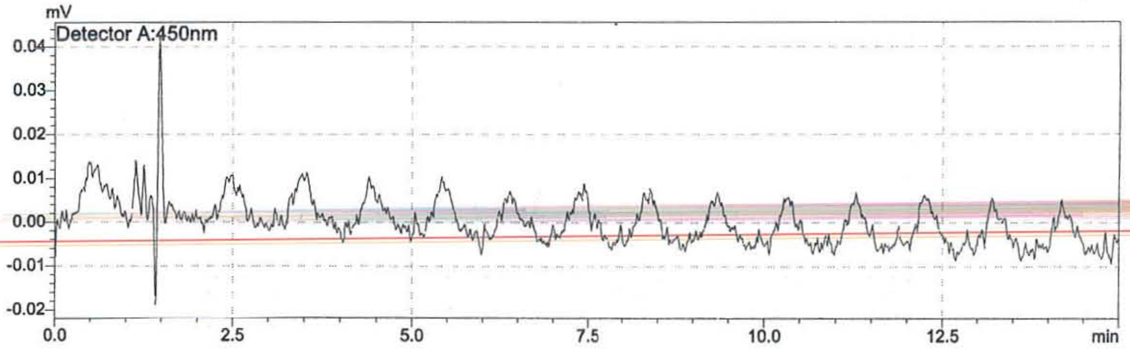
Appendix V: Fish meal composition

Ingredients	Percentage
Linseed	15%
Dicalcium phosphate	1.5%
Wheat bran	3.0%
Wheat grain flour	10%
Soya (fish) oil	0.2%
Vitamin premix	0.3%
Meat & bone meal	15%
Fish meal	55%
TOTAL	100

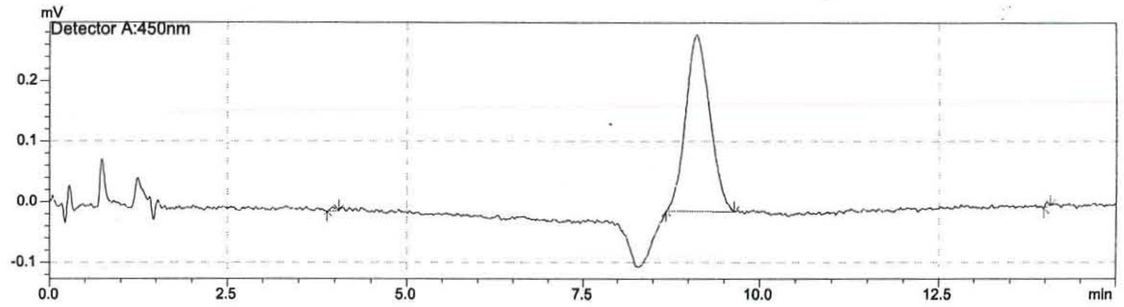
Appendix VI: Chromatogram of standard β-carotene (0.025ppm) with a retention time of 9 min.



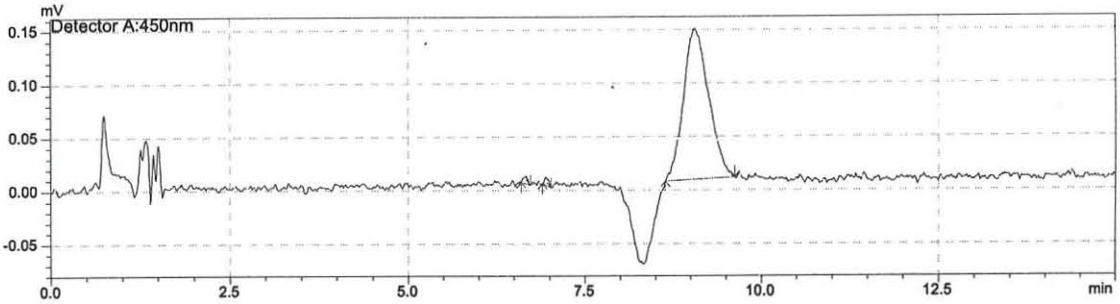
Appendix VII: Blank chromatogram indicating no interference of the mobile phase



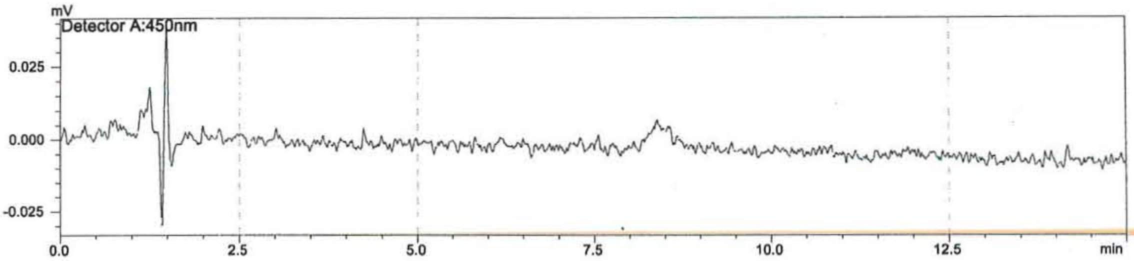
Appendix VIII: Beta-carotene chromatogramto determine Limit of Detection (LOD)



Appendix IX: Beta-carotene chromatogram to determine Limit of Quantification (LOQ)



Appendix X: Non-Detectable (ND) chromatogram below the LOD (0.0125ppm)



Appendix XI: Calibration curve for β-carotene to check linearity