Addis Ababa University
College of Natural and Computational Science
Center for Food Science and Nutrition

Nutritional Quality, Antioxidant Properties, Functionaland Oil Characteristics of Indigenous Okra (*Abelmoschus esculentus)* AccessionsGrown in Benishangul Gumuz Region, Ethiopia

A PhD Dissertation in Food Science and Nutrition

By:

Habtamu Fekadu Gemede

Submitted for the Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition

July 2017

Addis Ababa, Ethiopia

Supervisors:

1) Professor Gulelat Desse Haki (PhD)

Botswana University of Agriculture and Natural Resources

Department of Food Science & Technology

Private Bag 0027, Gaborone, Botswana

Email: hgulelat@bca.bw/ gulelatw@yahoo.com

2) Professor Fekadu Beyene (PhD)

Minister, Ministry of Livestock and Fisheries

Federal Democratic Republic of Ethiopia

P.O.BOX 170042, Addis Ababa, Ethiopia

Email: fekadu.beyene@yahoo.com

3) Dr.Ashagrie Zewdu (PhD)

Addis Ababa University, College of Natural Sciences

Center for Food Science and Nutrition

P.O.BOX 1176, Addis Ababa Ethiopia

Email: ashuyz1@yahoo.com/ ashagrie.zewdu@aau.edu.et

Dedication

This work is humbly dedicated to....

TheAlmighty GOD

my family

my parents

my supervisors

Habtamu... 

Acknowledgement

First and foremost, I gave thanks to the Almighty God for his innumerable love, protection and guidance over me and for giving me the strength and patience to complete this PhD dissertation. God is the secret of all my success. All honors and praises are for the almighty God.

I donot have enough words to express my deepest gratitude to my advisor Professor Gulelat Desse Haki for his unreserved guidance and encouragement from the beginning of this research. All the successes in my master thesis and this PhD dissertation are due to his unconditional support. His encouragement brought me a lot to run over the success truck and my entity at this stage is the result of his great efforts. I do not forget his entireeffortstowards my three months research stay at Lakehead University, Canada.

It is my immense pleasure to express my profound gratitude and sincere appreciation to my advisor Professor Fekadu Beyene for his constructive comments, valuable guidance and advice. The success of my whole career is due to his fingerprintand unreserved support. His kind approach, constructive comments, and share of experience are well appreciated. It is intellectually stimulating and rewarding to work under his supervision.

I would like to express my deepest gratitude and honor to my co-advisor Dr. Ashagrie Zewdu for his consistent support, follow up and valuable suggestions throughout this research. He is not only a good mentor but also a best friend and a father. His smiles, happiness, and encouragement at every stage of this work are really unforgettable.

I would also like to express my deepest gratitude to Professor Sudip K. Rakshit of Lakehead University, Canada for hosting me to accomplish part of my research work in his laboratory and making my stay in Canada comfortable. I am very much thankful for all the expenses he covered for my research and his dedication to following each step of my experiments in the lab. My stay was very short but it has been the greatest experience of my life time. I did not forget his strives during visa processing for my stay in Canada.

I would like to thank Mr. Bijaya K. Uprety for his support,the technical skill he taught me in adapting methodologies, preparing protocols and using instruments for my research work during my stay in Canada cannot be forgotten. I also would like to thank Ms. Fernanda A. Fiorda for her professional experience shared and guidance throughout my stay in Canada. It is a blessing to be

surrounded by so many wonderful people. I appreciate the hospitalities of members of staffs of LakeheadUniversity, Canada.

I would like to thank Assosa Agricultural Research Center (AARC) for allowing me to collect my study samples, especially Mr. Habtam Setu for sharing samples from his experiment and his support in facilitating the necessary equipment during sample preparation. I also want to thank the daily laborers and other AARC staffs who supported me during sample collection.

I would like also to express my deepest gratitude to Wollega University, for allowing me to proceed with my education and providing me with full sponsorship for my PhD study. I would like to thank Lakehead University, Nekemte Soil Research Center (NSRC), Ethiopian Public Health Institute (EPHI) and Ethiopian Food, Medicine and Healthcare Administration and Control Authority (EFMHACA) for their good will to use their laboratory space and facilities to conduct laboratory analyses. Unequivocally, I thank all laboratory staffs of Food Science and Nutrition laboratory, AAU, NSRC, EPHI and EFMHACA for their cooperation and technical support during the laboratory analysis.

I would like to thank Miss. Woinishet Abera, Mr. Adamu Belay, Mr. Seyoum Wolde, Mr. Debebe Hailu, Mr.Meseret Demissie,Ms. Mentewab Zeleke, Mr. Wosenyaleh Ambaw, Mr. Kebede Fufaand Mr. Addisu Legesse for all their laboratory assistance and other technical support in accomplishing this research work.

I would like to acknowledge Addis Ababa University for allowing me to continue my Ph.D. study. I would like to thank the Graduate Studies Office of Addis Ababa University for covering all the expenses of my Ph.D. study and providing the grant for my short term research stay in Canada. I would like also to acknowledge Dr. Kaleab Baye, Chair of the Center for Food Science and Nutrition for his unreserved support to facilitate administrative request during my stay in the center. I appreciate his keen interest to help others and positive outlook.

Words are not enough to express my special thanks to my darling wife, Ejigayehu and my adorable sons, Wanofi and Wabi for their patience, encouragement and unconditional love. I am the luckiest person to have them in my life. I would also like to express my special thanks to my mother, my father, my brothers and sisters for their love and help in using whatever resource

they have and always being affirmative towards my work. Also, I should not forget their care to me that brought me up to this success. I Love you!

I would like to express my special thanks to Dr. Dunkana Nugusa, Dr. Kassahun Tesfaye, Dr. Mulatu Geleta, Dr. Melaku Wakuma, Prof. Geremow Bultosa, Prof. Neguse Ratta, Dr. Melese Abdisa, Dr. Eba Mijana, Dr. Hirpa Leggese, Mr. Tilahun Abera, Mr. Belay Binatu, Mr. Alemu Lema, Mr. Tilahun Bekele, Dr. Paulos Getachew, Mr. Diriba Chawaka and Mr. Bekere Gemachu for their assistance in various tasks.

I want to acknowledge my friends, relatives and PhD students for their love and respect in all ways. My last but not least thanks go to all others who directly or indirectly contributed to the success of this work for their voluntary participation. Really, this is the fruit of our efforts.

Table of contents

List of Tables

No. **Pages**

List of Figures

No. **Pages**

List of Appendices

No. **Pages**

List of abbreviations/acronyms

AARC Assosa Agricultural Research Center

AAS	Atomic Absorption Spectrophotometer
AAU	Addis Ababa University
ABTS	2, 2'-azino-bis 3-ethyl-benzothiazoline -6-sulfonic acid
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
BD	Bulk Density
BG	Benishangul Gumuz
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
Ca	Calcium
CE	Catechin Equivalence
CHO	Carbohydrate
CRD	Completely Randomised Design
DPPH	2,2-diphenyl-1-picrylhydrazyl
DWB	Dry Weight Basis
EC_{50}	Effective Concentration
EDTA	2, 2-bipyridyl, disodium ethylenediaminetetracetate
EFMHACA	Ethiopian Food, Medicine and Healthcare Administration and Control Authority
EPHI	Ethiopian Public Health Institute
ES	Emulsion Stability
FC	Foaming Capacity
Fe	Iron
FS	Foam Stability
GAE	Gallic Acid Equivalent
GDP	Gross Domestic Production
IV	Indigenous vegetables
K	Potassium
Na	Sodium

ND	Non-Detected
NSRC	Nekemte Soil Research Center
OAC	Oil Absorption Capacity
Ox	Oxalate
P	Phosphorus
PC	Principal Component
PCA	Principal Component Analysis
Phy	Phytate
ROS	Reactive Oxygen Species
SE	Standard Error
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV-VIS	Ultraviolet-Visible
WAC	Water absorption capacity
Zn	Zinc

Abstract

Okra, a high nutritional potential, is one of the underutilized indigenous vegetables in Ethiopia. Lack of scientific information on the nutritional quality, phytochemical and oil properties of okra is a major constraint in its utilization in Ethiopia. This has worsened the already existing ever increase growing gap between human population and food supply. A food based-intervention specifically dietary diversification is an affordable and sustainable strategy to meet the demand of adequate food supply and population growth. One way of ensuring dietary diversity is to search and promote underutilized indigenous plant species such as okra.Therefore, the overall objective of this study was to determine nutritional quality, antioxidant properties, functionaland oil characteristics of pods and seeds of eight okra accessions grown in Assosa Agricultural Research Center in Benishangul Gumuz region, Ethiopia. The effect of different traditional processing methods on nutritional, antioxidant and functional properties of the pods and seeds of selected okra accessions was also evaluated.

The germplasm of okra accessions was collected from different agroecologicallocations in the region by Assosa Agricultural Research Center in 2012 and 2013 harvesting seasons andplanted on the research center plot under similar agronomic practice and management conditions during the 2014 main cropping season.The pods and seeds of eight okra accessions, namely OPA#1, OPA#2, OPA#3, OPA#4, OPA#5, OPA#6, OPA#7, and OPA#8 were collected from the center plots during the 2014 main okra harvesting season. The analyses were carried out using different official standard procedures and analytical grade chemicalreagents. The pods and seeds of okra accessions were then characterized for its proximate composition, mineral contents, anti-nutritional factors, phytochemical profiles, physicochemical properties, and functional properties.

The proximate composition in g/100gon dry weight basis of the pods and seeds of okra accessions varied significantly ($P<0.05$) from one accession to anotherand had the following ranges in pods and seeds respectively moisture 9.69-13.33 and 9.27-12.70; crude protein 10.25-26.16 and 22.51-38.09; crude fat 0.56-2.49 and 18.64-36.84; crude fibre11.97-29.93 and 1.94-5.96; total ash 5.37-11.30 and 4.53-6.05; utilizable carbohydrate 36.66-50.97 and 18.69-37.77. The gross energy ranges from 216.60-280.63 and 324.88-423.84 kcal/100g in pods and seeds respectively.The minerals in mg/100g ofpods and seeds of okra accessions also ranges from one

accession to another calcium 111.11-311.95 and 66.37-103.66; iron 18.30-36.68 and 8.33-20.29; potassium 122.59-318.20 and 90.00-187.92; zinc 3.83-6.31 and 3.92-6.42; phosphorus 25.62-59.72 and 516.94-1497.23 and sodium 3.33 to 8.31 and 15.06-27.81. The Na/K, Ca/P, Ca/K and Fe/Zn ratios of the pods and seeds of okra accessions, respectively were 0.025-0.290 and0.179-0.416; 2.605-11.312 and0.058-0.146; 0.507-1.753 and 0.399-0.999; and 3.239-9.611 and 1.297-4.806. The principal component analysis had shown a nutritional variability and five independent clusters in the pods and seeds of okra accessions and this may be useful to breeders for improvement of accessions based on the desired trait.The antinutrient in mg/100g of pods and seeds of okra accessions ranges from one accession to another. Phytate 0.83-0.87 and 0.39-0.46; tannin 4.93-9.90 and 0.71-3.78 and oxalate 0.04-0.53 and 0.74-0.75. The molar ratios of pods and seeds in this study were below the critical/ standard value.Particularly, pods and seeds of OPA#6 accession contained a significantly ((P<0.05) high amount of crude protein, ash, crude fat, calcium, iron and zinc contents.

The pods and seeds of okra accessions had total phenol (mg GAE/g) ranges from 28.10-95.21 and 21.28-57.34 and total flavonoid (mg CE/g) 8.18-18.72 and 10.73-29.04. The EC_{50} values (mg/ml) of pods and seeds of okra accessions are as follows: DPPH scavenging2.10-10.30 and 3.1->12; reducing power 1.20-4.20 and 1.18-4.30; metal chelating 0.50-1.52 and 0.32-1.11; and ABTS scavenging 0.31-1.33 and 0.07-1.5, respectively. The antioxidant activity of both pods and seeds of okra accession OPA#6 was high in all assays except ABTS scavenging activity for pod accessions with lower EC_{50} values and thus can be considered as a potentially rich source of natural antioxidants and used in functional food application.

The crude oil yield of the eight okra seedaccessions was significantly (P<0.05) varied and ranged from 19.25-38.19%. Compared with other vegetable oils, the present study revealed that okra seeds could be considered as potential sources of edible oil specifically the seed of OPA#2 accessions.The physicochemical properties of the okra seed oils varied significantly (P<0.05) from one accession to another except the saponification value. The values for the physicochemical properties are0.904-0.923 (specific gravity), 1.460-1.466 (refractive index), 1.315-5.055 (acid value), 2.990-9.060 (peroxide value), 100.45-132.92 (iodine value) and 188.97-194.78 (saponification value).Functional properties of the mucilage of okra pods varied significantly (P<0.05) and had respective ranges of bulk density 0.58 to 0.64 g/ml; water

absorption capacity 2.45 to 4.60 ml/g; oil absorption capacity 0.02 to 3.64 ml/g; emulsifying capacity 42.22 to 74.45%; emulsion stability 42.22 to 74.45%; foaming capacity 50.51 to 62.50% and foam stability 36.04 to 54.35%. Total phenolic and flavonoid contents of the mucilage of the pods of okra accessions ranged from 4.66 to 49.93 mg GAE/g and 8.18 to 18.72 mg CE/g, respectively. The effective concentration (EC_{50}) values (mg/ml) of mucilage of okra pods ranged from 3.15- 6.60 and 1.10-1.85 for DPPH scavenging and metal chelating effect, respectively.Particularly, mucilage of the pods from OPA#5 and OPA#7 had desirable water and oil absorption capacities, whereas the mucilage of accession OPA#1 and OPA#6 had high emulsifying and foaming properties.

Sun drying significantly ($P<0.05$) reduced the crude protein and crude fat contents of the pods by 7.53 and 34.94%, respectively,but increased the crudefibre and ash content of the pods by 27.90 and 20.44%, respectively. Soaking significantly ($P<0.05$) reducedthe crude protein, crude fibre, zinc and total phenolic content of the seeds by 3.68, 21.22, 9.03 and 21.03%, respectively. Germination significantly ($P<0.05$) increased the crude protein, crudefibre, total phenolic and total flavonoid content of pods by 3.68, 16.25, 17.32 and 15.12%, respectively, butreducedthe crude fat and zinc content of seeds by 17.65 and 34.89%, respectively. Roasting significantly ($P<0.05$) reduced crude protein,crude fat and crude fibre content seeds by 23.97, 31.49 and 50.86%, respectively, but it increased ash, total phenolic and total flavonoid content by 15.34, 14.89 and 14.12%, respectively.The flour of raw and processed pods and seeds of okra was found to exhibit good functional properties and can offer a great potential in various food applications.

Generally, the results of this research indicated that indigenous Ethiopian okra contains essential nutrients and phytochemicalsas compared to the commonly consumed green vegetables in Ethiopia such as cabbage, Ethiopian kale, lettuce, swiss chard, carrot, tomato, and celery. The mucilage of the pods and the oil characteristics of the seed accessions were found to exhibit good functional properties and can offer a great potential in various food systems. Hence, increasing the cultivation, promotion, and consumption of underutilized indigenous Ethiopian okra in the country couldhelp to mitigate food insecurity and alleviate malnutrition in the country. Further studies are recommended to determine the active ingredients and specific health benefits for the potential industrial use of the okra.

Key words: Okra; Pod; Seed, Accessions; Nutritional; Phytochemical; Oil; Mucilage; Processing

Chapter 1

Introduction

1.1 Background

Plants are the most important source of food for human beings mainly due to their availability and low cost compared to consumption of animal-source foods which are often unavailable because of economic and/or religious concerns (Gibson *et al*., 2006). The importance of plants to man, directly or indirectly, is demonstrated through their useful by-products from both wild and cultivated species. They are widely used as a source of food for man and livestock, as sources of energy, medicinal products, structural materials, and many other products. Plant crops can be classified into different types according to the way their product is used (cereals, pulses, root and tubers, fruit and vegetables, etc.) (Raven *et al*., 2005).

Among plant sources, vegetable crops play important role in human nutrition and health (Oguntibeju *et al*., 2013), this is because of their high vitamins, minerals, dietary fibre and phytochemicals, in addition to protein and energy (Grivetti & Ogle, 2000; Lyimo *et al*., 2003; Dias & Ryder, 2011). Regular consumption of vegetable-rich diets is recommended for overall better health, improvement of gastrointestinal health and vision, reduce risks of some chronic diseases such as cancer, heart disease, stroke, diabetes, anaemia, gastric ulcer and other chronic diseases (Thompson, 1993; Prior & Cao, 2000; Keatinge *et al*., 2010; Mullie & Clarys, 2011; Dias, 2012). Commonly, vegetables providethe basic useful properties providing health, nutrition and enzymes necessary for proper body function (Aman *et al*., 2005; Gemede *et al*., 2015b),while no single vegetable provides all the nutrient requirements (Uusiku *et al*., 2010).

Vegetables are of interest economically from several points of view: where there are good opportunities for sale, they are among the most profitable agricultural products (Aires*et al*., 2009). Vegetables are also resilient, adaptive and tolerant to adverse climatic conditions (Aires*et al*., 2009). Although they can be raised comparatively at lower management costs even on poor marginal lands, they have remained underutilized due to lack of awareness and popularization of technologies for utilization (Nath *et al*., 2009).Besides other crops, cultivation of vegetables will not only increase food production but also provide food security and poverty alleviation to the deprived section of a population (Sheela *et al*., 2004).

Vegetable crops are widely cultivated in Ethiopia (Fekadu & Dandena, 2006) and contribute 2.95% of the total crops production (Fanos & Derbew, 2015) and supporting a considerable portion of the country's population as a source of food. Among prominent vegetable crops produced in Ethiopia: pumpkin, tomato, carrot, cabbage, Ethiopian kale, pepper, onion, and garlic are the most popular (Gelmesa, 2010; Emana*et al*., 2015; Fanos & Derbew, 2015). Ethiopia has also the vast genetic diversity of plant species including important indigenous vegetables. The known indigenous vegetables which are native to Ethiopia are okra (*Abelmoschus esculents)* in Benishangul Gumuz Region, Jute mallow (*Corchorus olitorius)* in Afar region, moringa (*Moringa oliferain*) in Southern Nation Nationality People region and Anchote (*Coccinia abyssinica)* in Wollega(Simmone *et al*., 2004; Kumar*et al*., 2013;Olana, 2001; Gelmesa, 2010). The indigenous resources contribute considerably to the food, and the overall economy of a country (Demissie & Zerfu, 2009; Yang & Keding, 2009).

Okra (*Abelmoschus esculentus*) is a tall annual dicotyledonous vegetable plant indigenous to Ethiopia (Gelmesa, 2010; Kumar*et al*., 2013).It isan economically important vegetable crop (Ismail, & Ibn Idriss, 2013),grown in tropical, subtropical and warm temperate regions of the world (Arapitsas, 2008; Saifullah & Rabbani, 2009).Okra is the only vegetable crop of significance in the *Malvaceae* family(Abeykoon *et al*., 2010; Sharma & Prasad, 2010a).It is among the most heat and drought tolerant vegetable species in the world (Jarret *et al*., 2011;Priya *et al*., 2014).Okra is adapted to a wide range of climatic conditions(Akanbi *et al*., 2010) and grows best in hot weather (temperatures above 26◦C) (Ndunguru & Rajabu, 2004).

Okra can be grown on a wide range of soils (Tiamiyu*et al*., 2012; Priya *et al*., 2014),but well-drained fertile soils with adequate organic matter result in high yield (Akinyele & Temikotan, 2007).It can also grow well in poor soil with intermittent moisture (Ogungbenle & Omosola, 2015). The crop is widely cultivated throughout the year in the tropics (Singh *et al*., 2014), as a garden crop or on large commercial farms (Tripathi *et al*., 2011).

Okra is a multipurpose crop (Yonas*et al*., 2014) and its entire parts including the fresh leaves, buds, flowers, pods, stems, and seeds are edible and used as various food values (Maramag, 2013; Roy *et al*., 2014). Okra immature fruits (pods), which are consumed as vegetables, can be used in salads, soups, and stews, fresh or dried, fried or boiled (Ndunguru & Rajabu, 2004).Okra podscontain mucilage (thick slimy polysaccharides) that has the potential for use as food, non-

food products, and medicine (Kumar *et al*., 2010; Haruna *et al*., 2016). The extract of mucilage is often added to different recipes like soups, stews, and sauces to increase the consistency (Ahiakpa *et al*., 2014a; Biswal*et al*., 2014).

Okra seeds are a source of oil and protein (Oyelade *et al*., 2003) and they have been used on a small scale level for oil production (Anwar *et al*., 2010).Ithas also been called "a perfect villager's vegetable" because of its robust nature, dietary fibres and distinct seed protein balanced in both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) which it provides to diet (Kumar *et al*., 2010; Gemede *et al*., 2015a).Okra seed oilis also a richsource of antioxidants (Tian *et al*., 2015). Its seeds may be roasted and ground to form a caffeine-free substitute for coffee (Calisir *et al*., 2005).It also has industrial applications and is used in confectionery(Adetuyi *et al*., 2011).

Okra vegetable is one of the crop native to Ethiopia (Gelmesa, 2010; Kumar*et al*., 2013) and particularly originated in Benishangul Gumuz Regional State. In Ethiopia, the local name of okra is called Kenkase (Berta), Andeha (Gumuz), Bamuya (Oromiffa/Amharic). It is a major traditional vegetable food for the Berta community (one of the Ethnic groups in the region) and consumed as both fruit and vegetable (Gelmesa, 2010). According to Seyoum, (2013), the local farmers especially in the Berta community, consume it to assist the digestive system, to treat gonorrhea, tuberculosis, and tumor cancer. Traditionally, it is also believed that okra makes lactating mothers healthier and stronger. Therefore, okra the local name '*Kenkase'*, as used by the Berta community, describes the entire plant on one hand and the okra vegetable, on the other hand, are balanced(Seyoum, 2013).

1.2 Statement of the problem

A serious challenge to human survival, particularly in the developing world including Ethiopia, is the ever growing gap between human population and adequate food supply (Olana, 2001). A food-based intervention specifically dietary diversification is an affordable and sustainable strategy to meet the demand of adequate food supply and population growth. One way of ensuring dietary diversity is to search and promote underutilized indigenous plant species(Hussain *et al*., 2011) that are locally available and affordable as food and a source of nutrients (Adeyeye, 2006).Moreover, due to the high cost of animal food, attempts need to be

shifted to look into alternative food sources, especially underutilized and neglected plant sources including fruits and vegetables (Edwards & Parrett, 2003).

Ethiopia is the second most populous country in Africa with fast population growth rate (Mohammed *et al*., 2014) andhad food deficit (Gebreselassie, 2006).As a result, high malnutrition rates in Ethiopia pose a significant burden in economic and social development (Workneh *et al*., 2007; Regassa*et al*., 2015). About 40.2% of the total population was undernourished in the period 2010-2012 (FAO,WFP,& IFAD, 2012). Furthermore, in Ethiopia undernutrition is the major public health problem where40.4 % of children under the age of five years are stunted, 25 % are underweight, and 9 % are wasted (Gashu *et al*., 2016).

Diet in most part of Ethiopia is characterized asmonotonous andover-dependence on cereals and grains as the primary source of energy (Baye, 2014). Cereals and grains are the major food crops in terms of the area coverage (77%) at the national level and land allocated for vegetables is only 1%(Taffesse *et al*., 2012). The protection that fruits and vegetables provide against these maladies has been attributed to the presence of essential nutrients and several antioxidants (Olana, 2001; Garcia-Salas *et al*., 2010)and offer advantages over dietary supplements because of low cost and wide availability (Dias, 2012). Therefore, increasingvegetables utilization and consumption are critical to alleviating incidence of nutritional deficiencies (Yang *et al*., 2006).

As a remedial potential crop, okra is a powerhouse of valuable nutrients (Sharma & Prasad, 2010b)anda multiple-purpose crop in which the entire parts are edible and used to have several foods and nonfood applications (Lim, 2012; Maramag, 2013).There are also literature reports (Thampi & Indira, 2000; Oyelade *et al*., 2003; Aminigo & Akingbala, 2004; Goplana *et al*., 2007; Ndangui *et al*., 2010; Sharma & Prasad, 2010a; Sreeramulu & Raghunath, 2010; Adetuyiet al., 2008; Adetuyi *et al*., 2011;Adetuyi & Komolafe, 2011;Adelakun*et al*. 2012; Adetuyi& Stella, 2012; Ahiakpa *et al*., 2013; Nwachukwu*et al*., 2014; Hassan*et al*., 2015; Ogungbenle & Omosola, 2015; Adelakun*et al*., 2017)realizing the nutritional and phytochemical potentials of edible okra in different part of the world.Moreover, okra has been considered a minor crop and until recently no attention was paid to its huge untapped potential for improvement in the international research program (Kumar *at al*., 2010). In addition, Seyoum (2013) reported that despite the nativity of okra to Ethiopia and very important crop for the local Berta community in Benishangul Gumuz region, there is no significant research done in Ethiopia to promote and

enhance the food value of okra and thusconsidered as an orphan crop. Therefore, research strategies should give emphasis on promoting nutritional and phytochemical compositions of okra that could play a significant role in mitigating food insecurity and alleviating malnutrition in the country.

The demand for vegetable oils is rapidly increasing (Idouraine *et al*., 1996, Sorkhe*et al*., 2016)andtomeet the demand, there is a need not only to increase the production of the major oilseed crops but also to diversify the sources of oil by exploring and increasing the production of minor and neglected crops such as okra.Okra has also the potential to be cultivated as an oilseed crop because its seeds contain a high amount of oil (Kumar*et al*., 2010; Anwar *et al*., 2011).Although a number of studies have been reported on the characteristics of the oil and other components of okra vegetables (Camciuc *et al*., 1998b; Pham *et al*., 2003; Ndangui *et al*., 2010; Jarret *et al*., 2011; Ogungbenle & Omosola, 2015),to the best of our knowledge, there is no single published report currently available on physicochemical properties of Ethiopian okra seedoil.

Okra, like many other vegetable crops, is eaten in a raw or processed form. Like other fruits and vegetables, okra is also a perishable and seasonal plant food which is subjected to post-harvest losses (Tsado, 2015). Therefore, traditionally, the pods of okra are sun-dried in order to extend their shelf life and increase availability throughout the year, and other forms of traditional processing methods are also applied before consumption. Such processing methods can have both detrimental and beneficial effect on the nutritional, antioxidant and functional properties of food. The current study was, therefore, to evaluate nutritional quality, antioxidant properties, functional and oil characteristics of the pods and seeds of indigenous okra (*Abelmoschus esculentus*) accessions grown in Benishangul Gumuz region, Ethiopia. Also, the effect of different traditional processing methods on nutritional, antioxidant and functional properties of pods and seeds of selected okra accession was evaluated.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to determine the nutritional quality, antioxidant, functional properties and oil characteristics of indigenous okra (*Abelmoschus esculentus*) accessions grown in Benishangul Gumuz Region, Ethiopia.

1.3.2 Specific objectives

The specific objectives of the study were to:

a. Determine and comparethe proximate, mineral and antinutrient composition of the pods and seeds of okra in order to identify accessions with the bestnutritional and low antinutritional content.
b. Evaluate and compare the phytochemical profile and antioxidant activity of the pods and seeds of okra accessionsin order to identifying as the possible sources of antioxidants.
c. Characterizethe physicochemical,functional and antioxidant properties of okra seed oil and pod mucilage of accessionsin order to identify the possible sourcesof mucilage and oils
d. Determine the effect of traditional processing methods on nutritional, phytochemical and functional properties of the pods and seeds of selected okra accession.

1.4 Research questions

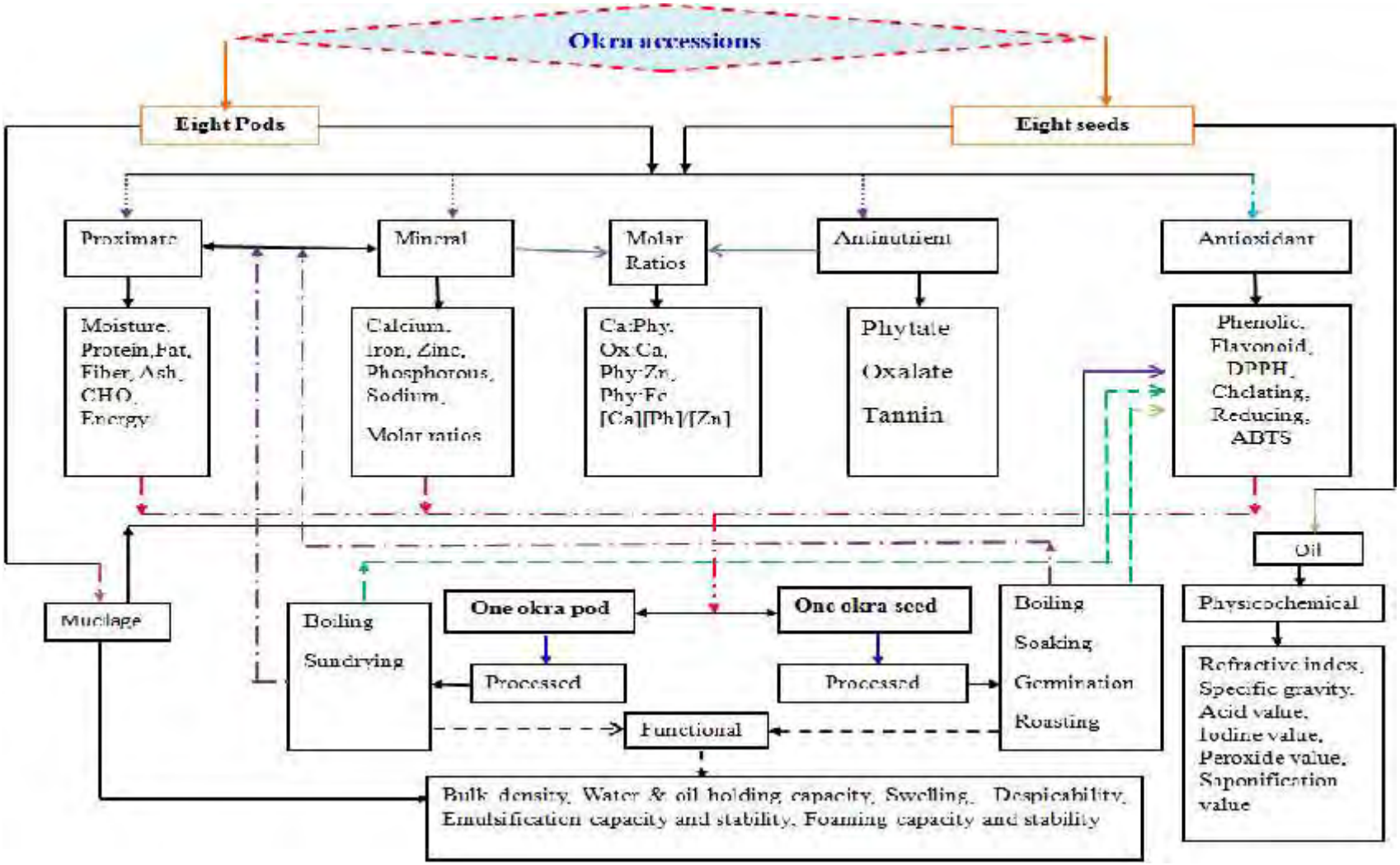
The research questions were:

a. Isthere anysignificant difference inproximate, mineral and antinutrient composition of the pods and seeds of okra accessions grown in Benishangul Gumuz region, Ethiopia?
b. Is there anysignificant difference in phytochemical profile and antioxidant activity of the pods and seeds of okra accessions?
c. Is there anysignificant difference inphysicochemical properties of the oil seeds of okra accessions?
d. is there a significant difference inthe functional and antioxidant properties of mucilage from the pods of okra accessions?
e. Do the traditional processing methods significantlyaffectthe nutritional, phytochemical and functional properties of the pods and seeds of okra accessions?

1.5 Significance of the study

The findings of this study are expected to fill the research gap concerning indigenous Ethiopian okra by providing scientific information on its nutritional composition, phytochemical and oil characteristics of the pods and seeds and would benefit researchers, producers, and consumers of okra based food in Ethiopia and the world at large. When the nutritional contents of the edible parts of Ethiopian okra are known, the society will use it as a staple food, and thereby, help in combating malnutrition, improving the health status, reducing poverty and ensuring food security in the country. It will provide the necessary information about antioxidant properties that would contribute to human health by protecting cells from oxidative stress and will be used as functional food ingredients. This study also will provide necessary information about quantity and quality of the oils from the seeds and as a result, okra oil will be used for nutritional, domestic processing and commercial oil prodution.

The information on the functional properties of the mucilage extracted from the pods of okra could be used in various food applications. The result of the study will help the society to identify the appropriate processing method, which will increase the absorbability of nutrients and antioxidants. It can also be used as a base line data for further research in the same area (enrich other food products, to develop new products etc).


Okra accessions
Eight Pods
Eight seeds
Proximate
Mineral
Molar Ratios
Antinutrient
Antioxidant
Moisture, Protein,Fat, Fiber, Ash, CHO, Energy
Calcium, Iron, Zinc, Phosphorous, Sodium, Molar ratios
Ca:Phy, Ox:Ca, Phy:Zn, Phy:Fe [Ca][Ph]/[Zn]
Phytate
Oxalate
Tannin
Phenolic, Flavonoid, DPPH, Chelating, Reducing, ABTS
Oil
Physicochemical
Refractive index, Specific gravity, Acid value, Iodine value, Peroxide value, Saponification value
Mucilage
Boiling
Sundrying
One okra pod
One okra seed
Processed
Processed
Boiling
Soaking
Germination
Roasting
Functional
Bulk density, Water & oil holding capacity, Swelling, Despicability, Emulsification capacity and stability, Foaming capacity and stability

Chapter 2

Literature review

2.1 Overview of okra

2.1.1 Origin of okra

Okra originated in Ethiopia (Olana, 2001; Simmone *et al*., 2004; Gelmesa, 2010; Kumar*et al*., 2013) and was distributed to North Africa, Mediterranean, Arabia and India in the 12th century (Nzikou *et al*., 2006). Considering the little contact between Ethiopia and the rest of the world within historic times, it is not surprising that little is known about the early history and distribution of okra. Therefore, the routes by which okra was takenfrom Ethiopia to the rest of the world is not documented (Tindall, 1983).

2.1.2 Taxonomy of okra

Okra (*Abelmoschus esculentus*) is one of the most widely known and utilized species of the family Malvaceae (Bayer & Kubitzki, 2003; Naveed *et al*., 2009). Okra plant was previously included in the genus *Hibiscus*. Later, it was designated to *Abelmoschus*, which is distinguished from the genus *Hibiscus*(Aladele *et al*., 2008).*Abelmoschus* was subsequently proposed to be raised to the rank of distinct genus by Kuwada (1974).

Scientific classification (Kumar *et al*., 2013)**:**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: Abelmoschus

Species: *A.Esculentus*

Binomial name: *Abelmoschus esculentus*

Okra is known by many local names in different parts of the world. It is calledKacang Bendi, qiukui, Okra, okura, Okro, Quiabos, Ochro, Quiabo, Gumbo, Quimgombo, Bamieh, Bamya, Quingumbo, Bamia, Ladies Fingers, Bendi, Bhindi and Kopi Arab (Kumar*et al*., 2013). In West and Central Africa, okra is called Gombo (French), Miyan-gro (Hausa), La (Djerma), Layre (Fulani), Gan (Bambara), Kandia (Manding), Nkruma (Akan), Fetri (Ewe) (Kumar *et al*., 2010).

In South East Asia okra is known as lady's fingers, bhindi in India, krajiabkheaw in Thailand, However, in Middle East it is known asbamia, bamya or bamieh and gumbo (Ndunguru & Rajabu, 2004).

In Portuguese and Angola, okra is known as quiabo, and as quimbombo in Cuba, gumbo in France, mbamia and mbinda in Sweden, and in Japan as okura(Lamont, 1999).In Taiwanit is called qiukui and in Nigeria as Igbo (McWhorter, 2000). In Ethiopia the local name of okra is called kenkase (Berta), andeha (Gumuz), bamiya (Oromiffa/Amharic). The name okra probabily derives from one of Niger-Congo group of Twi languages (ChutichudetBenjawan &Kaewsit, 2007).The term okra was in use the English by the late 18th century (Arapitsas, 2008).

2.1.3 Utilization of okra

Okra is a power house of valuable nutrients, nearly half of which is solublefibre in the form of gums and pectins and solublefibre helps to lower serum cholesterol, reducing the risk of heart diseases. The other half is insoluble fibre, which helps to keep the intestinal tract healthy (Adetuyi*et al*., 2008).Different part of okra seems to offer some useful purpose (National Research Council, 2006). It is a multipurpose crop due to its various uses of its edible parts like the fresh leaves, buds, flowers, pods, stems and seeds (Yonas *et al*., 2014). Immature fruits of okra (green pods), which are consumed as vegetables, can be used in salads, soups and stews, fresh or dried, fried or boiled (Ndunguru & Rajabu, 2004). It offers mucilaginous consistency after cooking. Often the extract obtained from the fruit is added to different recipes like soups, stews and sauces to increase the consistency. Okra is primarily used as a vegetable; its pods, seeds, leaves, and shoots, as well as the outer cover of the flowers (calyx)is all eaten as boiled greens (National Research Council, 2006).

Okra pods

Okra pods (Figure 2.1)are important as fresh fruits, and they can be consumed as boiled, fried or cooked (Akanbi *et al*., 2010; Akintoye *et al*. 2011).The pods are consumed as boiled vegetables; dried and used as soup thickeners or in stews (Yadev & Dhankhar, 2002; Ndunguru & Rajabu, 2004). The pods of okra have a unique flavor and mucilaginous texture. In Turkey, the young pods are strung together and allowed to dry for use in winter. In Africa the fruit is sliced, sun-

leading the production by 75.92% followed by Africa (23.41%) and America (0.54%) (Ahiakpa*et al*., 2014b). In West Africa, okra production is estimated at 500,000 to 600,000 tons per year (Farinde, *et al*., 2007). According to FAO database, the major okra producing countries in 2010-11 were India, Nigeria, Sudan, Iraq, Côte d'Ivoire and Pakistan (Oliver*aet al*., 2012)(Figure 2.3).

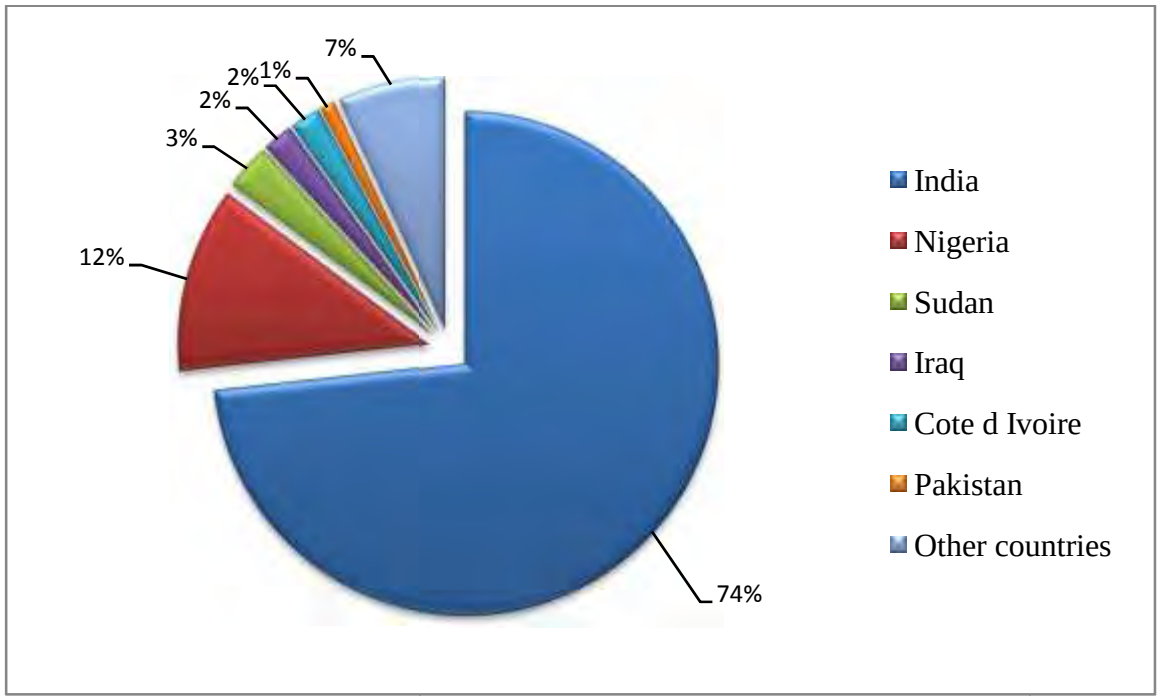


Figure 2.3 Major okra producing countries in 2010-11(Oliver*aet al*., 2012).

India is the leading producer of okra with over 70% of the total world production and has a vast potential as one of the foreign exchange earner crops, since it accounted for about 60% of the export of fresh vegetables excluding potato, onion, and garlic (Sankar *et al*., 2008; Gulsen *et al*., 2007; Ahiakpa *et al*., 2014b). In Ghana, it is the fourth most popular vegetable after tomatoes, pepper, and garden eggs (Sinnadurai, 1971). In West Africa, okra ranks second in vegetable production after tomato (Anonymous, 2002).

2.1.5 Growth and development of okra

Okra is perfect as a villager's crop which can be grown on a wide range of soil types, although rich, sandy loam soils are optimum (Sharma & Prasad, 2010b). It is also easy to grow, robust, and adapts to difficult conditions and can grow well where other food plants prove unreliable (National Research Council, 2006).Flowering begins about two months after planting. Each

flower then develops rapidly into a pod, which is typically harvested just 3-6 days after the flower was formed. Pods harvested at this stage are tender, flavorful, and about half was grown. Any that remain on the plant quickly turn fibrous and tough (National Research Council, 2006).

2.1.6 Harvesting and yields of okra

Okra plant is usually grown for its green tender pods that are harvested over multiple times. Okra podis usually harvested at least three times a week. Pods can be harvested by hand and the seeds extracted when the pods become dry and brittle. The pods have a high respiration rate and should be cooled quickly. Those in good condition can be kept satisfactorily for 7 to 10 days at 7 to 10°C. A relative humidity of 90 to 95 percent helps prevent shriveling (National Research Council, 2006).About 10 to 15 ton per hectare of the pod yields can be obtained under good management (NARP, 1993),but yields of over 40 tons per hectare can be realized under optimal conditions (Kumar *et al*., 2013).

An average yield of okra varies from 6.5-7.5 t/ha of pods during the dry season and 11.5-12.5 t/ha during the rainy season (Ahmad *et al*., 2015). Seed yields approaching 500 kg per picking per hectare (0.5 kg per plant) may be produced during a harvest period of 30-40 days. On a commercial scale, it is possible to get 1500kg of seeds per hectare but subsistence cultivation yields are in the region of 500kg/ha (Schippers, 2000). Kumar*et al*. (2013)reported that the seed yields of okra are in the range of 500-1000 kg/ha.

2.1.7 Economic importance of okra

Okra is the only vegetable crop of economic importance in the Malvaceae family and cultivated throughout the tropic and sub-tropic regions (Sharma & Prasad, 2010a). It is tolerant to a wide range of climatic conditions (Akanbi *et al*., 2010). It provides good yields and possibly more products than any other vegetables and economically speaking, its products are within almost everyone's reach (National Research Council, 2006). Okra is an important vegetable as its contribution to Ghana's agricultural Gross Domestic Production (GDP) in 2010 was 32, 309, 445, 183, US Dollars (Attigah *et al*., 2013). In India okra has a vast potential as one of the foreign exchange earner crops, since it accounted for about 60% of the export of fresh vegetables excluding potato, onion, and garlic (Sankar *et al*., 2008).To the best of our knowledge there is no publishedreport on economic importance of okra grown in Ethiopia.

2.2 Nutritional value and use of okra

Nutritional value is the main concern when a crop is considered as a food source (Hussain *et al*., 2009.Proximate and nutrient analysis of edible fruit and vegetables plays a crucial role in assessing their nutritional significance (Pandey *et al*., 2006). The nutritional composition of plant-based foods can vary depending on the environment, climate, soil nutrition and agronomic practices(Wood & Grusak, 2007).

2.2.1 Proximate composition of okra

Proximate composition gives an information on the basic chemical composition of food (Tsado *et al*., 2013), which involves moisture, ash, crude fat, crude protein, crudefibre, and carbohydrate (Aja *et al*., 2015). It also provides a good initial impression of relative nutritive value and utility of an agricultural product and allows basis for comparison between different species, plant parts and cultivation conditions (Hussain *et al*., 2009).

Okra is highly perishable because of its high moisture content and respiratory activities, thus it is necessary to dry them for prolonged use(Falade & Omojola, 2010). The high moisture content (89 g/100g) in okra fruits is also reported by Goplana *et al*. (2007) and Nwachukwu *et al*. (2014)(88.47%). The maximum water content varies between individual vegetables because of structural differences and cultivation condition that influence structural differentiation and may also have a marked effect on water levels of vegetables (Florkowski *et al*., 2009). Nzikou *et al*. (2006) reported thatokra seed moisture content ranged from 9.6 to 11.7 g/100g.The commonly consumed vegetables in Ethiopia have been reported to have moisture contentof 93.30, 87.60, 95.50, 91.50, 89.10, 92.50and 86.70 g/100gfor cabbage (*Brassica oleracea*), Ethiopian kale (*Brassica carinata*), lettuce (*Lactuca sativa*), swiss chard (*Beta vulgaris*), carrot (*Daucouscarota*), tomato (*Lycopersicum esculentum*) and celery (*Appiumgraveolens*), respectively (EHNRI, 1997) (Appendix 3.2).

Proteins may be found in a variety of foods. The proteins from plant sources are considered to be of low biological value because an individual plant source does not contain all of the essential amino acids. Therefore, combinations of plant sources must be used to provide these nutrients (Nelson & Cox, 2005). Okra pods have been reported to have crude protein contents (g/100g) of 13.61 to 16.27 (Adetuyi *et al*., 2011), 18 to 27 (Sharma & Prasad, 2010a), 4.81 (Nwachukwu*et*

al., 2014)and23.4 (Ogungbenle & Omosola, 2015).Okra seed is known to be rich in high-quality protein (Oyelade *et al*., 2003). Okra seeds have been reported to have appreciable protein contents of 21 g/100g (Aminigo & Akingbala, 2004),24.85 g/100g (Ndangui *et al*., 2010) and 22.30 to 26.81 g/100g (Hassan*et al*., 2015).The commonly consumed vegetables in Ethiopia have been reported to have crude protein content (g/100g) of 1.10 (cabbage), 2.80 (Ethiopian kale), 1.00 (lettuce), 2.20 (swiss chard), 0.04 (carrot), 1.30 (tomato) and 3.30 (celery) (EHNRI, 1997) (Appendix 3.2).

Dietary fats are used to increase the palatability of food by absorbing and retaining flavors (Antia *et al*. 2006). Excess consumption of fat has been implicated in certain cardiovascular disorders such as atherosclerosis, cancer, and aging whereas a diet providing 1-2% of its energy as fat is said to be sufficient to human beings (Blessing*et al*., 2011). Cert *et al*. (2000) reported thatagronomic and climatic conditions, fruit or seed quality, oil extraction system and refining procedures can cause variation in the content and composition of the constituents of vegetable oil. The oil content of some okra varieties of the seed can be quite high, about 40% (Deeplata & Rao, 2013). Okra oil has a pleasant taste and odor and is high in unsaturated fats such as oleic acid and linoleic acid (Franklin *et al*., 1982). Okra pods have been reported to have crude fat contents of 0.18 g/100g (Nwachukwu *et al*., 2014) and 9.22 to 10.57 g/100g(Adetuyi *et al*. (2011). Okra seeds have also been reported to have crude fat content of 23.44 g/100g (Ndangui *et al*., 2010) and 16.00 g/100g (Aminigo & Akingbala, 2004).The commonly consumed vegetables in Ethiopia have been reported to have crude fat content (g/100g) of 0.10 (cabbage), 0.80 (Ethiopian kale), 0.20 (lettuce), 0.04 (swiss chard), 0.02 (carrot), 0.07 (tomato) and 0.05 (celery) (EHNRI, 1997) (Appendix 3.2).

The ash content is a measure of the nutritionally important mineral contents present in the food material(Nesamvuni*et al*., 2001).Ash is the inorganic residue remaining after the water and organic matter has been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food (Kwenin*et al*., 2003).Okra pods have been reported to have crude ash content ranging from 7.19 - 9.63 g/100g(Adetuyi *et al*., 2011). Hassan*et al*. (2015)reportedthatokra seeds havea crude ash content of 9.02 g/100g.The commonly consumed vegetables in Ethiopia have been reported to have crude ash contentof 0.09, 1.90, 0.05, 2.10, 1.90, 0.70 and 2.00 g/100gfor cabbage (*Brassica oleracea*), Ethiopian kale

(*Brassica carinata*), lettuce (*Lactuca sativa*), swiss chard (*Beta vulgaris*), carrot (*Daucouscarota*), tomato (*Lycopersicum esculentum*) and celery (*Appiumgraveolens*), respectively (EHNRI, 1997) (Appendix 3.2).

Okra is considered as a rich source of dietary fibre. Nearly half of the okra pod is soluble fibre in the form of gums and pectin, which helps in lowering serum cholesterol (Jenkins *et al*., 2005) and thus reducing the risk of coronary heart disease (Jeff, 2002). The other half is insolublefibre, which helps to keep the intestinal tract healthy and prevents the symptoms of irritable bowel syndrome (Jeff, 2002). The high value of fibre reported for okra fruit can improve its digestibility and absorption processes in a large intestine, help to stimulate peristalsis and thereby preventing constipation (Olaofe*et al*., 2008).The fibre in okra helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract (Sabitha *et al*., 2011).Adetuyi *et al.* (2011)reported that okra pods had crude fibre contents ranged from 10.15 to 11.63 g/100g. Okra seeds have been reported to have crude fibre contents of 9.7 g/100g (Ndangui *et al*., 2010)and 13.00 to 17.00 g/100g (Hassen *et al*., 2015).The commonly consumed vegetables in Ethiopia have been reported to have crude fibre content (g/100g) of 1.30 (cabbage), 1.50 (Ethiopian kale), 0.07 (lettuce), 1.10 (swiss chard), 1.30 (carrot), 1.50 (tomato) and 1.90 (celery) (EHNRI, 1997) (Appendix 3.2).

Carbohydrate constitutes a major class of naturally occurring organic compounds which are essential for the maintenance of life in plant and animals and also provide raw materials for many industries.Plants are a good source of carbohydrate when consumed because they meet the recommended dietary allowance values (Mlitan *et al*., 2014). Okra is the most important vegetable crop and a source of calorie (4550 kcal/kg) for human consumption. It ranks first before other vegetable crops (Babatunde *etal*., 2007). Carbohydrates are mainly present in the form of mucilage (Kumar *et al*., 2009).Ogungbenle & Omosola (2015) confirmed that the edible portion of okra fruit is rich in carbohydrate.The commonly consumed vegetables in Ethiopia have been reported to have utilizable carbohydrate content (g/100g) of 23.70 (cabbage), 46.00 (Ethiopian kale), 15.40 (lettuce), 27.60 (swiss chard), 27.80 (carrot), 30.70 (tomato) and 47.70 (celery) (EHNRI, 1997) (Appendix 3.2).

2.2.2 Mineral content of okra

Minerals are considered to be essential in human nutrition.All form of living matter requires many minerals for their life processes. The human body requires more than 22 mineral elements that can be supplied by an appropriate diet in varying amounts for proper growth, health maintenance, and general well-being (World Health Organization, 1998). Plant-derived foods have the potential to serve as dietary sources of all human-essential minerals, and with a well-balanced diet. These minerals are vital for the overall mental and physical well-being and are important constituents of bones, teeth, tissues, muscles, blood and nerve cells (Soetan *et al*., 2010). They also help in the maintenance of acid-base balance, the response of nerves to physiological stimulation and blood clotting (Hanif *et al*., 2006). Plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle (Valvi & Rathod, 2011).

Calcium (Ca) is one of the important minerals more than 99% of which is found in the bones, and teeth to keep them strong, and support their structure (Shils, 1999). The rest is stored in blood, muscles, and cells. Sources of calcium include beans, lentils, nuts, leafy vegetables, dairy products, small fishes including sardines, bones, etc (Soetan *et al*., 2010).The green tender fruits of okra are highly nutritious; contain 107 mg/ 100 g of calcium per edible portion (Thampi & Indira, 2000). Okra pods havecalcium contents of 58.22 to 58.31 mg/100g (Adetuyi *et al*. 2011) and 107 mg/100g(Thampi & Indira, 2000). The calcium content of okra seeds reported by Ndangui*et al*. (2010)is78.65 mg/100g and Rao (1985)also reported that okra seeds contain245 mg/100g of calcium. The commonly consumed vegetables in Ethiopia have been reported to have calciumcontent (g/100g) of 43.00 (cabbage), 20.00 (Ethiopian kale), 22.00 (lettuce), 85.00 (swiss chard), 31.00 (carrot), 9.00 (tomato) and 317.00 (celery) (EHNRI, 1997) (Appendix 3.2).

Iron (Fe) is involved in many vital functions in the human body. In humans, iron is an essential component of hundreds of proteins and enzymes (Wood & Ronnenberg, 2006). Hemoglobin and myoglobin are heme-containing proteins that are involved in the transport and storage of oxygen (Walker *et al*., 2007). Iron nutrition is particularly important during the complimentaryperiod when the infant is growing rapidly and has a high demand for iron (Lorenz *et al*., 2007). One mole of phytic acid binds 6 mol ferric iron so that even relatively small quantities of residual phytate are still strongly inhibitory. Studies indicated that adding 10 mg/100g phytic acids to

bread rolls decreased iron absorption by 20%, and that adding 20 mg/100g decreased iron absorption by 40%. Phytate: Iron molar ratios greater than 0.15 are regarded as indicative of poor iron bioavailability.Absorption of iron from cereals can be increased by the degradation or removal of phytic acid with simple technologies like fermentation (Hurrell, *et al*., 2003). The best natural sources of iron are red meat, spleen, heart, liver, kidney, fish, egg yolk, sea vegetables, clams, cockles, mussels, oysters, yeast, molasses, beans, nuts, seeds, and cereals, legumes, dark green leafy vegetables (Soetan *et al*., 2010).The iron values of okra pods reported by Adetuyi *et al*. (2011) varied from 0.87 to 0.96 mg/100g. The green tender fruits of okra are highly nutritious, containing 8.9 mg of iron for every 100g edible portion (Thampi & Indira, 2000).The commonly consumed vegetables in Ethiopia have been reported to have ironcontent (g/100g) of 0.07 (cabbage), 4.10 (Ethiopian kale), 1.60 (lettuce), 3.60 (swiss chard), 0.05 (carrot), 0.90 (tomato) and 5.20 (celery) (EHNRI, 1997) (Appendix 3.2).

Zinc (Zn) is an essential trace element for all forms of life (Adeyeye *et al*., 2000).It is known to be an essential component of over 200 enzymes, and necessary for normal collagen synthesis, and mineralization of bones. The metal has also been found to be involved in vital processes such as mitosis, synthesis of DNA, protein, gene expression, and activation (Walingo, 2009).Diets have been classified into high, medium and low-Zinc availability based on the phytate- zinc molar ratio. Phytate: zinc molar ratio is used to estimate the likely absorption of zinc from a mixed diet. Diets with a phytate-zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with the phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009). Phytate; Zinc molar ratio play a major role in inhibiting zinc absorption such that zinc absorption is typically less than 15% in high phytate meals (Adeyeye *et al*.,2000).Zinc content of okra podsreported by Adetuyi *et al*. (2011)was1.29-1.37 mg/100g.

Phosphorus (P) is an essential mineral that is required by every cell in the body for normal function. It is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. It also helps to maintain normal acid-base balance by acting as one of the body's most important buffers (Knochel, 2006).Dairy products, meat, poultry, eggs, fish, nuts, and legumes are generally good sources of highly available phosphorus. However, the main form of phosphorus from plant material is phytate which is resistant to digestion unless enzymatically

degraded by phytase (Golden, 2009). Thus, phosphorus from phytate is only absorbed to a minor degree under normal conditions, and the phytate fraction of phosphorous should, therefore, be discounted from the calculations of the total phosphorous requirements. Important sources of phosphorus include phosphate food additives, green leafy vegetables, and fruits, especially banana (Soetan *et al*., 2010). Okra pods and seeds are reported having aphosphorus contentof60.05 to 62.17 mg/100g (Adetuyi *et al*., 2011)and1450mg/100g (Ndangui *et al*., 2010), respectively. The commonly consumed vegetables in Ethiopia have been reported to have phosphorus content (g/100g) of 37.00 (cabbage), 64.00 (Ethiopian kale), 31.00 (lettuce), 41.00 (swiss chard), 20.00 (carrot), 29.00 (tomato) and 52.00 (celery) (EHNRI, 1997) (Appendix 3.2).

Potassium (K) is an essential dietary mineral. It is a very significant body mineral in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle and cell membrane function (Soetan *et al*., 2010). High concentration of potassium in the body was reported to increase iron utilization and beneficial to people taking diuretics to control hypertension and those who suffer from excessive excretion of potassium through the body fluid (Arinanthan, 2003).Sources include vegetables, fruits, nuts (Soetan *et al.* 2010).Okra seeds have been reported to have a potassium content of 109.76 mg/100g (Ndangui *et al*., 2010).

Sodium (Na) is the principal cation in extracellular fluids. It regulates plasma volume and acid-base balance, involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function, maintenance ofmembrane potentials, transmission of nerve impulses and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts (Murray *et al*., 2000). The daily value for sodium is 2400mg for adults and children aged 4 and older. However, there is a need to judiciously consider diets prepared from okra since high dietary sodium is implicated in cardiovascular and renal disorders (Aletor & Adeogun, 1995). Similarly, people who suffer from or are prone to hypertension are discouraged from high dietary sodium. Okra seeds have been reported to have a sodium content of 54.78 mg/100g (Ndangui *et al*., 2010).

2.3. Antinutritional factor in okra

Antinutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some of theantinutrients have been shown to be evidently advantageous to human and animal health if consumed in appropriate amounts (Ugwu & Oranye, 2006).

Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) (Loewus, 2002).The major concern about the presence of phytate in the diet is its negative effect on the mineral uptake (Greiner & Konietzny, 2006).Also, phytate is reported to interact with carbohydrates (starch) and reduce their bioavailability and digestion. At the same time, phytate may have beneficial roles at a low level as an antioxidant, and anticarcinogen (Jenab & Thompson, 2002).Depending on the amount of plant-derived foods in the diet, and the grade of food processing, the daily intake of phytate can be as high as 4500 mg. On average, daily intake of phytate was estimated to be 2000- 2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries, and 150- 1400 mg for mixed diets (Golden, 2009).The phytate content of okra reported by Adetuyi *et al*. (2011)was 2.64-3.90 mg/100g.

Oxalate is a common and widespread component of most plant families(Liebman, 2002). While its levels in many plants are generally low, it is found in high concentrations in the leaves, and conus of plants consumed daily that are of concern. When oxalic acid is consumed, it irritates the lining of the gut and can prove fatal in large doses. Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50- 60 mg per day (Massey *et al*., 2001).Okra pods have been reported to have oxalate content of 0.32-0.506 mg/100g(Adetuyi *et al*., 2011).

Tannins are found almost in all plants all over the world(Anonymous, 1973). The antinutritional factors of tannins depend upon their chemical structures and dosage and the total acceptable tannin daily intake for a man is 560 mg.For example, tannins are found in tea and coffee and consuming too much of these beverages without milk may lead to calcium and iron deficiency in the body and often lead to osteoporosis and anemia (Stéphane, 2004).In order to counter these problems, it is advised that one should take tea or coffee between meals and not consecutively. In

addition, adding milk or lemon juice to the tea helps in reducing or neutralizing tannins' adverse actions on iron intake. Similarly, consuming food that is rich in vitamin C also helps in neutralizing tannin's effects on iron absorption (Osada *et al*., 2004**)**.To the best of our knowledge there is no published study available on tannin contents of okra.

2.4 Antioxidant properties of okra

Antioxidants are substances capable of inhibiting oxidation, reducing the concentration of free radicals in the body and/or chelating metal ions, and preventing lipid peroxidation (Ozsoy *et al*., 2008)and when added to food tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life (Fukumoto & Mazza, 2000).According to Hamid *et al*. (2010)antioxidants are categorized into many groups based on their sources and mechanism of reactions. Based on their source antioxidants are categorized into two groups namely: natural antioxidants and synthetic antioxidants. Natural antioxidants are found naturally and are extracted from plant and animal sources. The antioxidants such as superoxide dismutase, an enzyme that metabolize reactive oxygen species, superoxide reductase that catalyzes direct reduction of superoxide, catalases that catalyze dismutation of hydrogen peroxide to water and molecular oxygen, glutathione-related systems, selenium compounds, lipoic acid, and ubiquinones are other examples of naturally occurring antioxidants (Valdés *et al*., 2015).Synthetic antioxidants are prepared synthetically in the laboratory.Several synthetic antioxidants, such as BHA (Butylated Hydroxy Anisole) and BHT (Butylated Hydroxy Toluene) are widely used in the food industry due to their abilities to prevent food deterioration and to extend the shelf life of foods (Hotta *et al*., 2002). Unfortunately, the usage of synthetic antioxidants was restricted due to their side effects, such as an increase in the risk of cancer and liver damage in humans (Hue *et al*., 2012).

Based on their mechanism of reaction, antioxidants are categorized into two groups namely: primary antioxidantsand secondary antioxidants. The primary antioxidants are the chain breaking antioxidants which are co-factor of antioxidant enzymes in which their absence will definitely affect the metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron and manganese and antioxidant vitamins needed for most body metabolic functions, i.e. vitamin C, vitamin E, vitamin B(Hamid *et al*., 2010). Secondary antioxidants are also regarded as preventive antioxidants. These offer their antioxidant activity through various

mechanisms to slow the rate of oxidation reactions. The main difference with primary antioxidants is that secondary antioxidants do not convert free radicals into stable molecules (Chandran *et al*., 2013).

Total phenolics and flavonoids

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents and donating hydrogen atoms to free radicals (Adetuyi & Komolafe, 2011). Plants rich in phenolics are being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Saeed *et al*., 2012).Okra seed is a good source of total phenol as reported by Adetuyi & Komolafe (2011). Sreeramulu & Raghunath (2010)evaluated the antioxidant activity and phenolic content of nineteen vegetables commonly consumed in India and okra fruits ranked third in their phenolic content (167.70 mg GAE/100 g), behind red cabbage and broad beans.

Flavonoids can act as antioxidants in various ways, including direct trapping of reactive oxygen species, inhibition of enzymes responsible for superoxide anions formation, chelation of transition metals involved in the processes of free radical formation, and prevention of the peroxidation process (Rice-Evans *et al*., 1996).Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases (Baba & Malik, 2015). Flavonoids have been reported to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, antiallergic and vasodilatory actions (Baba & Malik, 2015).In addition, flavonoids reduce free radicals by quenching, up-regulating, or protecting antioxidant defenses and chelating radical intermediate compounds (Ndhlala*et al*., 2010).Flavonoids suppress reactive oxygen formation, chelate trace elements involved in the free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati *et al*., 2012). Ahiakpa *et al*. (2013) reported thatokra pods possess high amounts of total flavonoids.

Antioxidant activity assays

DPPH scavenging activity

DPPH *(2,2'-diphenyl-1 - picrylhydrazyl)* is a stable radical compound frequently used to examine free radical scavenging activity of natural compounds (Amarowicz *et al*. 2004). The DPPH radical has strong absorbance at 517 nm due to its unpaired electron and giving the radical

a purple color (Kalava & Menon, 2012). But upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH-H (Gursoy *et al*., 2010). This purple color generally disappears when an antioxidant is present in the medium/ low.Thus, antioxidant molecules can quench DPPH free radicals and convert them to a colorless/ bleached product resulting in a decrease in absorbance at 517 nm (Gursoy *et al*., 2010; Woldegiorgis *et al*., 2014). Therefore, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract.

Ferric reducing power

Reducing power is a novel antioxidation defense mechanism that affects the property of electron transfer ability (Suttirak & Manurakchinakorn, 2014)and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod *et al*., 2007). The Fe^{3+}–Fe^{2+} reducing power of the extract may serve as a significant indicator of its potential antioxidant activity (Dastmalchi *et al*., 2007). The yellow color of the test solution change to various green and blue shade depending on the reducing power of each compound (Ferreira *et al*., 2007).The amount of Fe^{2+} can be monitored and determined by measuring the generation of Perl's Prussian blue at 700 nm and a higher absorbance indicates higher activity (Zarena & Sankar 2009).The higher ferric reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential (Lee *et al*., 2007). The ferric reducing power property indicated that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod *et al*., 2007).

Metal chelating effect

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Woldegiorgis *et al*., 2014).Ferrous ions, the most effective pro-oxidants, are commonly found in food systems. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in the reduction of the intensity of the red color of the complex (Chandran *et al*., 2013). Measurement of color reduction, therefore, allows estimation of the chelating activity of the coexisting chelator (Middha *et al*., 2013).Ferrous ions could stimulate lipid peroxidation by Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) in which iron participates as a catalyst in the body (Li, 2011) and also accelerate

peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Torreggiani *et al*., 2005).Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions (Lin *et al*., 2014).

ABTS scavenging activity

ABTS (*2, 2'-azino-bis 3-ethyl-benzothiazoline -6-sulfonic acid)* radical scavenging activity is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (a scavenger of lipid peroxyl radicals) (Leong & Shui, 2002). The $ABTS^{\bullet+}$ is initially formed by reacting 2, 2'-azino-bis-(3- ethylbenzthiazoline-6-sulphonic acid) (ABTS) with oxidants.Hydrogen peroxide in an enzymic system and potassium persulphate are two of the most commonly used oxidants.

2.5 Physicochemical properties of okra oil

Oil yields

Vegetable oils are essential in meeting global nutritional demands and are utilized with many foods and other industrial purposes. Okra, which is currently grown mainly as a vegetable crop, has the potential for cultivation as an oilseed crop because its mature pods contain high quantity of seeds containing a considerable amount of oil which could be characterized and utilized for commercial purposes (Anwar*et al*., 2011). According to Andras *et al*. (2005), oil yields of okra seeds from Greece were found to be 15.9 to 20.7%, depending on the extraction method. The oil of okra seed appears to be as good as cottonseed oil (Aminigo & Akingbala, 2004). Deeplata & Rao, (2013)reported that the oil content of the seed of some okra varieties can be quite high, about 40% and was comparable with some common edible oils reported by Nichols & Sanderson (2003)for cottonseed (22-24%), safflower (30-35%), soybean (18-22%), rapeseed (40-48%), and olive (12-50%). The variation in oil yields may be due to the differences in a variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used (Mohamed& Girgis, 2005).

Specific gravity

Specific gravity is the ratio of the density of a substance to that of water at a specific temperature and is used as a simple means of obtaining information about the concentration of solutions

(Inekwe *et al*., 2012). It can also reveal the extent of adulteration (Akinhanmi *et al*., 2008). Okra oil has been reported to have a specific gravity of 0.913 g/cm^3(Ogungbenle & Omosola, 2015). The specific gravity (g/cm^3) of cottonseed, soybean, corn, sunflower, sesame safflower oils are 0.917, 0.9175, 0.918, 0.920, 0.918 - 0.921, 0.919 - 0.924, respectively (Amoo *et al*., 2008; Khanzadeh *et al*., 2012).

Refractive index

Refractive index is the degree of refraction of a beam of light that occurs when it passes from one transparent medium to another and is used by most processors to measure the change in unsaturation as the fat or oil is hydrogenated (Roger *et al*., 2010). The refractive index of oils depends on their molecular weight, fatty acid chain length, the degree of unsaturation, and degree of conjugation (Kolakowska & Sikorski, 2003; Gohari Ardabili *et al*., 2011). Refractive index is also an important optical parameter to analyze the light rays traversing through materials medium and can be used as a tool for determination of the adulteration of oils (Ariponnammal, 2012). Refractive index increases as the double bond increases and vice versa (Eromosele & Paschal, 2003; Omari *et al*., 2015).

The refractive index (1.463) reported by Ogungbenle & Omosola (2015)for okra seed oil is comparable to the conventional oils like soybean (1.466- 1.470) and palm kernel (1.449- 1.451) (Falade *et al*., 2008), cotton seed (1.468-1.472), safflower (1.473-1.476) and soybean (1.4728) (Khanzadeh *et al*., 2012). It is alsoin agreement with those reported by Olubaet *al.* (2011) for egusi melon seed oil (1.45) and by Ardabili*et al.* (2011) for pumpkin seeds (1.4662). However, okra seed oils contain less refractive index compared to most drying oils whose refractive indices are between 1.48 and 1.49 (Nichols & Sanderson, 2003; Akinhanmi *et al*., 2008).

Acid value

The acid value is a direct measure of the percentage content of free fatty acid content in a given amount of oil due to enzymatic activity (Ochigbo & Paiko, 2011).It is a measure of the extent to which the triglycerides in the oil have been decomposed by lipase action into free fatty acids; acid value depends on the degree of rancidity which is used as an index of freshness and is also often used as general indicators of the condition and edibility of oils (Sani*et al*., 2014; Ouattara *et al*., 2015).

The acid value of oil from 0.00 to 3.00 mgKOH/g is recommended for oil in the application of cooking (Barkatullah *et al*., 2012). The acid value (3.39 mgKOH/g) reported by Sani*et al*. (2014)for okra seed oil is lower than the acid value for benniseed oil (4.76 mgKOH/g) (Oshodi *et al*., 1999);calabash seed oil (5.92 mg/KOH); lump-in-neck oil (4.59mgKOH/g) and bottle gourd seed (5.21 mgKOH/g) (Olaofe *et al*., 2012). Ogungbenle & Omosola, (2015) reported that okra oil is used for cooking and in the formulation of pomades and margarine.Nzikou *et al*. (2006) also reported that okra oils could probably be good edible oils that may be stored for a long time without spoilage via oxidative rancidity. The lower the acid values the more its acceptability for edibility purpose (Sani*et al*., 2014).

Peroxide value

Peroxide value is a measure of oxidative rancidity and deterioration of oil level that could be used as an indication of the quality and stability of fats and oils (Ekwu & Nwagu, 2004). Oxidative rancidity is the addition of oxygen across the double bonds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds. Thus, the high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage (Mohammed & Hamza, 2008). The maximum acceptable peroxide value set by Codex Alimentarius Commission for groundnut oil is 10 mgEquiv.O_2/Kg (Codex Alimentairus Commission, 1993).On the other hand, according to the Codex Alimentarius Commission, the peroxide value for unrefined olive oil may be a maximum of 20 mgEquiv.O_2/kg oil (Gohari Ardabili*et al*., 2011).

The peroxide value for okra seed oils (7.31 mgEquiv.O_2/Kg) (Ogungbenle & Omosola, 2015)is higher than the peroxide valueof legume oils (5.63- 6.63 mgEquiv.O_2/Kg) (Olaofe *et al*., 2012) and lower than that of quinoa oil (2.44 mgEquiv.O_2/Kg) (Ogungbenle, 2003). Peroxide value is a measure of oxidative rancidity and deterioration of oil level that could be used as an indication of the quality and stability of fats and oils (Ekwu & Nwagu, 2004). Oxidative rancidity is the addition of oxygen across the double bonds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds. Thus, the high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage (Mohammed & Hamza, 2008).

Iodine value

The higher the iodine value, the more unsaturated is the oil (Ziyada & Elhussien, 2008). However, when the iodine value becomes too high, the stability of the oil reduces because it is

most likely to undergo oxidation. Vegetable oils are classified as drying, semi-drying, and non-drying regarding their iodine values (Khanzadeh*et al*., 2012).The iodine value obtained is less than <100 suggesting the absence of unsaturated fatty acids and this places the oil in the non-drying groups.This shows that the oil could be nutritionally beneficial, especially now that vegetable oils rich in polyunsaturated fatty acids and naturally occurring antioxidants are being sourced and recommended to patients that are hyperlipidemic or are suffering from any other lipid disorder (Njoku *et al*., 2001).

In addition,the high unsaturated fatty acid content of the seeds oil is of importance since they offer protective role against atherosclerotic cardiovascular disease (Ejikeme *et al*., 2010). The high degree of unsaturation of semi-drying oil further suggests that the oils can be used for the manufacture of cosmetics and oil paints (Peace & Aladesanmi, 2008) as well as shoe polish and varnishes (Akintayo, 2004). Ogungbenle & Omosola (2015)is also reported that the iodine value of okra seed oil enables it to be employed in the manufacture of soaps, lubricants, and candles. The iodine value of okra seed oil reported by Ogungbenle & Omosola (2015)is112.16 mgI_2/100g. The iodine value in mgI_2/100g of unsaturated fatty acid rich oils such as soybean, corn, sunflower, and cotton seed oils are 120-143, 103-128, 125-136, 100.0 - 123.0, respectively (Khanzadeh*et al*., 2012).

Saponification value

Oils with high saponification values are desirable in the soap making industry (Akanni *et al*., 2005). Ogungbenle & Omosola (2015) also reported that dry okra seed oil has a potential as an ingredient in the industrial manufacture of soap and cosmetics. These properties make them useful as sources of essential fatty acids required in the body (Akanni *et al*., 2005). The saponification value for okra seed oil reported by Ogungbenle & Omosola (2015)is182.20 mgKOH/gandbyNdangui *et al*. (2010)is183.1 mgKOH/g. The saponification value (mgKOH/g) of common oils such as soybean, peanut, and cotton seed oils range from 189- 195, 18 - 196 and 189- 198, respectively(Codex Alimentairus Commission, 1993).

2.6 Mucilage in okra

Mucilages are water-soluble polysaccharides inherent in several plant species and in some microorganisms (Ahiakpa *et al*., 2014a). Mostly plants belonging to the Malvaceae family and other related species such as baobab (*Adansonia digitata L.*), cotton (*Gossypium spp*), ambrette

The food applications include a whipping agent for reconstituted egg whites, an additive in the formulation of flour-based adhesives and additive for clarifying sugarcane juice. It is also used to modify the food quality in terms of food stability, texture and appearance properties by acting as emulsifiers, thickeners, gelling agents or texture modifiers (Noorlaila *et al*., 2015). Okra mucilage also contributes to improved functionality, especially water holding, emulsifying and foaming properties of food products (Jideani, & Bello, 2009). The mucilaginous extract of okra is often used to clarify sugarcane juice from which jaggary or brown sugar is manufactured (Prasad & Nath, 2002).

Industrially, okra mucilage is used to glace/ preserve certain papers and also useful in confectionery among other uses (Farinde *et al*., 2007).The chemical compositions, molecular structures, monosaccharide sequences, glycoside linkages configuration and position in the backbone and side chains are some of the factors that can affect the functional properties of natural plant mucilage (Mirhosseini & Amid, 2012). The mucilage content reported by Adetuyi & Dada (2014) for Nigerian okra (1.4 g/100g) is comparable with the finding reported by Hong & Ibrahim, (2012)forrose cactus (1.10-2.55%) and lower than the finding reported by Fedeniuk & Biliaderis (1994) for linseed (3.6-9.4%) and Kaewmanee *et al*. (2014) for flaxseed (1.80 to 6.65%).The variation in mucilage yield was attributed to species type, maturity at harvesting time, effect of drying, genetic factor, the season of collection and topographic variation like rain distribution, temperature, soil type, etc. (Gebresamuel & Gebre-Mariam, 2011).Moreover,Kaewmanee*et al*. (2014)pointed out that the extraction yield of mucilage can vary as a function of environmental factors, such as the climatic condition and crop age.

2.7 Functional properties of okra flour

Bulk density

Bulk density is a measure of the flour heaviness (Adejuyitan *et al*., 2009).The products with high bulk density are known to exhibit better packaging properties than those with low bulk density.Arinola *et al*. (2016)reported that high bulk density is desirable in that it offers greater packaging advantage as greater quantity may be packaged within a constant volume. A higher bulk density is desirable for a greater ease of dispersibility and a reduction of paste thickness. On the other hand, lower bulk density implies less quantity of the food samples which could be packaged in a constant volume, ensuring an economical packaging (Osundahunsi & Aworh,

2002). The okra seed has been reported to have a bulk density of 0.68 g/ml (Bryant *et al*., 1988).Adelakun *et al*. (2017)also reported that the bulk density of five okra seed varieties ranged from 0.12- 0.216 g/cm^3.The bulk density ofAfrican breadfruit kernel, tigernut and wheat flour is 0.54, 0.62 and 0.71 g/ml, respectively(Oladele& Aina, 2007). The raw and fermented wheat flour has been also reported to have a bulk density of 0.80 and 0.86 g/ml, respectively(Steve, 2011).

Water absorption capacity

Water absorption capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations (Obiegbuna *et al*., 2014)and is the amount of water available for gelatinization (Edema *et al*., 2005).It shows whether the protein can be incorporated into aqueous food formulations, especially those involving dough handling such as processed cheese, sausages and bread dough (Osungbaro *et al*., 2010).Water absorption capacity of 1.25 ml/g and above is an indication of good bakery property (Giami & Alu, 1994).Adelakun *et al*. (2017)reported that the water absorption capacity of five okra seed varieties ranged from 1.93- 3.2 g/cm.

Oil absorption capacity

Oil absorption capacity (OAC) is of significant, since fat acts as flavor retainer and also increases soft texture to mouth feel of foods (Aremu*et al*., 2006). They are also important because of their storage stability and particularly in the rancidity development (Siddiq*et al*., 2010).Oil absorption is the physical entrapment of oil in foods, especially by proteins. Oil absorption plays a major role in flavor retention by interacting with hydrophobic groups of flavor compounds and trapping them in the food matrix. A high oil absorption capacity is valuable in ground meat formulations, meat replacers and extenders, doughnuts, pancakes and baked foods (Amandikwa & Chinyere, 2012).Adelakun *et al*. (2017)reported that the oil absorption capacity of five okra seed varieties ranged from 1.80- 2.94 g/cm.

Emulsifying capacity

The emulsion capacity (EC) reflects the ability of a protein to aid in the formation of an emulsion and is related to the protein's ability to absorb at the interfacial area of oil and water in an emulsion (Fennema, 1996).The capacity of proteins to enhance the formation and stabilization of emulsion is critical for many applications in food products like chopped and comminuted meat, cake batter, coffee whitener, milk, mayonnaise, salad dressing, and frozen dessert (Elbaloula *et al*., 2014). Emulsion capacity is governed by the hydrophilicity and hydrophobicity of proteins as

they create electrostatic repulsion on oil surface. Proteins with high oil and water binding capacities are desirable for use in meats, sausages, bread and cakes, while proteins with high emulsifying capacity are good for sausages, bologna, soups and salad dressing (Fennema, 1996).Adelakun *et al*. (2017)reported that the emulsion capacityof five okra seed varieties ranged from 2.92- 3.44%.

Emulsion stability

Emulsion stability (ES) is important in food emulsions as it indicates the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing and flocculation (Singh *et al*., 2010). Increasing emulsion stability and fat binding during processing are primary functional properties of protein foods such as comminuted meat products, salad dressing, frozen desserts, and mayonnaise.Adelakun *et al*. (2017)reported that the emulsion stability of five okra seed varieties ranged from 1.33- 1.81%.

Foaming capacity

The foaming capacity of a food material depends on the surface active properties of its protein (Udensi& Okoronkwo, 2006).Foams are used to improve foods texture, consistency and appearance (Akubor, 2007).Flours with high foaming ability could form large air bubbles surrounded by thinner less flexible protein film. This air bubbles might be easier to collapse and consequently lower the foam stability (Jitngarmkusol *et al*., 2008).Foaming formation is governed by three factors: transportation, penetration, and reorganization of the molecule at the air–water interface. For good foaming, therefore, the protein should be capable of migrating at the air–water interface, unfolding and rearranging at the interface (Elbaloula *et al*., 2014).Adelakun *et al*. (2017)reported that the foaming capacity of five okra seed varieties ranged from 5.73- 6.44%.

Foam stability

Foam stability is important when the agent's usefulness whipping depend on their ability to maintain the whip as long as possible (Oulai*et al*., 2014).The ability to form stable foam is an important property in whipped toppings, frozen desserts, and sponge cakes. Adelakun *et al*.(2012)reported that the flour of roasted okra seeds cannot be used in these formulations. Foam stability is governed by the ability of the film formed around the entrapped air bubbles to remain intact without draining. Stable foams can only be formed by highly surface-active solutes

(Oulai*et al*., 2014). Adelakun *et al*. (2017)reported that the foam stabilityof five okra seed varieties ranged from 3.78-4.50%.

2.8 Effect of processing on nutritional, antioxidant and functional properties

Okra, like many other fruit and vegetable crops, is eaten in raw or processed form(Ndunguru & Rajabu, 2004). Traditionally, some forms of processing methods such as boiling, soaking, and germination are applied before consumption.In addition, okra is perishable and seasonal plant food which is subjected to post-harvest losses. Therefore, traditionally the pods of okra are sund-ried in order to extend their shelf life and increase availability throughout the year (Tsado, 2015).The seeds of mature okra are also reported to be roasted, ground and used as a coffee substitute (Calisir *et al*., 2005). Roasting has been reported to improve flavor (Akingbala *et al*., 2003). Such different processing methods can have both detrimental and beneficial effect on the nutritional, antioxidant and functional properties of food(Oghbaei & Prakash, 2016).

Effect of processing on proximate and mineral composition

Several studies reported that food processing have effects on nutritional contents like roasting to decrease moisture content of peanut (Adegoke *et al*., 2004); boiling to increasemoisture content of okra seed,whichmight be due to the water absorption capacity of fibres and other natural chemical components during heating (El Sohaimy, 2013); sun drying decrease moisture content of African spinach and okra species(Ukegbu & Okereke, 2013), boiling reduce crude protein content, due to leaching and denaturation of protein caused by boiling (Jayewardenapura,2000); roasting reducesprotein content of maize varieties to the denaturation and loss of protein (Onyango *et al*., 2004). In contrast to this, Olanipekun*et al*. (2015)reported that roastingincreases the protein value of the processed kidney bean seeds due to break down of crude protein to amino acids during processing. It has been earlier reported that when food is subjected to roasting, the activity of proteolytic enzymes is increased (Mbah *et al*., 2012),which hydrolyze inherent proteins to their constituent amino acids and peptides.Hooda & Jood (2003) reported that increases in protein content due to germination might be due to the reduction of seed nitrates into protein or ammonium compound.

Pandey & Awasthi (2015)reported that there is a loss of fat during germination which may be due to its consumption as an energy source in the process of germination (Pandey & Awasthi,

2015). Reduction in fat content upon roasting may be due to loss of volatile oils on open dry heat treatment (Mathur & Chaudhary, 2009). This decreased trend is also reported by Kiin-Kabari & Akusu (2014), who stated that roasting decreased the oil content of watermelon seed flour; that may bedue to volatilization or melting out of the fat as earlier observed by Kiin-Kabari & Akusu (2014). Akingbala *et al.* (2003)reported that the fat content of okra seed was reduced significantly from 31.04% in untreated okra seed flour to 17.22% after 40 min of roasting. The decrease might be due to the fact that direct heat helps to separate out oil from the cells of nuts and oil seed and subsequent removal during milling (Mohini & Eram, 2005).However, such decreasing trend is in contrast to the finding reported by Oboh *et al*. (2010),who stated that roasting will increase significantly the crude fat content of yellow and white maize varieties;which may be associated with heat-induced break down of the bonds that exist between the fat and matrix of the maize, resulting in efficient release ⁄ mobilisation of the oil reserve in the maize grain after roasting.

Ali*et al*.(2010)reported thatthefat content of vegetables decreased during boiling thatcould be attributed to leaching of the fats into the boiling water. On the other hand,Adeniyan *et al*. (2013)reported thatboiling significantly increased the crude fat contentofbeniseed, that could be attributed to the disruption of the cell structures and membrane partitions of the seeds by heat during boiling causing the fat to melt and be easily released from the seeds.Mathur & Chaudhary (2009) reported that there isadecrease in the fibre content of soaked seeds, which might be attributed to enzymatic degradation of seeds during soaking.The increase in crude fibre content upon germination is also reported by Pandey & Awasthi (2015), whichmight be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicellulose during germination.

Reduction in crude fibre content during roastingis also reported by Mathur & Chaudhary (2009), which might be due to retrogradation of starch during roasting.Obohet *al*. (2010) also reported that the structural alteration in the cell wall structure as a result of increased roasting temperature may lead to breakage of weak bonds between polysaccharide chains and glycosidic linkages in thefibre. A decreased association between fibre molecules and ⁄ or depolymerisation of the fibre results in solubilisation, hence, the observed decrease in crude fibre after roasting. In contrast to this finding, Akingbala *et al*. (2003) reported that the fibre content of okra seeds pretreated by roasting had been reported to increase compared with the untreated one. The thick hull of the

matured okra seeds consists mainly of fibre. D'souza (2013) reported that during cooking/ boiling of vegetables thecrude ash content was reduced may be due to the leaching of minerals into the cooking/boiling water andwater absorption during the boiling.

Effect of processing on antioxidant properties

The heat-induced increase in total phenolics has also been reported in roasted common kidney and pinto beans (Siddhuraju & Beckar, 2001)and barley (Gallegos-Infante *et al*., 2010), that could be due to the formation of heat-induced and extractable phenolics (Manzocco *et al*., 2000). Boateng *et al*. (2008) explained that disruption of the cell wall through heating or by the breakdown of insoluble phenolic compounds as a function of thermal treatments could lead to better extractability of phenolic compounds in dry beans. Thermal processing may also release more bound phenolic acids due to the breakdown of cellular constituents (Dewanto *et al*., 2002). Furthermore, the bound phenolics with larger molecular weight might have been liberated into simple free forms by heat treatment leading to enhanced over all total phenolic content of the samples.

Several studies also reported that heat treatment is effective in increasing the total phenolic content in different foods such as dry beans (Boateng *et al*., 2008), carob powder (Win *et al*., 2011), vegetables (Sultana*et al*., 2008), and grape seeds (Kim*et al*., 2006). Also, roasting induces the Maillard reaction with the resultant production of many compounds which have an antioxidant activity (Manzocco *et al*., 2000). Yu *et al*. (2005) investigated that Maillard reaction products might lead to increase in the amounts of total phenolics or phenolic-like complexes.The increase in total phenolic content during germination of okra seeds is reported by Duenas *et al*. (2009) that might be due to mainly endogenous enzymes activation and the complex biochemical metabolism of seeds during the process.

An increase of total phenols after germination has also been reported by Khattak *et al*. (2007) for chickpea, Duenas *et al*., (2009)for lupines andoat. Duenas *et al*. (2009) reported that significant increase in the phenolic compositionduring germination was mainly due to endogenous enzymes activation and the complex biochemical metabolism of seeds during this process.The increase in the total flavonoid content during roasting has been reported by Thidarat *et al*. (2016) thatcould result from the release of bound polyphenols or from Maillard reaction products formed during

roasting, which then exhibited scavenging activity on the reactive oxygen species.The heat-induced increase in flavonoid content has also been associated with deactivation of endogenous oxidative enzymes, thereby preventing enzymatic oxidation which causes loss of the antioxidant compounds in the raw plant materials (Jeong *et al*., 2004; Jannat *et al*., 2010).The increase in the DPPH scavenging activity of the roasted okra seed extracts, us reported by Dewanto *et al*.(2002),might be due to the better solubility of non-phenolic compounds (such as Maillard reaction products) following the thermal treatments, which may further enhance the free radical-scavenging properties of processed foods.

Effect of processing on functional properties

Food processing may affect the functional properties of the food products (Chukwuma *et al*.,2016).Enujiugha *et al*. (2003)reported thatbulk density is reduced due to heat application.Chauhan & Sing(2013) reported that there is an increase in water absorption capacity during germination which may be due to increased levels of protein and quality of protein, which enhance interactions with water.There is also an increase in water absorption capacity during roasting could be attributed to increased level of damaged starch which was induced by gelatinization of starch during roasting (Sharma & Gujral, 2013). The formation of a porous structure which imbibes and holds water by capillary action is also a reason for an increase in water absorption capacity (Sharma *et al*., 2016). Abbey & Ibeh (1988)also reported that theincrease in the water absorption capacity has always been associated with anincrease in the amylose leaching and the solubility and loss of starch crystalline structure.

Reduction in emulsion capacities of the samples that may be probably influenced by their respective oil contents has been reported by Ihemeje *et al*. (2015). Another possible cause of the reduction could be the thermal denaturation of the protein caused by heating has also been reported by Chandra*et al*. (2015).The effect of heat processing on foam capacity and stability of winged bean flour has been reported (Ihemeje*et al*., 2015). Kouakou*et al*. (2013) also reported that native protein provides higher foam capacity than denatured protein. Since proteins are heat labile, the reduced foaming capacity and stability of heat processed flours can be explained on the basis of protein denaturation, hence the flour of raw seeds gave a higher foam capacity than the processed one.

Chapter 3

Proximate, Mineral and Antinutrient Composition ofthe Pods and Seeds ofOkra (*Abelmoschus esculentus)* Accessions Grownin Benishangul Gumuz Region,Ethiopia

3.1 Abstract

Proximate, mineral and antinutritional composition and variability of pods and seeds of eight indigenous Ethiopian okra accessions were analyzed. The accessions were also further investigated for their molar mineral ratios in order to predict the implications of mineral interaction and bioavailability. The results of pods and seeds of okra accessions revealed that the proximate composition (g/100g) varied significantly ($P<0.05$) and had respective ranges (g/100g) of moisture 9.69-13.33 and 9.27-12.70; crude protein 10.25-26.16 and 22.51-38.09; crude fat 0.56-2.49 and 18.64-36.84; crude fibre11.97-29.93 and 1.94-5.96; total ash 5.37-11.30 and 4.53-6.05; utilizable carbohydrate 36.66-50.97 and 18.69-37.77; gross energy 216.60-280.63 and 324.88-423.84 kcal/100g. The pods and seeds of okra accessions had respective ranges (mg/100g) of calcium 111.11-311.95 and 66.37-103.66; iron 18.30-36.68 and 8.33-20.29; potassium 122.59-318.20 and 90.00-187.92; zinc 3.83-6.31 and 3.92-6.42; phosphorus 25.62-59.72 and 516.94-1497.23; sodium 3.33 to 8.31 and 15.06-27.81.Principal component analysis showed a nutritional variability and five independent clusters in the pods and seeds of okra accessions. The first two principal components explained 61.60% and 60.90% of the total variation in the pods and seeds of the accessions, respectively. The pods and seeds of okra accessions had respective ranges (mg/100g) of phytate 0.83-0.87 and 0.39-0.46; tannin 4.93-9.90 and 0.71-3.78; oxalate 0.04-0.53 and 0.74-0.75. The molar ratios of pods and seeds in this study were below standard value andwhich indicate the high bioavailability of minerals in all accessions. The results of the study showed that okra pods and seeds contain an appreciable amount of vital nutrients and are low in antinutrient contents with high mineral bioavailability as compared to the commonly consumed green vegetables in Ethiopia such as cabbage, Ethiopian kale, lettuce, swiss chard, carrot, tomato, and celery.Particularly, pods and seeds of OPA#6

accession contained a significantlyhigh amount of crude protein, ash, crude fat, calcium, iron, and zinc, whereas seeds of accession OPA#8 was high in calcium, iron and potassium.

Keywords: Okra, Seed, Pod, Accessions, Proximate, Mineral, Antinutrient, Bioavailability

3.2 Introduction

Indigenous vegetables have long been part of traditional diets in communities worldwide, yet many of these crops are underutilized and their nutritional value is unknown (Keatinge, 2012; Kamga *et al*., 2013). Okra is an indigenous vegetable crop originated in Ethiopia (Simmone *et al*., 2004). It is a multipurpose crop (Alba *et al*., 2013) and consumed as vegetable salads, soups and stews, fresh or dried, fried or boiled (Gemede *et al*., 2015a). Okra is a powerhouse of valuable nutrients (Adetuyi *et al*., 2011) and affordable source of protein, carbohydrates, minerals, vitamins and dietary fibre(Gemede *et al*., 2015b). Okra seeds also contain appreciable protein content (Akingbala *et al*., 2003). Evaluating the nutritional importance of indigenous edible vegetables can lead to a better understanding of the value of the plants (Pandey *et al*. 2006) and plays a crucial role in assessing their nutritional significance (Pandey *et al*., 2006; Hussain *et al*., 2009).

Increasing vegetable utilization is critical to alleviating worldwide incidence of nutritional deficiencies (Bamishaiye *et al*., 2011). However, many of the local vegetables are underexploited because of inadequate scientific knowledge of their nutritional potentials (Awobajo *et al*., 2010). Therefore, promoting the consumption of indigenous vegetables including okra could provide cheap sources of nutrients that can improve the nutritional status and reduce the prevalence of malnutrition especially among resource-constrained households and can also be used as a means of dietary diversification. However, okra has been considered as a minor crop and there is no published study on proximate and mineral contents of pods and seeds of indigenous Ethiopian okra vegetable. Improving the nutritional quality of indigenous vegetables should be a priority of crop research (Gockowski *et al*., 2003).

In addition, one of the major drawbacks limiting the nutritional qualities of plant source foods is the presence of anti-nutritional factors (Kathirvel & Kumudha, 2011). In this regard, okra does not only have beneficial nutrients but might also contain traces of antinutritional factors, whichhave adverse effects on bioavailability of some minerals like calcium, iron, and zinc. There

is also a scarcity of information regarding antinutritional contents of pods and seeds of indigenous Ethiopian okra vegetable. Therefore, the aim of this study was to evaluate the proximate, mineral and anti-nutrient composition and variability of pods and seeds of eight okra accessions grown in Benishangul Gumuz Region, Ethiopia.

3.3 Materials and Methods

3.3.1 Description of sampling site and initial collection areas

Pods and seeds of eight okra accessions (candidate breeding lines that have not yet officially been released) namely: OPA#1, OPA#2, OPA#3, OPA#4, OPA#5, OPA#6, OPA#7, and OPA#8 were collected from Assosa Agricultural Research Center during 2014 main okra harvesting season (Appendix 3.1). Assosa Agricultural Research Center is located in Benishangul Gumuz Regional State in Assosa zone, Assosa district. It is situated at a distance of 3km from the regional capital city, Assosa and 664 km from west of Addis Ababa, country's capital city (BGRIO, 2010).Currently, the center is undertaking several research activities which enhance production and productivity of the mandate areas and different agro-ecologies. The map of sampling site is shown inFigure 3.1.

Source: Benishangul Gumuz Finance and Economic Development Bureau

Figure 3.1Map of Ethiopia and sampling site.

Initially, Assosa Agricultural Research Center collected okra accessions in 2012 and 2013 harvesting seasons from differentagroecologicallocations in the regions(Table 3.1)and grown on their own plot under similar agronomic practice and management conditions during the 2014

main cropping season.Benishangul Gumuz Regional State is located in the part of Ethiopia between 9º 30' to 11º 39" N and 34º 20' to 36º 30" E covering a total land area of 50,000 square kilometers (km^2) (BGRIO, 2010). The detail initial collection areas and climatic conditions of the accessions are shown in Table 3.1.

Table 3.1Okra accessions along with their initial sources or collection areas

Sample No.	Accessions	Region	Zone	Alt. (m)	Lat.	Long.	Genetic status	Source of collection	Soil colour	Year
1	OPA#1	BG	Assosa	1301	$10^0$32'09.36"N	$034^0$30'38.21"E	Primitive cultivar/landrace	Field	Redish	2012
2	OPA#2	BG	Assosa	1300	$10^0$32'09.31"N	$034^0$30'38.24"E	Primitive cultivar/landrace	Field	Redish	2012
3	OPA#3	BG	Kamash	1307	$10^0$32'09.35"N	$034^0$30'38.29"E	Primitive cultivar/landrace	Field	Redish	2012
4	OPA#4	BG	Assosa	1317	$10^0$32'09.33"N	$034^0$30'38.25"E	Primitive cultivar/landrace	Field	Redish	2012
5	OPA#5	BG	Assosa	1132	$10^0$30'20.49"N	$034^0$23'38.58"E	Primitive cultivar/landrace	Field	Redish	2012
6	OPA#6	BG	Assosa	1405	$10^0$18'51.51"N	$034^0$38'04.19"E	Primitive cultivar/landrace	Field	Redish	2013
7	OPA#7	BG	Metekel	1406	$10^0$18'51.54"N	$034^0$38'04.16"E	Primitive cultivar/landrace	Field	Redish	2013
8	OPA#8	BG	Kamash	1050	$09^0$36'37.0"N	$035^0$58'76.0"E	Primitive cultivar/landrace	Field	Pale	2013

Source: Assosa Agricultural Research Center, 2014

3.3.2 Sample collection and preparation

The pods (immature fruit) and seeds (fully mature fruit) of eight okra accessions (OPA#1, OPA#2, OPA#3, OPA#4, OPA#5, OPA#6, OPA#7 and OPA#8) grown under the same agronomic and management practices were harvested randomly from Assosa Agricultural Research Center plots, Benishangul Gumuz Regional State, Ethiopia (Figure 3.1)in the 2014 harvesting seasons(Appendix 3.1). The pods and seeds of each okra accessions were coded, packed in polyethylene bags, kept in an ice box (to prevent moisture loss), and transported to Food Technology and Process Engineering Research laboratory of Wollega University, Ethiopia. Once the samples

arrived in the laboratory, each of the pod accessions was washed with distilled water and sliced to a uniform thickness of 5 mm using a stainless steel knife. The moisture content of the pods was determined immediately after slicing to a uniform thickness. The seeds of the fully matured fruits weremanually removed from the pods, sorted and sun dried. The sliced okra pods were sun dried and then followed by oven drying at 45 ^{o}C. The dried pods and seeds were milled separately into a fine powder using an electric grinder until it could pass through 0.425 mm sieve size. Finally, the powder was packed into airtight polyethylene plastic bag and was stored in a desiccator until required for further analysis. All chemicals used were of analytical grades.

3.3.3 Determination of proximate composition of okra

3.3.3.1 Determination of moisture content

The moisture content of the samples was determined according to approved AOAC (2000) method 925.09. Briefly, a clean empty aluminum dishes and its lids were dried in drying oven (DHG- 9055A) at 100 ^{o}C for 1 hour and cooled in a desiccator (CSN-SIMAX) with fresh granular silica gel desiccants for about 30 minutes and weighed. The prepared samples were mixed thoroughly and about 5.000 g of samples were weighed. The dishes and their contents were placed in the drying oven and dried for 3 hr at 105 ^{o}C. After drying, the samples were cooled in desiccators for 30 min and reweighed until a constant weight is achieved. The amount of water lost from the sample was considered to be directly proportional to the loss of weight due to drying of the sample.

3.3.3.2 Determination of crude protein content

The protein content of the samples was determined according to approved AOAC (2000) method 979.09. About 0.5000 g of samples were taken in a Tecator tube and 6 ml of acid mixture of concentrated orthophosphoric acid and concentrated sulfuric acid (5 parts of concentrated orthophosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed thoroughly and then, 3.5 ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken for 3 min and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000 g of copper sulfate with 100 g of potassium sulfate) was added to each tube and allowed to stand for about 10 min before digestion. The mixture was digested in the digester stove (HYP-1008 eight holes) at 370 ^{o}C for 4 hrs. The digestion was continued for about 1 hr until a clear solution was obtained. The tubes in the rack

were transferred into the fume hood for cooling and 15 ml of distilled water was added to dissolve the precipitate and to avoid further precipitation of sulfate in the solution.

A 250 ml conical flask containing 25 ml of the boric acid indicator solution was placed under the condenser of the distiller (KDN-102F, nitrogen analyzer distillation device) with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5 ml distiller water and the rinses were added to the solution. About 25 ml of 40% sodium hydroxide solution was added to the compartment and washed down with a small amount of water and the steam switched on. A 100 ml solution of the sample was distilled and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150 ml is collected. The tip was rinsed with a 3 ml of distilled water before the receiver was removed.The distillated solution was titrated with 0.1 N hydrochloric acid to a reddish color and the amount of hydrochloric acid was recorded.

3.3.3.3 Determination of total ash content

Total ash content of the samples was determined according to approved AOAC (2000)method 923.03. About 2.000 g of samples were added todish. The dishes were placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The ashed samples were placed inside the Muffle Furnace (Carbolite CSF 1200) and ashedat 550 ^{o}C for 3 hrs. The charred samples were removed from a Muffle Furnace and cooled, seen to be clean and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried and returned to a Muffle Furnace. Then, it was checked until traces of carbon are fully ashed. Finally, it was taken out of the Muffle Furnace and were placed immediately in a desiccator till cooled to room temperature and each dish plus ash was reweighed. The weight of total ash was calculated by difference and expressed as a percentage of a sample.

3.3.3.4 Determination of crude fibre content

Crude fibre content of the samples was determined according to approved AOAC (2000)method 962.09. About 1.5000 g of sampleswere placed into a 600 ml beaker and about 200 ml of 1.25% H_2SO_4 was added and the mixture was boiled gently for 30 minutes, placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with

hot distilled water. After 30 min of boiling, 20 ml of 28% KOH was added and boiled gently for a further 30 minute with occasional stirring.

The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a 2 ml of the distilled water. The solution was poured from the beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water four times and washings were transferred to a crucible and filtered.

The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H_2SO_4 and filtered and then washed with hot distilled water and filtered. It is washed again with 1% NaOH and filtered. Finally, the residue was washed with water free acetone.

The crucible with its content was dried in an electric drying oven at 130 ^{o}C for 2 hrs and cooled for 30 min in the Desiccator and then weighed. The crucible was transferred to a Muffle Furnace (Gallenkamp, size 3) and incinerated for 30 min at 550 ^{o}C. Finally, it was cooled in desiccators and re-weighed.

3.3.3.5 Determination crude fat content

The crude fat content of the samples was determined according to approved AOAC (2000) method 920.39. Briefly, the cleaned extraction flasks with boiling chips were dried in drying oven (DHG-9055A) at 90 ^{o}C for 1 hr, cooled in desiccators for 30 min and then weighed. The bottom of the extraction thimble was covered with about 2 cm layer of fat-free cotton. About 2.000 g of samples were added into the extraction thimbles and then covered with about 2 cm layer of fat-free cotton. The thimbles containing the sample were placed into Soxhlet (Shanghai Qianjian Instrument Co., Ltd) extraction chamber. The cooling water was switched on and 50 ml of diethyl ether was added to the extraction flask through extraction cylinder. The extraction was conducted for about 3 hrs. The extraction flasks with their content were removed from the extraction chamber and were placed in the drying oven at 90 ^{o}C for about 30 min, cooled to room temperature in the Desiccator for about 30 min and re-weighed.

3.3.3.6 Determination of utilizable carbohydrates

Utilizable carbohydrate content was calculated by difference i.e.100 – (% crude protein + % crude fibre + % total ash+ %crude fat).

3.3.3.7 Determination of gross energy

The gross energy content was determined by calculation from fat, carbohydrate and protein contents using conversion factors; 4kcal/g for protein, 9 kcal/g for fat and 4 kcal/g for carbohydrates (Guyot *et al*., 2007).

3.3.4 Determination of mineral content of okra

To reduce the risk of contamination, glass-wares were washed with 10 % HNO_3 acid and crucibles were soaked in 6N HCl for 24 hrs after being washed with detergent and water. All materials were then rinsed with distilled-deionized water and dried in an oven before use.

3.3.4.1 Determination of calcium, iron, zinc, potassium and sodium

The minerals were determined according to the standard method of AOAC (2000). Calcium, iron, and zinc were determined by using atomic absorption spectrophotometer (AAS), while, sodium and potassium contents were determined using flame photometer (Jenway, PF 7, Essex UK). About 2.000 g of samples were weighed into the dish and then placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. When the samples become thoroughly charred, the dishes were placed in a Muffle Furnace, as near to the center as possible and ashed at 550 ^{o}C for 3 hrs. The dishes were removed from a muffle furnace, cooled, seen to be clean, and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried, and return to a Muffle Furnace when the ash appears black and not ignited well. Then dishes were checked until traces of carbon are fully ashed and then taken out of the muffle furnace placing immediately in desiccators till cooled to room temperature.

The ash of the sample was made wet completely with 5 ml of 6 M HCl and was carefully dried on a low-temperature hotplate. 7 ml of 3 M HCl were added and the dish was heated on a hot plate until the solution just boils. Then it was cooled and filteredthrough a Whatman no.1 filter paper into a 50 ml volumetric flask retaining as much of the solids as possible in the dish. Again 7 ml of 3 M HCl was added to the dishesand heated until the solution just boils. Then, the solution was cooled and filtered into a volumetric flask. The dishes were then washed with water, and filtered into the volumetric flask. The filter paper was washed thoroughly and collected in the flask. Since calcium is to be determined 2.5 ml of 10 % Lanthanum chloride solution were added to the flask. Finally, the solution was diluted to the mark (50 ml) with freshly de-ionized water. Since estimation of the mineral concentration of the blank is important

for the determination of the detection limit of the analytical method. The reagent blanks were prepared by taking the same amount of reagents through all steps and they were analyzed for their metal content of the sample.

$$\text{Metal content (mg/100g)} = \frac{(A - B)xV}{10W}$$

Where:
W : wight of the sample (g)
V : volume of the extract (ml)
A : concentration («g/ml) of sample solution
B : concentration («g/ml) of blank solution

3.3.4.2 Determination of phosphorus

Phosphorus was determined by the colorimetric method using Ammonium Molybdate (AOAC, 1984). About 1 ml of clear extract solution was taken and diluted to 100 ml with deionized water in a 100 ml volumetric flask. 5 ml of the sample dilution was added into test tubes. 0.5 ml of molybdate and a 0.20 ml aminonaphthol-sulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. 0.20 ml aminonaphthol-sulphonic acid was added into the test tube repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minutes. The absorbance of the solution was measured at 660 nm against distilled water. Simultaneously, with sample phosphorous, the standard and blank analysis was carried out. The standard and blank solutions were prepared as described above, but 5 ml of working standard and 5 ml of deionized water in place of the sample dilution were used, respectively. A standard curve was made from absorbance versus concentration. The phosphorus contents were calculated by using the following formula:

$$\text{Phosphorus (mg/100g)} = \frac{(A - B) \times 50 \times 100}{\text{Slope} \times W_f \times 10}$$

Where:
A : reading of the sample solution
B : reading of the blank solution
W_f : weight of sample.

3.3.5 Determination of mineral ratios

The mineral ratios are often more important than individual mineral levels themselves because they are useful in determining nutritional interrelationships and also provide information regarding the many possible factors that may be represented by a disruption of their relationships such as disease states, physiological and developmental factors, the effects of diets etc (Hoskin &

Ireland, 2000). The mineral ratio was calculated by dividing the first mineral level to the second mineral level (Jacob *et al*., 2015).

3.3.6 Determination of antinutritional factors in okra

3.3.6.1 Determination of phytate content

Phytate was determined by the method described by Vantraub & Lapteva (1988).About 0.100 g of samples were extracted with 10 ml of 2.4% HCl in a mechanical shaker (Eberbach) for 1 hour at a room temperature. The extract was centrifuged at 3000 rpm for 30 minutes. The clear supernatant was used for phytate estimation. 1 ml of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalcilic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. The absorbance of the sample solutions was measured at 500 nm using UV- VIS spectrophotometer (Beckman DU-64-spectrophotometer, USA).

A series of standard solution were prepared to contain 0, 4.5, 9, 18, 27 and 36 µg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3 ml of the standard was added into 15 ml of centrifuge tubes with 3 ml of water which were used as a blank. 1 ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The mixture was centrifuged for 10 minutes and the absorbance of the solution (both the sample and standard) was measured at 500 nm by using de ionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. The sodium salt of phytic acid was used as a standard for construction of calibration curve (Absorbance = -115.01 phytic acid mg + 57.592, R^2= 0.9915).The phytate content was calculated by using the following formula:

Phytic acid (µg/100g) = [(absorbance - intercept)/ (slope * density * weight of sample)] * [10/3]

3.3.6.2 Determination of oxalate content

Oxalate was analyzed using the method originally used by Ukpabi & Ejidoh (1989). About2.000 g of samples were suspended in 190 ml de-ionized water contained in a 250 ml volumetric flask; 10 ml of 6 M HCl was added and the suspension was digested at the boiling point of water for 1 hr that followed by cooling. Then, the solution was made up to 250 ml and filtered and 125 ml of filtrate were measured into a beaker. Four drops of methyl red indicator were added to the filtrate

and followed by the addition of concentrated NH_4OH solution drop wise until the test solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was heated to 90 ^oC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was heated again to 90 ^oC and 10 ml of 5 % $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight in the refrigerator. The solution was then centrifuged at a speed of 2500 rpm for 5 min and the supernatant was decanted and the precipitate was completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution.

The total filtrate was made up to 300 ml. Aliquots of 125 ml of filtrate were heated until near boiling, and then titrated against 0.05 M standard $KMnO_4$ solution to a faint pink color which persists for 30 seconds. The oxalate content was calculated by using the following formula:

$$\text{Oxalate (mg/100g)} = \frac{T * V_1 * Df * 10}{V_2 * W}$$

Where :
T is normality of potassium permanganate
V_1 is volume of potassium permanganate
Df is dilution factor which is 26.8
V_2 is volume of extract oxalate
W is weight of sample in gram

3.3.6.3 Determination of condensed tannin content

Tannin content was determined according to the method described by Maxson & Rooney (1972).About 1.000g of the sample was weighed and mixed with 10 ml of 1% HCl solution in methanol in a screw cap test tube. Then the tube was shaken for 24 hr at room temperature on a mechanical shaker. The solution was centrifuged at 1000 rpm for 5 minutes. One ml of supernatant was transferred to another test tube and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol). D-catechin was used as a standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl solution in methanol, which was used as stock solution. 0, 12, 24, 36, 48 and 60 ml of stock solution was taken in a test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm. The blank sample consisted 1 ml of extract solution with 5 ml of 1 % HCl without vanillin-HCl reagent. (+) catechin (0.5-12 mg /100 ml) was used as standard for construction of calibration curve (Absorbance = 0.009 (+) -catechin mg

+ 0.015, R^2=0.9961).A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. The condensed tannin content was calculated by using the following formula:

$$\text{Condensed Tannin (mg/100g)} = \frac{(A_s - A_b) - \text{Intercept}}{\text{Slope x d x W}}$$

Where:
A_s is sample absorbance
A_b is blank absorbance
d is density of solution (0.791g/ml)
W is weight of sample in gram

3.3.7 Determination of molar ratio of antinutrients to minerals

The molar ratio of the antinutrients (phytate and oxalate) to minerals (Ca, Zn and Fe) was predicted by dividing the mole of antinutrient (phytate: 660 g/mol; oxalate: 88 g/mol) to the mole of minerals (Ca: 40 g/mol; Zn: 65 g/ mol; Fe: 56 g/mol) (Norhaizan & Norfaizadatul, 2009).The calculated values of the molar ratios were also compared with the reported critical toxicity values.

3.3.8 Determination of phytate phosphorus and non-phytate phosphorus content

Phytate phosphorus was calculated by assuming 28.18% of phytate ($C_6P_6O_{24}H_{18}$) is phosphorus. The non-phytate phosphorus was determined from the difference between phytate phosphorus and total phosphorus, whereas, the proportion ofphosphorous as phytate was calculated byphytate phosphorus divided by total phosphorus. The phytate phosphorus, non-phytate phosphorus, andphosphorous as phytate content was calculated by using the following formula:

Phytate phosphorus (mg/100g) = phytate content (mg/100g) x 28.18%

Non-phytate phosphorus (mg/100g) = phytate phosphorus (mg/100g) - total phosphorus (mg/100g)

Phosphorous as phytate (%) = phytate phosphorus (mg/100g)/ total phosphorus (mg/100g)

3.3.9 Statistical analysis

The Completely Randomized Design (CRD) was used with two replicates. All the statistical analyses were performed for the result obtained using SPSS version 20.0 for windows. Data were evaluated by using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means and the result was reported as a mean ± standard error (SE). A p-value of 0.05 or less was considered as the statistically significant difference. Principal component

analysis (PCA) was performed using Minitab® software (Minitab Inc., State College, PA) version 14 to evaluate the existence of clusters grouping okra accessions according to their nutritional (proximate and mineral compositions) variability.

3.4 Results and Discussions

3.4.1 Proximate Composition

Moisture content

The moisture content of pods and seeds of okra accessions are presented in Table 3.2. As fresh okra pods vary considerably in water content, moisture contents were calculated on a dry-weight basis, which allows a greater consistency of data. Okra pod accession, OPA#2 was significantly ($P<0.05$) high in dry matter (13.33 g/100g) while OPA#7, OPA#8, and OPA#6 accessions were significantly ($P<0.05$) low in their dry matter content (10.38 g/100g; 10.22 g/100g and 9.69 g/100g, respectively). In the seed accessions, OPA#5 was significantly ($P<0.05$) high in moisture content (12.7 g/100g) while accession, OPA#2 was significantly ($P<0.05$) low (9.27 g/100g).

The moisture content of fresh pods of okra accessions ranged from 87.98 to 90.60 g/100g water. The mean moisture content (89.29 g/100g) of the pods werein agreement with the finding of Adetuyi *et al*. (2011) (87.59-90.13 g/100g); Goplana*et al*. (2007) (89 g/100g) and Nwachukwu*et al*. (2014) (88.47 g/100g). The mean moisture contents of the pods of okra was also high like the commonly consumed vegetables in Ethiopia such as cabbage (*Brassica oleracea*) (93.30 g/100g), Ethiopian kale (*Brassica carinata*) (87.60 g/100g), lettuce (*Lactuca sativa*) (95.50 g/100g), swiss chard (*Beta vulgaris*) (91.50 g/100 g), carrot (*Daucouscarota*) (89.10 g/100g), tomato (*Lycopersicum esculentum*) (92.50 g/100g) and celery (*Appiumgraveolens*) (86.70 g/100g) (EHNRI, 1997)(Appendix 3.2).

The mean moisture content (11.22 g/100g) of the seed accession was comparable to the value reported for okra seed cultivars; 9.6 to 11.7 g/100g (Nzikou *et al*., 2006); mango seeds; 12.50 g/100g (Etong *et al*., 2013), but higher than the value reported for raw okra seeds; 7g/100g (Aminigo & Akingbala, 2004); melon seeds; 4.78- 5.21 g/100g (Abiodun & Adeleke, 2010) and pumpkin seeds; 5.00 g/100g (Elinge *et al*., 2012).Ifmoisture content is low keeping quality is good(Ijeh *et al*., 2004; Edem *et al*., 2009).Low moisture contents of the seeds observed in this work also confer good stability (keeping quality) and high yield.

Crude protein

The results showed that okra is a good source of protein which ranged from 10.25 g/100g to 26.16 g/100g in the pods and 22.51 g/100g to 38.09 g/100g in the seeds (Table 3.2). The crude protein content of the pod was significantly (P<0.05) high in OPA#6 (26.16 g/100g) and was followed by OPA#1 (20.75 g/100g) and OPA#4 (17.16 g/100g) and in that order,while low in OPA#2 (10.25 g/100g) accession. In the seeds, OPA#6 and OPA#4 accessions were significantly (P<0.05) high (38.09 g/100g and 36.22 g/100g, respectively) in crude protein content while low (22.51 g/100g) inOPA#3 accession.

The mean crude protein content of the pod (16.45 g/100g) was lower by half than the seed (31.88 g/100g) accessions (Figure 3.2).The crude protein values of pods of some okra accessions such as OPA#3, OPA#7 and OPA#4 obtained in this study were in the range of those reported for okra pods by Adetuyi *et al*. (2011) (13.61 to 16.27 g/100g)and OPA#1 and OPA#6 accessions were also in the range of the finding reported by Sharma & Prasad, (2010a) (18 to 27 g/100g). However, the crude protein contents of the pods of all the accessions obtained in this finding were far higher than the values reported for okra pods byNwachukwu*etal*. (2014) (4.81 g/100g).Ogungbenle & Omosola, (2015) also reported that the crude protein content of okra pod was23.4 g/100g which was higher than almost all okra accession studied except OPA#6 (26.16 g/100g).

The mean crude protein content (16.45 g/100g) of okra pod in this study areatleastfour times higher than commonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (1.10 g/100g), Ethiopian kale (*Brassica carinata*) (2.80 g/100g), lettuce (*Lactuca sativa*) (1.00 g/100g), swiss chard (*Beta vulgaris*) (2.20 g/100g), carrot (*Daucous carota*) (0.40 g/100g), tomato (*Lycopersicum esculentum*) (1.30 g/100g) and celery (*Appium graveolens*) (3.30 g/100g) (EHNRI, 1997) (Appendix 3.2).Okra pods can also be considered as high protein vegetable when compared with *Moringa oliefera (*4.2 g/100g), *Amarantus* (6.1 g/100g), *Gnetum Africanum* (1.5 g/100g)and *Pterocarpus* (2.0 g/100g) (Nzikou *et al*., 2006) and this implies that okra pod can serve as a good source of protein. Nwofia *et al*., (2012) reported that diet is nutritionally satisfactory if it contains high caloric value and a sufficient amount of protein. It has been shown that any plant foods that provide about 12% of their calorific value from protein are considered a good source of protein (Aberoumand, 2010; Effiong *et al*., 2009). The pods of these okra

accessions meet this requirements and this implies that okra pods can serve as a good source of protein.

The mean crude protein (31.88 g/100g) of the seeds was higher than those reported for raw okra seed (21 g/100g) (Aminigo & Akingbala, 2004); whole okra seed (24.85 g/100g) (Ndangui *et al*., 2010); okra seed varieties (22.30 to 26.81 g/100g) (Hassan*et al*., 2015); Colocynthis citrullus seeds (28.63 g/100g) (Bankole *et al*., 2005); Cucurbitapepo seeds (27.48 g/100g) (Elinge *et al*., 2012); gourd seeds (30.9 g/100g) and quinoa seeds flour (13.5 g/100g) (Ogungbenle, 2003). Akingbala *et al*. (2003) reported that okra seeds contain appreciable protein and this study also revealed that okra seeds are a good source of protein for human nutrition. Hence, consumption of the seeds should be encouraged to alleviate protein malnutrition in the country.

Table 3.2Proximate composition (g/100g, dwb) of pods and seeds of eight okra accessions

	Accessions	Moisture content	Crude protein	Crude fat	Crude fibre	Total ash	Utilisable carbohydrate	Gross Energy (Kcal/100g)
Pods	**OPA#1**	$10.61 \pm 0.27^{c,d}$	20.75 ± 0.52^{b}	$1.39 \pm 0.28^{b,c}$	16.58 ± 0.05^{e}	$6.05 \pm 0.25^{c,d,e}$	44.62 ± 0.23^{b}	274.02 ± 3.66^{a}
	OPA#2	13.33 ± 0.28^{a}	10.25 ± 0.69^{e}	1.67 ± 0.02^{b}	17.13 ± 0.39^{e}	6.66 ± 0.03^{c}	50.97 ± 0.00^{a}	259.88 ± 2.79^{b}
	OPA#3	12.17 ± 0.16^{b}	13.94 ± 0.02^{d}	1.14 ± 0.01^{c}	21.95 ± 0.03^{d}	10.20 ± 0.28^{b}	40.56 ± 0.49^{c}	228.23 ± 1.88^{c}
	OPA#4	11.29 ± 0.26^{c}	17.16 ± 0.65^{c}	1.69 ± 0.01^{b}	24.35 ± 1.17^{c}	5.37 ± 0.01^{e}	40.15 ± 1.55^{c}	244.44 ± 4.82^{c}
	OPA#5	$10.66 \pm 0.24^{c,d}$	12.97 ± 0.25^{d}	0.56 ± 0.01^{d}	21.69 ± 0.19^{d}	$10.60 \pm 0.17^{a,b}$	43.52 ± 0.85^{b}	230.98 ± 2.37^{c}
	OPA#6	9.69 ± 0.29^{e}	26.16 ± 0.12^{a}	2.49 ± 0.28^{a}	11.97 ± 0.83^{f}	11.30 ± 0.19^{a}	$38.41 \pm 0.56^{c,d}$	280.63 ± 4.29^{a}
	OPA#7	$10.38 \pm 0.26^{d,e}$	14.16 ± 0.14^{d}	0.58 ± 0.01^{d}	26.42 ± 0.21^{b}	$5.62 \pm 0.45^{d,e}$	42.87 ± 1.06^{b}	233.10 ± 3.64^{c}
	OPA#8	$10.22 \pm 0.22^{d,e}$	16.24 ± 0.94^{c}	0.56 ± 0.01^{d}	29.93 ± 0.09^{a}	$6.39 \pm 49^{c,d}$	36.66 ± 0.85^{d}	216.60 ± 3.21^{d}
Seeds	**OPA#1**	$11.67 \pm 0.11^{b,c}$	27.66 ± 0.63^{c}	$29.36 \pm 0.46^{d,e}$	$4.05 \pm 0.76^{a,b}$	3.65 ± 0.27^{c}	23.62 ± 0.05^{b}	469.37 ± 5.29^{d}
	OPA#2	9.27 ± 0.70^{e}	29.49 ± 0.34^{c}	36.84 ± 0.38^{a}	1.94 ± 0.72^{c}	6.05 ± 0.61^{a}	16.43 ± 0.63^{c}	515.16 ± 0.78^{a}
	OPA#3	10.59 ± 0.01^{d}	22.51 ± 0.98^{d}	18.64 ± 0.83^{g}	5.96 ± 0.75^{a}	$4.53 \pm 0.01b^{c}$	37.77 ± 1.06^{a}	408.89 ± 4.39^{h}
	OPA#4	$11.09 \pm 0.13^{c,d}$	36.22 ± 0.40^{a}	33.85 ± 0.72^{b}	3.64 ± 0.20^{c}	$4.24 \pm 0.28b^{c}$	10.97 ± 0.02^{d}	493.40 ± 6.57^{b}
	OPA#5	12.7 ± 0.10^{a}	32.99 ± 0.26^{b}	$31.43 \pm 0.12^{c,d}$	$5.12 \pm 0.49^{a,b}$	$5.00 \pm 0.49ab^{c}$	12.78 ± 0.48^{d}	465.87 ± 1.54^{e}
	OPA#6	$11.3 \pm 0.30^{c,d}$	38.09 ± 0.31^{a}	22.77 ± 0.30^{f}	$5.23 \pm 0.68^{a,b}$	3.92 ± 0.55^{c}	18.69 ± 2.14^{c}	432.09 ± 6.57^{g}
	OPA#7	$12.48 \pm 0.08^{a,b}$	33.83 ± 0.79^{b}	$33.53 \pm 1.51^{b,c}$	$3.89 \pm 0.54^{a,b,c}$	5.32 ± 0.40^{ab}	10.89 ± 0.42^{d}	480.61 ± 10.13^{c}
	OPA#8	$10.71 \pm 0.11^{c,d}$	34.25 ± 0.66^{b}	27.24 ± 0.11^{e}	$4.53 \pm 0.31^{a,b}$	$4.91 \pm 0.12^{a,b,c}$	18.53 ± 0.83^{c}	456.22 ± 4.41^{f}

Values not followed by the same superscript letters in each column of the pods and seeds are significantly ($P<0.05$) different from each other. Data are expressed as a mean ± standard error of replicate determinations (n=2).

Crude fat

The crude fat content of okra accessions varied from 0.56 g/100g (OPA#5 and OPA#8) to 1.14 g/100g (OPA#3) in the pods and 18.64 g/100g (OPA#3) to 36.84 g/100g (OPA#2) in the seeds(Table 3.2).The crude fat content of the pods of the accession OPA#6 was significantly ($P<0.05$) high (2.49 g/100g) whereas the pods of OPA#8 (0.56 g/100g), OPA#5 (0.56 g/100g) and OPA#7 (0.58 g/100g) accessions were low. The seeds of accession OPA#2 had the high crude fat content (36.84 g/100g) and was followed by OPA#4 (33.85 g/100g), OPA#7 (33.53 g/100g), OPA#5 (31.43 g/100g) in that order. However, seeds of OPA#3 accession had the lowest (18.64 g/100g) on a dry weight basis. Cert *et al*. (2000) reported thatagronomic and climatic conditions, fruit or seed quality, oil extraction system and refining procedures can cause variation in the content and composition of the constituents of vegetable oil. The mean crude fat content of the pods (1.26 g/100g) was very lower than the seed (29.21 g/100g) accessions (Figure 3.2). In this finding, the crude fat content of the pods of all accessions are higher than the value reported by Nwachukwu *et al.* (2014) (0.18 g/100g)but is far lower than the values reported by Adetuyi *et al*. (2011) (9.22 to 10.57 g/100g).

The mean crude fat content (1.26 g/100g) of okra pod in this study is low similar tothecommonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (0.10 g/100g), Ethiopian kale (*Brassica carinata*) (0.80 g/100g), lettuce (*Lactuca sativa*) (0.20 g/100g), swiss chard (*Beta vulgaris*) (0.04 g/100g), carrot (*Daucous carota*) (0.20 g/100g), tomato (*Lycopersicum esculentum*) (0.70 g/100g) and celery (*Appium graveolens*) (0.05 g/100g) (EHNRI, 1997) (Appendix 3.2).Dietary fats are used to increase the palatability of food by absorbing and retaining flavors (Antia *et al*. 2006). Excess consumption of fat has been implicated in certain cardiovascular disorders such as atherosclerosis, cancer, and aging whereas a diet providing 1-2% of its energy as fat is said to be sufficient to human beings (Blessing*et al*., 2011), in this regard, the consumption of okra pods should be encouraged.

The mean crude fat (29.21 g/100g) content of the seeds was higher than those reported for okra seeds by Ndangui *et al*. (2010) (23.44 g/100g) and Aminigo & Akingbala (2004) (16.00 g/100g) but lower than those reported for four varieties of melon seeds; 40.26-45.21% (Abiodun & Adeleke, 2010); Citrullus lanatus seeds; 57.26% (Edidiong *et al*., 2013) and Colocynthis citrullus seeds; 53.85% (Bankole*et al*., 2005). The fat content is a high energy nutrient and does add to

the bulk of the diet (Atasie *et al*., 2009). The high crude fat values obtained to give an indication that okra seed accessions could be used in improving the palatability of foods in which they are incorporated. The high crude fat values also signify that these seed accessions are viable sources of oil. The high-fat content of the seeds gave the okra flour an oily and compacted appearance instead of a smooth powdery appearance (Kajihausa *et al*., 2014).

Crude fibre

The crude fibre content of the accessions of okra ranged from 11.97 g/100g to 29.93 g/100g in the pods and 1.94 g/100g to 5.96 g/100g in the seeds (Table 3.2). Crude fibre content of the pod of OPA#8 accession was significantly ($P<0.05$) high (29.93 g/100g) and was followed by OPA#7 (26.42 g/100g) and OPA#4 (24.35 g/100g), in that order. However, pods of OPA#6 accession was low (11.97 g/100g) on a dry weight basis. The seeds of accessions, OPA#3 (5.96 g/100g) was high in crude fibre but was not significantly ($P>0.05$) different from OPA#6 (5.23 g/100g), OPA#5 (5.15 g/100g), OPA#8 (4.53 g/100g), OPA#1 (4.05 g/100g) and OPA#7 (3.89 g/100g) accessions, on the other hand OPA#2 was low in its fibre content (1.94 g/100g) but was not significantly ($P>0.05$) different from OPA#4 (3.64 mg/100g) and OPA#7 (3.89 g/100g) accessions on dry weight basis.

The mean crude fibre content of the pod (21.25 g/100g) was five times higher than the seed (4.29 g/100g) accessions (Figure 3.2). Adetuyi *et al.* (2011)reported that the fibre content of okra pod ranges from 10.15 to 11.63 g/100g, which is lower than the crude fibre content of all the accessions obtained in this study.The mean crude fibre content (21.25 g/100g) of okra pod in this study are atleast ten times higher than commonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (1.30 g/100g), Ethiopian kale (*Brassica carinata*) (1.50 g/100g), lettuce (*Lactuca sativa*) (0.70 g/100g), swiss chard (*Beta vulgaris*) (1.10 g/100g), carrot (*Daucous carota*) (1.30 g/100g), tomato (*Lycopersicum esculentum*) (1.50 g/100g) and celery (*Appium graveolens*) (1.90 g/100g) (EHNRI, 1997) (Appendix 3.2). Adetuyi *et al*. (2011) also reported that the fibre content of okra is high when compared with *Amarantus hybridus* (1.6 g/100g) and *Laurea taraxifolia* (2.0 g/100g) but very low in comparison with *Gnetumn africanum* (3.0 g/100g).

The mean crude fibre (4.29 g/100g) content of okra seed accession was lower than those reported for okra seed by Ndangui *et al*. (2010) (9.7 g/100g) and Hassen *et al*. (2015) (13.00 to 17.00

g/100g) but higher than those reported for pigeon pea (3.80 g/100g), cowpea (2.6 g/100g), pearl millet (3.1 g/100g), pumpkin seed (2 g/100g) (Ogungbenle & Omosola, 2015), melon seeds (1.66-2.16 g/100g) (Abiodun and Adeleke,2010) and *Mangifera indica* kernels (2.22-3.95 g/100g) (Kayode *et al*., 2011).

Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin and other components of this type present in food (Eshun*et al*., 2013). The interest in fibre evaluation has increased due to the recent information on the potential role of dietary fibre in human nutrition (Mensan *et al*., 2008). Evidences from epidemiological studies suggest that high fibre consumption may contribute to a reduction in the incidence of certain diseases like diabetes, coronary heart disease, colon cancer, high blood pressure, obesity and various digestive disorders (Ponka *et al*., 2005; Dawczynski *et al*., 2007; Ikewuchi *et al*., 2008). Moreover, dietary fibre is known to alter the coronary environment in such a way as to protect against colorectal diseases (Ekumankama, 2008). It provides protection by increasing faecal bulk to relieve constipation(Dillard *et al*., 2000; Zhao *et al*., 2007; Appiah *et al*., 2011). When found in excess, it may bind some essential trace elements leading to deficiency of some minerals such as iron and zinc (Adamma*et al*., 2014).

The fibre in the diet is also important as it helps to maintain human health by reducing cholesterol level in the body (Bello *et al*., 2008). Diet low in crude fibre is undesirable as it could cause constipation and such diets have been associated with diseases of the colon like piles, appendicitis, and cancer (Atasie *et al*., 2009).This finding revealed that okra pod diet is considered as a main source of crudefibre. Kumar*et al*., (2013) alsorevealed that okra seed contains fibre which controls blood sugar levels and acceptable for the bowels.

Crude ash

The crude ash content of the okra pods varied from 5.37 g/100g (OPA#4) to 11.30 g/100g (OPA#6) and in the seeds, it varied from 3.65 g/100g (OPA#1) to 6.05 g/100g (OPA#2) (Table 3.2). The level of the ash content of the pod was significantly ($P<0.05$) high in OPA#6 (11.30 g/100g) and low in OPA#4 (5.37 g/100g), OPA#7 (5.62g/100g) and OPA#1 (6.05g/100g) accessions on dry weight basis. The crude ash content of the seeds of the okra accession OPA#2 was significantly ($P<0.05$) high (6.05g/100g) but was not significantly($P>0.05$)differentfrom accession OPA#7 (5.32 g/100g), OPA#5 (5.00 g/100g), and OPA#8 (4.91 g/100g). On the other

hand, OPA#1 was low (1.94 g/100g) but was not significantly (P>0.05) differentfrom accession OPA#6 (3.92 g/100g), OPA#4 (4.24 g/100g), OPA#3 (4.53 g/100g), OPA#8 (4.91 g/100g) and OPA#5 (5.00 g/100g) on a dry weight basis.

The mean ash content (7.78 g/100g) of the okra pods in this study agrees with the findings reported by Adetuyi *et al*. (2011) (7.19- 9.63 g/100g). The mean ash content of the seed accession (4.70 g/100g) was higher than those reported for melon seed varieties; 3.35- 4.89% (Elinge *et al*., 2012), but in agreement with that reported for okra seed (5.68 g/100g) (Ndangui *et al*, 2010). In addition, the ash values reported for okra seeds in the present study are lower than the value reported by Hassan*et al*. (2015) for a variety of okra seeds (9.02 g/100g).The ash content is a reflection of the nutritionally important mineral contents present in the food sample (Omotoso, 2006; Nnamani *et al*., 2009). The high amounts of mineral content in foods enhance growth and development and catalyze metabolic processes in the human body. The mean ash content of the pod (7.78 g/100g) was higher than the seed (4.70 g/100g) accessions (Figure 3.2).

The mean ash content (7.78 g/100g) of okra pod in this study wereatleast of three times higher than thecommonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (0.09 g/100g), Ethiopian kale (*Brassica carinata*) (1.90 g/100g), lettuce (*Lactuca sativa*) (0.50 g/100g), swiss chard (*Beta vulgaris*) (2.10 g/100g), carrot (*Daucous carota*) (1.90 g/100g), tomato (*Lycopersicum esculentum*) (0.70 g/100g) and celery (*Appium graveolens*) (2.00 g/100g) (EHNRI, 1997) (Appendix 3.2).The okra pods contained fairly high ash content which is an indication that the pods would provide essential minerals needed for body development.

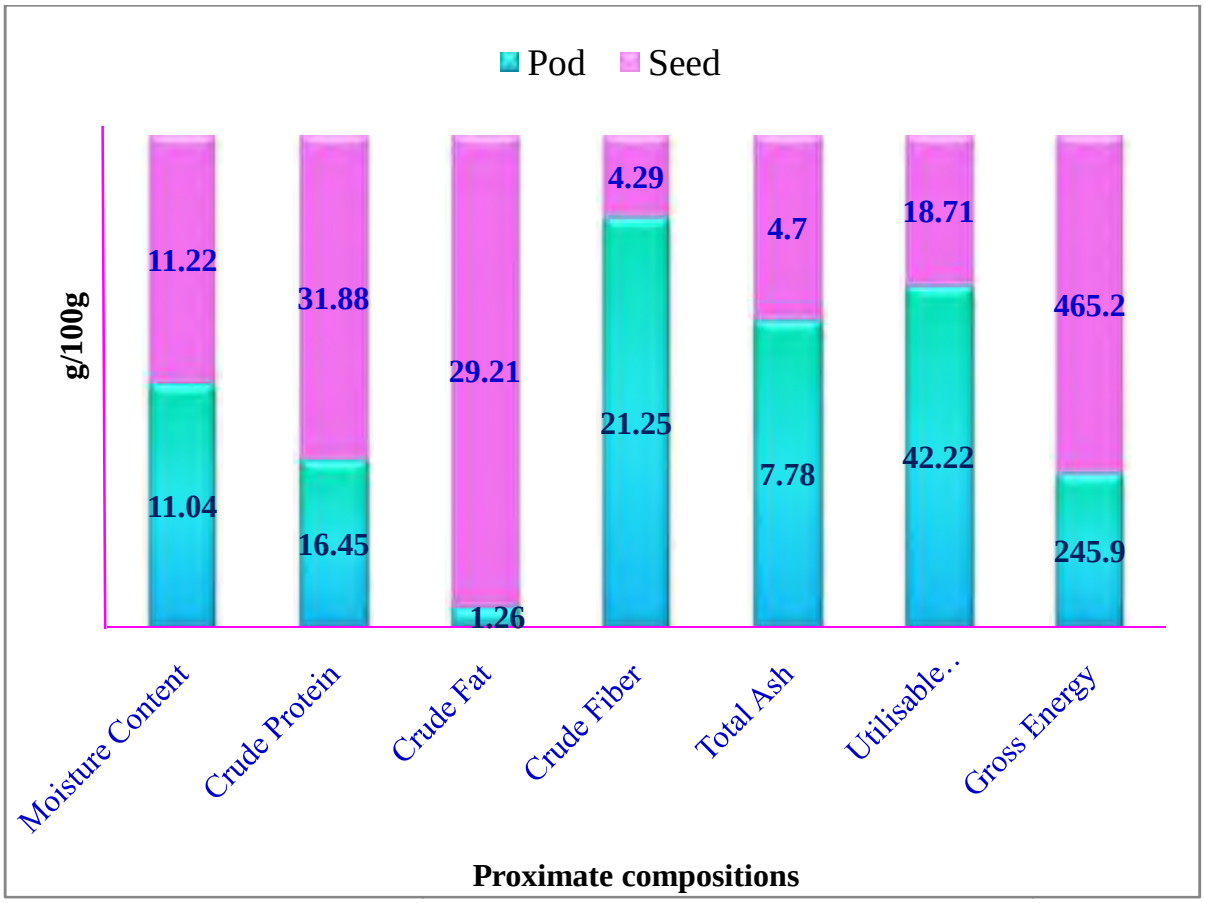


Figure 3.2 Mean proximate compositions of the okra pod and seed accessions

Utilizable carbohydrate

The utilizable carbohydrate content of the pods varied from 36.66 g/100g (OPA#3) to 50.97 g/100g (OPA#1) and it ranged from 10.89 g/100g (OPA#7) to 37.77 g/100g (OPA#2) for okra seeds (Table 3.2). The utilizable carbohydrate content of okra pod was significantly ($P<0.05$) high for accession OPA#2 (50.97 g/100g), whereas OPA#6 (38.41 g/100g) and OPA#8 (36.66 g/100g) accessions were low in their carbohydrate content. The seed of okra accessions OPA#3 was significantly ($P<0.05$)high in utilizable carbohydrate content (37.77 g/100g) and was followed by OPA#1 (23.62 g/100g), OPA#6 (18.69 g/100g) in that order. However, accession, OPA#7 recorded the lowest (18.64 g/100g) but it was not significantly ($P>0.05$) differentfrom OPA#4 (10.97 g/100g) and OPA#5 (12.78 g/100g). The mean utilizable carbohydrate content of the okra pods (42.22 g/100g)was atleast about two times higher than the seeds (18.71 g/100g) (Figure 3.2).

The mean utilizable carbohydrate content (42.22 g/100g) of okra pod in the present study were higher than that of commonly consumed vegetables in Ethiopians such ascabbage (*Brassica oleracea*) (23.70 g/100g), lettuce (*Lactuca sativa*) (15.40 g/100g), swiss chard (*Beta vulgaris*) (27.60 g/100g), carrot (*Daucous carota*) (27.80 g/100g) and tomato (*Lycopersicum esculentum*) (30.70 g/100g) but lower than Ethiopian kale (*Brassica carinata*) (46.00 g/100g) and celery (*Appium graveolens*) (47.70 g/100g) (EHNRI, 1997) (Appendix 3.2).

The mean utilizable carbohydrate content (18.71 g/100g) of the okra seed in this finding was lower than the value reported for okra seed varieties by Manal *et al*. (2015) (29.44 - 36.13 g/100g) and Ndangui*et al*. (2010) (36.83 g/100g). The utilizable carbohydrate content of the pods is low when compared to some conventional sources of carbohydrate like cereals with 72-90 g/100g (Elinge *et al*., 2012).

Gross energy

The gross energy content of the pods ranged from 216.60 kcal/100g in OPA#8 to 280.63 kcal/100g in OPA#6, whereas in the seeds it ranged from 408.99kcal/100g (OPA#3) to 515.16 kcal/100g (OPA#2) (Table 3.2). The gross energy content of pods of okra OPA#6 (280.63 kcal/100g) was high but was not significantly (P>0.05)different from OPA#1 (274.02 kcal/100g), while OPA#8 (216.60kcal/100g) was the lowest. The seeds of okra accessionOPA#2 was significantly (P<0.05)high in gross energy content (423.84 kcal/100g) and was followed by OPA#4 (493.40 kcal/100g), OPA#7 (480.61 kcal/100g), OPA#1 (469.37 kcal/100g) in that order. However, the accession OPA#3 had the lowest gross energy content (408.89 kcal/100g). The mean gross energy content of the pod (245.90 kcal/100g) was lower than the seed (465.20 kcal/100g) (Figure 3.2).Manal *et al*. (2015) and Ndangui *et al*. (2010) also reported that okra seed contains high gross energy (379 to 440 kcal/100g and 385.13 kcal/100g). The high gross energy values obtained in the present study also indicate that okra seed could be a major source of energy.

3.4.2 Mineral composition

Minerals are considered to be essential in the human diet (Isaac & Ekpa, 2009; Valvi & Rathod, 2011) because of their physiological and metabolic function in the body (Amon *et al*., 2014). Specifically, minerals are vital for the overall mental and physical well-being and are important constituents of bones, teeth, tissues, muscles, blood and nerve cells (Soetan *et al*., 2010). They

also help in the maintenance of acid-base balance, a response of nerves to physiological stimulation and blood clotting (Hanif *et al*., 2006). The mineral composition of pods and seeds of the eight accessions of okra are shown in Table 3.3on a dry weight basis.

Calcium

The calcium concentration of the pods ranged from 111.11 mg/100g to 311.95 mg/100g while in the seeds it ranged from 66.37 mg/100g to 103.66 mg/100g.Okra pod accession OPA#3 (311.95 mg/100g) and OPA#6 (311.35 mg/100g) were significantly (P<0.05) high in calcium content whereas accession OPA#1 was the lowest (111.11 mg/100g) (Table 3.3). The calcium content of seed of accession OPA#8 (103.66 mg/100g) was significantly (P<0.05) high while accession OPA#1 (66.37mg/100g) was the lowest. The mean calcium concentration of the pods (224.72 mg/100g) was 2.5 times higher than the seeds (81.77 mg/100g) accessions (Figure 3.3). The result of all the pod accessions obtained in this study was higher than the calcium contents of okra variety reported by Adetuyi *et al*. (2011)(58.22 mg/100g to 58.31 mg/100g) whereas the value of accession OPA#1 (111.11 mg/100g) was relatively comparable with the finding of Thampi & Indira (2000) (107 mg/100g).

The mean calcium content (224.72 mg/100g) of okra pod in this study wereatleast of 2.5 times higher than thecommonly consumed vegetables in Ethiopia (cabbage (*Brassica oleracea*) (43.00 mg/100g), lettuce (*Lactuca sativa*) (22.00 mg/100g), swiss chard (*Beta vulgaris*) (85.00 mg/100g), carrot (*Daucous carota*) (31.00 mg/100g) and tomato (*Lycopersicum esculentum*) (9.00 mg/100g) but lower than Ethiopian kale (*Brassica carinata*) (260.00 mg/100g) and celery (*Appium graveolens*) (317.00 mg/100g)) (EHNRI, 1997) (Appendix 3.2).The mean calcium content (81.77 g/100g) of the seeds was higher than those reported for okra seeds by Ndangui *et al*. (2010)(78.65 mg/100g) but lower than those reported by Rao (1985)(245 mg/100g).

Iron

The iron content of the pods ranged from 18.30 mg/100g in OPA#7 to 36.68 mg/100g in OPA#5 accession while in the seeds it varied from 8.33 mg/100g (OPA#6) to 20.08 mg/100g (OPA#1) (Table 3.3). The iron content of okra pods of accession OPA#5 was high (36.68mg/100g) but this was not significantly (P>0.05) differentfrom accession OPA#6 (32.90mg/100g). Accession OPA#7 had the lowest (18.30 mg/100g) iron content but it did not significantly (P>0.05) different from accession OPA#2 (20.98 mg/100g) on dry weight basis. In the seed, iron content

was significantly (P<0.05) high in OPA#8 (20.29 mg/100g) and OPA#1 (20.08 mg/100g) while it was low in OPA#5 (9.14 mg/100g) and OPA#6 (8.33 mg/100g) accessions on dry weight basis.

The mean iron level of the okra pods (27.23 mg/100g) was higher than the seeds (14.04 mg/100g) accessions (Figure 3.3). The iron values of okra pods obtained inthis study was far higher than the value reported by Adetuyi *et al*. (2011)(0.87to 0.96 mg/100g).The mean iron content (27.23 mg/100g) of okra pod in the present study wereatleast of five times higher than thecommonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (0.07 mg/100g), Ethiopian kale (*Brassica carinata*) (4.10 mg/100g), lettuce (*Lactuca sativa*) (1.60 mg/100g), swiss chard (*Beta vulgaris*) (3.60 mg/100g), carrot (*Daucous carota*) (0.05 mg/100g), tomato (*Lycopersicum esculentum*) (0.90 mg/100g) and celery (*Appium graveolens*) (5.20 mg/100g) (EHNRI, 1997) (Appendix 3.2).

Table 3.3Mineral contents (mg/100g, dwb)of pods and seeds of eight okra accessions

	Accessions	Calcium	Iron	Zinc	Phosphorous	Potassium	Sodium
Pods	OPA#1	111.11 ± 0.37^{g}	25.41 ± 1.36^{c,d}	4.13 ± 0.04^{d,e}	33.02 ± 0.46^{e}	169.82 ± 8.25^{c}	5.01 ± 0.54^{b,c}
	OPA#2	276.29 ± 0.96^{b}	20.98 ± 0.75d^{e}	4.61 ± 0.01^{c}	42.17 ± 0.78^{c}	318.20 ± 6.67^{a}	8.31 ± 0.51^{a}
	OPA#3	311.95 ± 0.57^{a}	31.77 ± 0.37^{b}	6.30 ± 0.09^{a}	27.61 ± 0.91^{f}	177.96 ± 2.89^{c}	3.97 ± 0.56^{b,c}
	OPA#4	140.88 ± 1.39^{f}	23.30 ± 0.48^{d}	4.16 ± 0.09^{c,d,e}	54.11 ± 1.62^{b}	277.82 ± 9.62^{b}	5.61 ± 1.13^{b,c}
	OPA#5	253.52 ± 4.02^{c}	36.68 ± 0.84^{a}	3.83 ± 0.24^{e}	59.72 ± 0.55^{a}	122.59 ± 11.00^{d}	3.91 ± 0.57^{b,c}
	OPA#6	311.35 ± 0.27^{a}	32.90 ± 2.65^{a,b}	6.31 ± 0.19^{a}	36.32 ± 0.68^{d}	263.12 ± 1.06^{b}	6.06 ± 0.57^{a,b}
	OPA#7	188.79 ± 3.30^{e}	18.30 ± 0.18^{e}	5.65 ± 0.05^{b}	25.62 ± 0.83^{f}	183.52 ± 7.79^{c}	4.99 ± 0.57^{b,c}
	OPA#8	203.89 ± 1.08^{d}	28.49 ± 1.77^{b,c}	4.35 ± 0.19^{c,d}	58.48 ± 1.21^{a}	174.04 ± 2.75^{c}	3.33 ± 1.11^{c}
Seeds	OPA#1	66.37 ± 0.50^{e}	20.08 ± 1.48^{a}	4.18 ± 0.02^{c,d}	797.90 ± 16.58^{e}	166.22 ± 1.99^{a}	27.60 ± 0.57^{a}
	OPA#2	75.58 ± 0.92^{d}	15.26 ± 0.86^{b}	4.56 ± 0.02^{c}	516.94 ± 15.19^{g}	97.17 ± 0.86^{c}	19.16 ± 0.59^{b}
	OPA#3	84.31 ± 0.89^{c}	14.46 ± 0.32^{b}	6.19 ± 0.08^{a,b}	633.09 ± 18.30^{f}	174.87 ± 3.11^{a}	15.06 ± 0.56^{c}
	OPA#4	72.66 ± 1.04^{d}	11.75 ± 0.67^{c}	4.16 ± 0.12^{c,d}	1038.59 ± 24.89^{d}	165.27 ± 10.5^{a}	27.98 ± 1.05^{a}
	OPA#5	85.62 ± 3.57^{b,c}	9.14 ± 0.76^{d}	3.92 ± 0.23^{d}	1221.07 ± 4.88^{c}	125.49 ± 11.74^{b}	26.88 ± 0.54^{a}
	OPA#6	89.89 ± 0.45^{b}	8.33 ± 0.18^{d}	6.42 ± 0.19^{a}	1048.21 ± 12.83^{d}	90.00 ± 1.15^{c}	17.40 ± 0.62^{b,c}
	OPA#7	76.10 ± 1.85^{d}	13.06 ± 0.14^{b,c}	5.79 ± 0.04^{b}	1304.27 ± 5.82^{b}	187.92 ± 7.58^{a}	27.81 ± 0.59^{a}
	OPA#8	103.66±1.33^{a}	20.29 ± 0.91^{a}	4.38 ± 0.21^{c,d}	1497.23 ± 25.69^{a}	175.01 ± 3.41^{a}	25.70 ± 1.15^{a}

Means not followed by the same superscript letters in each column of the pods and seeds are significantly ($P<0.05$) different from each other. Data are expressed as mean ± standard error of replicate determinations (n=2)

Zinc

Zinc level of okra pods varied from 3.83 mg/100g in OPA#5 to 6.31 mg/100g in OPA#6 and for okra seeds, it ranged from 3.92 mg/100g (OPA#5) to 6.42 mg/100g (OPA#6) (Table 3.3). The pod of the accession OPA#6 was the highest (6.31 mg/100g) in zinc content but this was not significantly (P>0.05)differentfrom accession OPA#3 (6.30 mg/100g). Accession OPA#5 was the lowest (3.83 mg/100g) and was not significantly (P>0.05) differentfrom pods of the accessions OPA#1 (4.13 mg/100g) and OPA#4 (4.16 mg/100g). Zinc content of the seed of accession OPA#6 was the highest (6.42 mg/100g) but did not significantly (P>0.05) differentfrom OPA#3 (6.19 mg/100g). The seed of accessions OPA#5 was the lowest (3.92 mg/100g) but this also did not significantly (P>0.05) differentfrom accessions OPA#8 (4.38 mg/100g), OPA#1 (4.18 mg/100g) and OPA#4 (4.16 mg/100g). The mean zinc level of the pods of okra accessions (4.92 mg/100g) was comparable with that of the seeds (4.95 mg/100g) (Figure 3.3).Zinc content of the pods obtained in this study is higher than the values reported by Adetuyi *et al*. (2011) (1.29 mg/100g -1.37 mg/100g).

Phosphorus

In this study, the phosphorus content varied from 25.62 mg/100g (OPA#7) to 59.72 mg/100g (OPA#5) in the pods and in the seeds, it ranged from 516.94 mg/100g (OPA#2) to 1497.23 mg/100g (OPA#5) (Table 3.3). The phosphorus content of the pods of okra accession OPA#5 was the highest (59.72mg/100g) but this was not significantly (P>0.05)differentfrom accession OPA#8 (58.48 mg/100g). The pods of okra accession OPA#7 had the lowest (25.62 mg/100g) phosphoruscontent but which was not significantly (P>0.05) differentfrom accession OPA#3 (27.61 mg/100g). The phosphorus level of the seeds of okra accession OPA#8 (1497.23 mg/100g) was significantly (P<0.05)high and was followed by OPA#7 (1304.27mg/100g), OPA#5 (1221.07 mg/100g), OPA#6 (1048.21 mg/100g) in that order. However, the phosphoruscontent of the seeds of the okra OPA#2 (516.94 mg/100g) was the lowest. The mean phosphorus level of the pods of okra accessions (42.13 mg/100g) was much lower than the seeds (1007.16 mg/100g) (Figure 3.3).

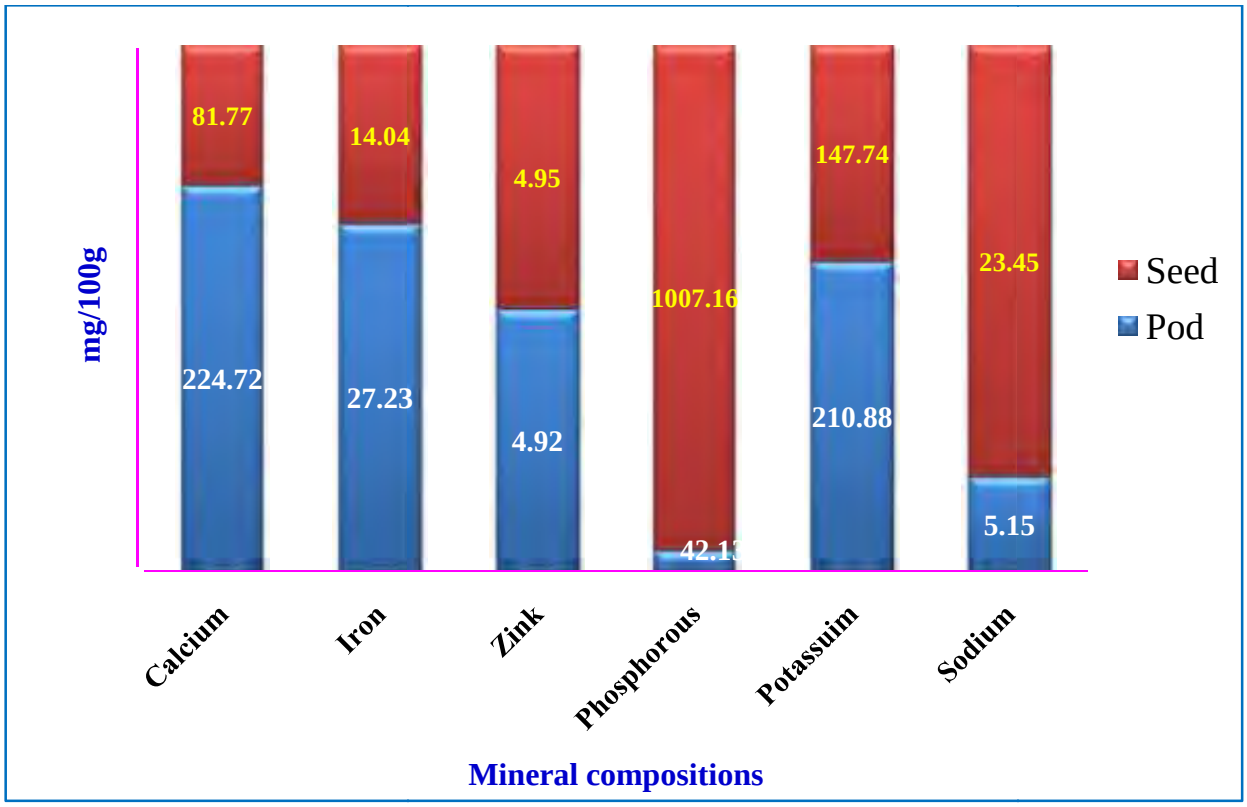


Figure 3.3 Mean mineral compositions of the pod and seed accession

The mean phosphoruscontent of the pods of okra accessions in this study was lower than the mean values reported for okra pods by Adetuyi *et al*. (2011) (60.05 mg/100g to 62.17 mg/100g).The value of phosphorus (42.13 mg/100g) of okra pod in this studyare higher than thecommonly consumed vegetables in Ethiopia such ascabbage (*Brassica oleracea*) (37.00 mg/100g), lettuce (*Lactuca sativa*) (31.00 mg/100g), carrot (*Daucous carota*) (20.00 mg/100g) and tomato (*Lycopersicum esculentum*) (29.00 mg/100g) but lower than Ethiopian kale (*Brassica carinata*) (64.00 mg/100g) and celery (*Appium graveolens*) (52.00 mg/100g)and comparable toswiss chard (*Beta vulgaris*) (41.00 mg/100g), (EHNRI, 1997) (Appendix 3.2).

The mean concentration (1007.16 mg/100g) of phosphorus in okra seeds was lower than the value reported by Ndangui *et al*. (2010) (1450 mg/100g). This value was very high in comparison with the phosphorus value of 47.68 mg/100g reported for pumpkin seeds but much higher than the phosphoruscontent reported for Juglans regia seeds, 0.87 mg/100g (Elinge *et al*., 2012). Phosphorus is closely linked with calcium and the two minerals combine to form calcium phosphate, which gives bones their strength and rigid structure (Eshun *et al*., 2013).

Potassium

The potassium content of the pods varied from 122.59 mg/100g to 318.20 mg/100g while in the seeds it varied from 90.00 to 187.92 mg/100g (Table 3.2). The potassium content of pod accession OPA#2 was significantly (P<0.05) high (318.20mg/100g) while accession OPA#5 had the lowest (122.59 mg/100g). In the seeds, potassium content of OPA#7 was high (187.92 mg/100g) but this was not significantly (P>0.05) differentfrom OPA#8 (175.01 mg/100g), OPA#3 (174.87 mg/100g) and OPA#4 (165.27 mg/100g) accessions. The potassium content of the seeds of OPA#6 was the lowest (90.00 mg/100g) but this was not significantly (P>0.05) differentfrom seeds of accession OPA#2 (97.17 mg/100g).The mean potassium content of the okra pods (210.88 g/100g) was much higher than the seeds (147.74 g/100g) (Figure 3.3).

The mean value of the seeds was higher than the values reported for okra seed (109.76 mg/100g) (Ndangui *et al*., 2010). Potassium is a very significant body mineral that is important to both cellular and electrical function (Soetan *et al*., 2010). High concentration of potassium in the body was reported to increase iron utilization (Adeyeye, 2002; Elinge *et al*., 2012) and beneficial to people taking diuretics to control hypertension and those who suffer from excessive excretion of potassium through the body fluid (Arinanthan, 2003).

Sodium

The sodium content varied from 3.33 mg/100g to 8.31 mg/100g in the pods while in the seeds it varied from 15.06 mg/100g to 27.98 mg/100g.The sodium content of pods of accession OPA#2 was high (8.31 mg/100g) but this was not significantly (P>0.05) differentfrom accession OPA#6 (6.06 mg/100g) (Table 3.3). Accession OPA#8 had the lowest (3.33 mg/100g) but this was not significantly (P>0.05) differentfrom the other five remaining accessions on dry weight basis. The sodium content of the seed of OPA#4 was high (27.98 mg/100g) but was not significantly (P>0.05)differentfrom accession OPA#1 (27.60 mg/100g), OPA#1 (27.60 mg/100g) and OPA#6 (26.88 mg/100g). The seeds of the accession OPA#3 had the lowest (15.06 mg/100g) sodium content and it was not significantly (P>0.05)differentfrom the seed of OPA#6 (17.40 mg/100g) on dry weight basis. This finding was lower than the value reported for okra seed; 54.78 mg/100g(Ndangui *et al*., 2010**).**

3.4.3 Mineral ratios

Vegetables are considered to be the most affordable and sustainable sources of several minerals that are essential for physical and mental development, immune system functioning, and various metabolic processes in humans. Minerals are essentially required for tissue functioning in human beings and their presence in plants can have a positive contribution as a source of essential nutrients or even as active principles, or a negative effect because of the accumulation of high concentrations of potentially toxic elements (Prasad *et al*., 2008). However, their effectiveness as dietary sources of minerals is influenced by mineral- mineral interactions that may either enhance or reduce the absorption of certain micronutrients in the body (Soetan *et al*., 2010).

The awareness of such interactions, therefore, is useful when selecting vegetables that could help meet specific dietary criteria for improving micronutrient status. The mineral ratios are often more important than individual mineral levels themselves because they are useful in determining nutritional interrelationships and provide information regarding the many possible factors that may be represented by a disruption of their relationships such as disease states, physiological and developmental factors, the effects of diets etc. (Watts, 2010).Therefore, understanding of mineral ratios is extremely exciting and much more revealing than analyzing mineral levels alone. The mineral ratios of the pods and seeds of eight okra accessions are shown in Table 3.4.

Sodium to Potassium ratio

Sodium-potassium (Na/K) ratio plays a very important role in the diet as it reduces high blood pressure and risk of stroke in the body (Jacob*et al*., 2015). It is important because an imbalance causes several chronic diseases, including hypertension and osteoporosis (Khan *et al*., 2015). According to Alinnor & Oze (2011), Na/K ratio is also of great importance for the prevention of high blood pressure if the Na/K ratio of the food value is less than one. The lower sodium and higher potassium intake help to reduce high blood pressure in hypertensive patients (Perez & Chang, 2014). The Na/K ratios of the pods and seeds of okra accessions are shown in Table 3.4. The Na/K ratios of the accessions of okra ranged from 0.025 to 0.290 in the pods and 0.179 to 0.416 in the seeds. The mean Na/K ratio of the pods was 0.030 while in the seed it was 0.416(Table 3.4).

The recommended Na/K ratio should be less than one (Jacob*et al*., 2015). Ijarotimi*et al*. (2013) also reported that the Na/K ratio less than one is recommended for diets, particularly for hypertensive patients. Therefore, the observed Na/K molar ratio of pods and seeds of okra in this investigation revealed that regular consumption of pods and seeds of okra would help to prevent hypertension and might lower blood pressure in hypertensive patients and may be also suitable for people who have the risk of high blood pressure. This result agrees with the finding of Aremu *et al*., (2006) who reported that Nigerian underutilized legumes are good sources of diets for lowering blood pressure.

Table 3.4Mineral ratios of the pods and seeds of eight okra accessions

Accessions	Okra Pods				Okra seeds			
	Na:K	**Ca:P**	**Ca:K**	**Fe:Zn**	**Na:K**	**Ca:P**	**Ca:K**	**Fe:Zn**
OPA#1	0.030	3.366	0.656	6.156	0.416	0.083	0.399	4.806
OPA#2	0.026	6.555	0.869	4.551	0.253	0.146	0.778	3.348
OPA#3	0.022	11.312	1.753	5.042	0.179	0.133	0.482	2.335
OPA#4	0.020	2.605	0.508	5.601	0.385	0.070	0.441	2.833
OPA#5	0.033	4.246	2.088	9.611	0.314	0.070	0.691	2.330
OPA#6	0.023	8.575	1.183	5.231	0.194	0.086	0.999	1.297
OPA#7	0.027	7.372	1.030	3.239	0.365	0.058	0.405	2.255
OPA#8	0.019	3.489	1.172	6.543	0.248	0.069	0.592	4.657
Mean	0.025	5.940	1.16	5.74	0.290	0.09	0.60	2.98
Standard	<1	>0.5	<4	>2	<1	>0.5	<4	>2

Calcium to Phosphorous ratio

A higher calcium-phosphorous (Ca/P) levels in foods are required for favorable calcium absorption in the intestine for bone formation (Adeyeye *et al*., 2012). According to Adeoti *et al*. (2013), diets rich in protein and phosphorus may promote the loss of calcium in the urine. The Ca/P ratio of the pods and seeds of okra accessions are given in Table 3.4. The Ca/P ratio of the accessions varied from 2.605 to 11.312 in the pods while in the seeds it varied from 0.058 to 0.146. The mean Ca/P ratio of the pod accessions (5.940) is far higher than the seed accessions (0.090) (Table 3.4). Therecommended Ca/P ratio should be greater than 0.5 (Jacob *et al*., 2015). Ca/P ratio greater than 2 also contributes to the absorption of calcium in the small intestine (Adeyeye & Aye, 2005; Alinnor & Oze, 2011). Furthermore, food is considered as good if Ca/P ratio is greater than 1 and poor if this ratio is less than 0.5 (Alinnor & Oze, 2011). Chitsa*et al*. (2014) also reported that the Ca/P ratio must be close to 1 for a good Ca and P intestinal utilization. The Ca/P ratio in this study indicates that the pod accessions would help calcium

absorption in the body. The high Ca/P ratio observed in this study is also of nutritional benefit, particularly for children and the aged who need higher intakes of calcium and phosphorus for bone formation and maintenance. It is well known that diets with a high value of Ca/P ratio are considered good, particularly for growing children who require a high intake of calcium and phosphorus for bone and teeth formation (Oluwole *et al*., 2013). On the other hand, the Ca/P ratio of the seed of okra accessions was lower than the standard of 0.5 and this indicates that okra seed would not help calcium absorption in the body. Therefore the meals based solely on the okra seeds would have to be supplemented with calcium to avoid mineral and osmotic imbalance (Fasasi *et al*., 2004). In addition, to bring this ratio high, food rich in calcium should be consumed together with okra seeds.

Calcium to Potassium ratio

The Calcium-Potassium (Ca/K) ratio is called the thyroid ratio because calcium and potassium play a vital role in regulating thyroid activity (Olagbemide *et al*., 2016). This ratio would also be associated with the adrenal activity.Calcium is affected by several hormones and is considered to be under parasympathetic (an involuntary nervous system that serves to slow the heart rate, increase intestinal and glandular activity) control. The elevation of the Ca/K ratio can be indicative of reduced thyroid expression. In contrary, a low Ca/K ratio would indicate an elevation of thyroid expression (Watts, 2010).Watts (2010)also reported that the ideal ratio ofCa/K is 4:1 with an acceptable ideal range of 2.2 to 6.2. A higher Ca/K levels in foods is required for favorable calcium absorption in the intestine for bone formation (Jacob *et al*., 2015). The Ca/K ratio of the pods and seeds of okra accessions are shown in Table 3.4. Ca/K ratios among the accessions of okra ranged from 0.507 to 1.753 in the pods and 0.399 to 0.999 in the seeds. The mean Ca/K ratio of the pods was 1.160, while it was 0.600 in the seeds (Table 3.4). Since the Ca/K ratios of the pods and seeds are low, both accessions are considered good for thyroid activity however for bone formation it should be consumed with calcium rich food for favorable calcium absorption.

Iron to Zinc ratio

The iron-zinc (Fe/Zn) ratio of the pods and seeds of okra accessions are shown in Table 3.4.The Fe/Zn ratio of the accessions varied from 3.239 to 9.611 in the pods while in the seeds it varied from 1.297 to 4.806. The mean Fe/Zn ratio of the pods (5.740) was far higher than the seed accessions (2.890) (Table 3.4). Pérès*et al*. (2001) reported that iron did not impair zinc

absorption up to an iron: zinc ratio of 2:1; then a dose-dependent effect was observed up to a ratio of 5:1; when the ratio was increased from 5:1 to 10:1, no further inhibition of zinc occurred. Beside on this report, one can conclude that the iron present in the pods and seeds of okra accessions did not impair zinc absorption.

3.4.4 Principal component analysis

Principal component analysis is a technique used to determine the variables containing the maximum possible variance and to reduce the information of a multidimensional data set displayed in a scatter plot (Bozokalfa *et al*., 2011). The nutritional (proximate and mineral) variability of the pods and seeds of the eight okra accessions were explained by four principal components (PC1, PC2, PC3 and PC4) (Figure 3.4), with eigenvalues higher than one (Figure 3.4), but only the first two (PC1 and PC2) had a significant contribution to the total variability of the accessions distribution. Therefore, the third and fourth principal component factors (PC3 and PC4) capture less variability and are thus not further discussed. The cumulative variation of PC1 and PC2 in the pods and seeds were 61.60% and 60.90%, respectively. The numerical value of a given variable loading on a principal component indicates how much the variable has in common with that component (Hrastar *et al*., 2009). In this finding, the variable/ loading plot of principal component analysis of pods and seeds of eight okra accessions revealed the existence of the wide range of proximate and mineral variability (Figure 3.6) and that could be selected by their traits desired for crop improvement proposes.

According to Chahal & Gosal (2002), characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. PC1 in the pods explained 34.80% of the total variation and showed that these accessions were highly variable for crude fat, crude fibre, gross energy, sodium, potassium and phosphorous while in the seeds PC1 explained 37.00% of the total variation indicating that crude fat, crude fibre, utilized carbohydrate, gross energy, sodium, and zinc were important contributing variables in distinguishing the accessions. PC2 in the pods explained 26.80% of the total variation and showed that these accessions were highly variable for crude protein, crude fat, crude ash, utilized carbohydrate, calcium, iron, and zinc while in the seeds PC2 explained 23.90% of the total variation and mainly influenced by moisture content, crude protein, crudefibre, crude ash, utilized carbohydrate and zinc (Table 3.5).

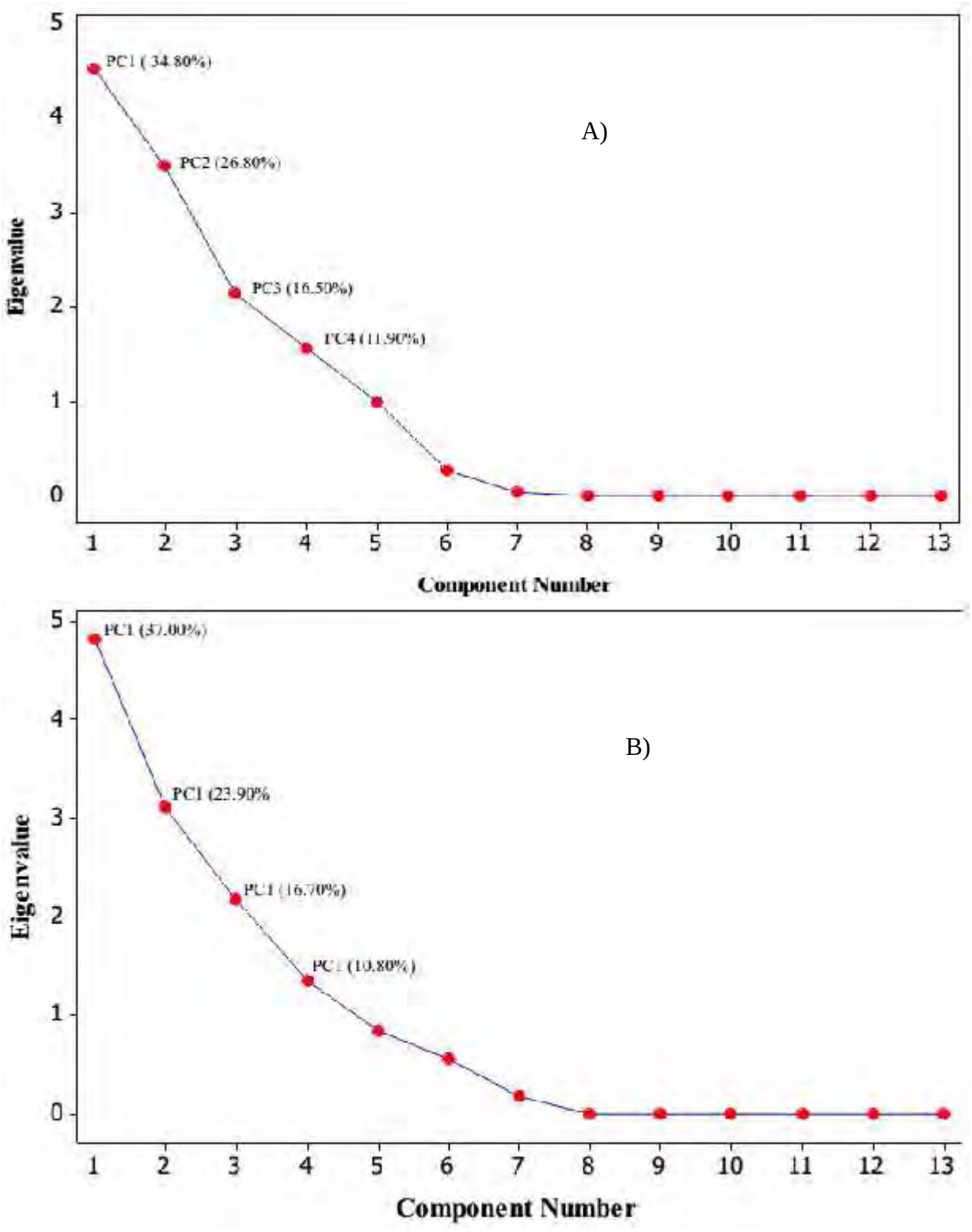
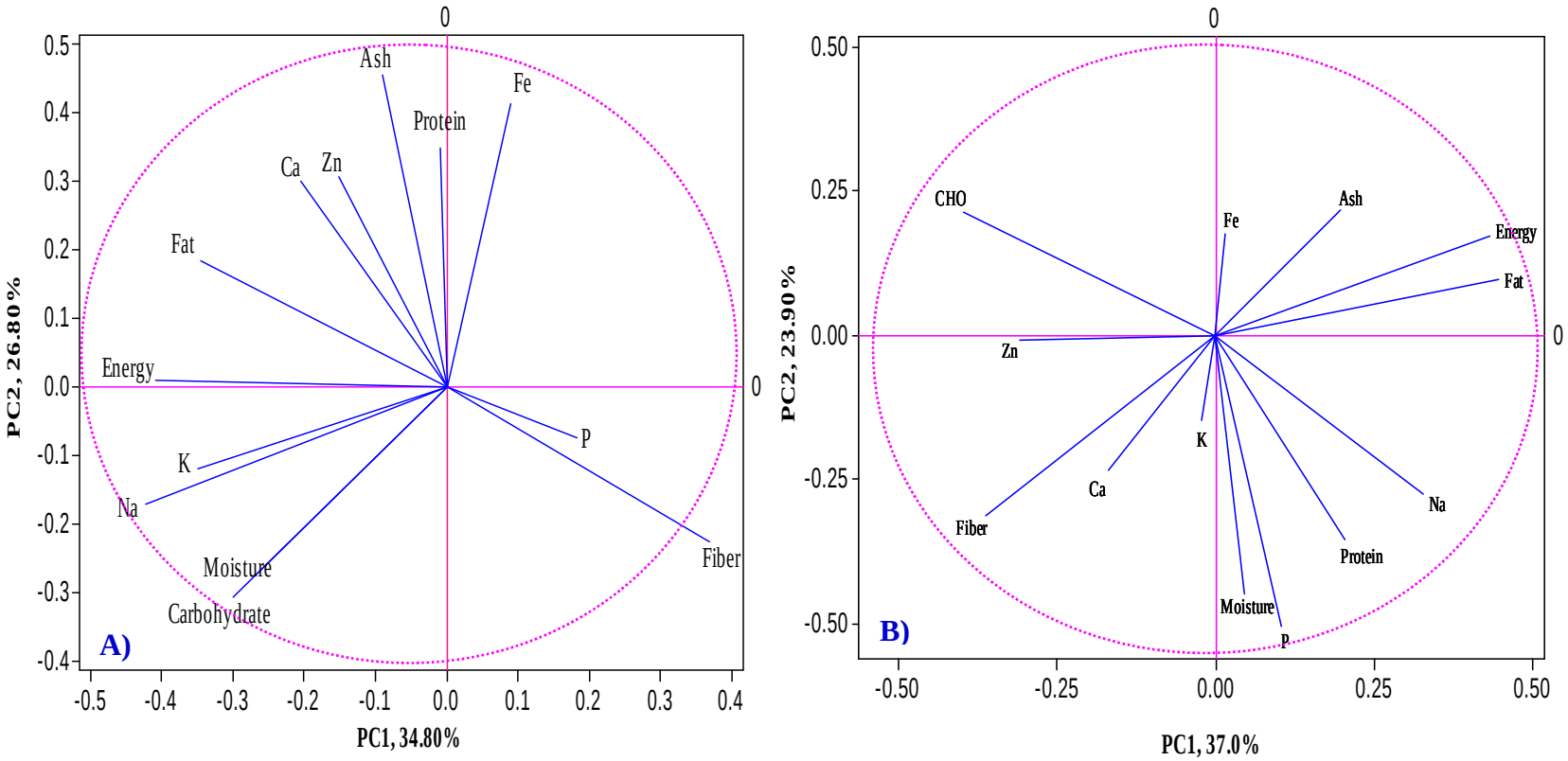


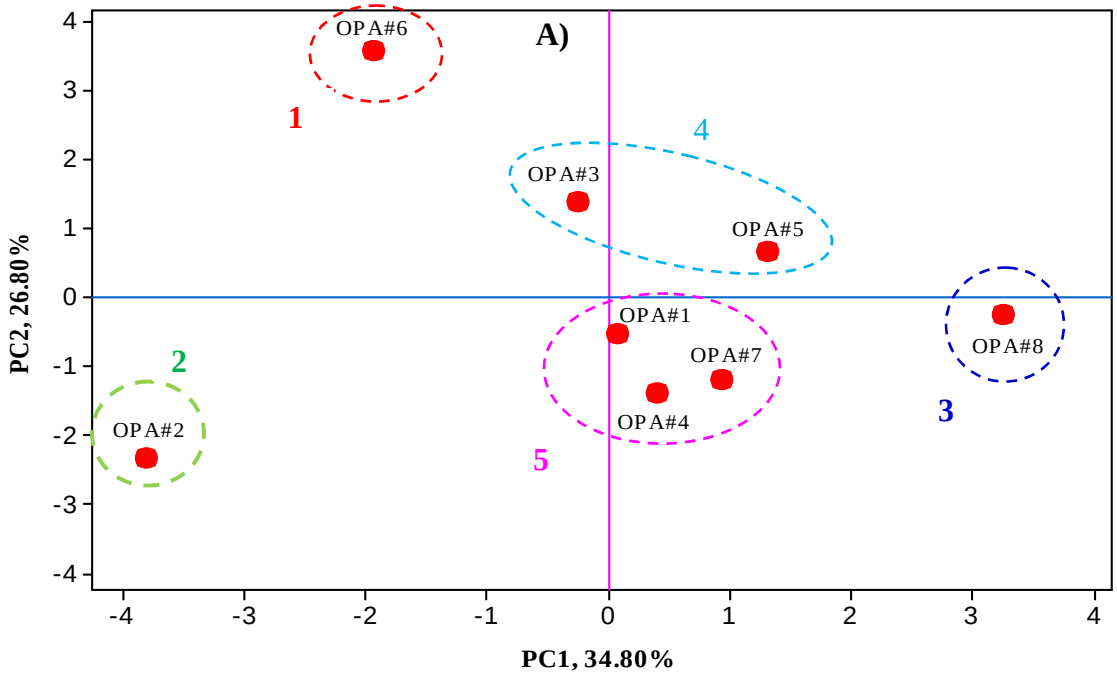
Figure 3.4 Eigenvalues of each principal component of A) pods and B) seeds of the eight okra accessions

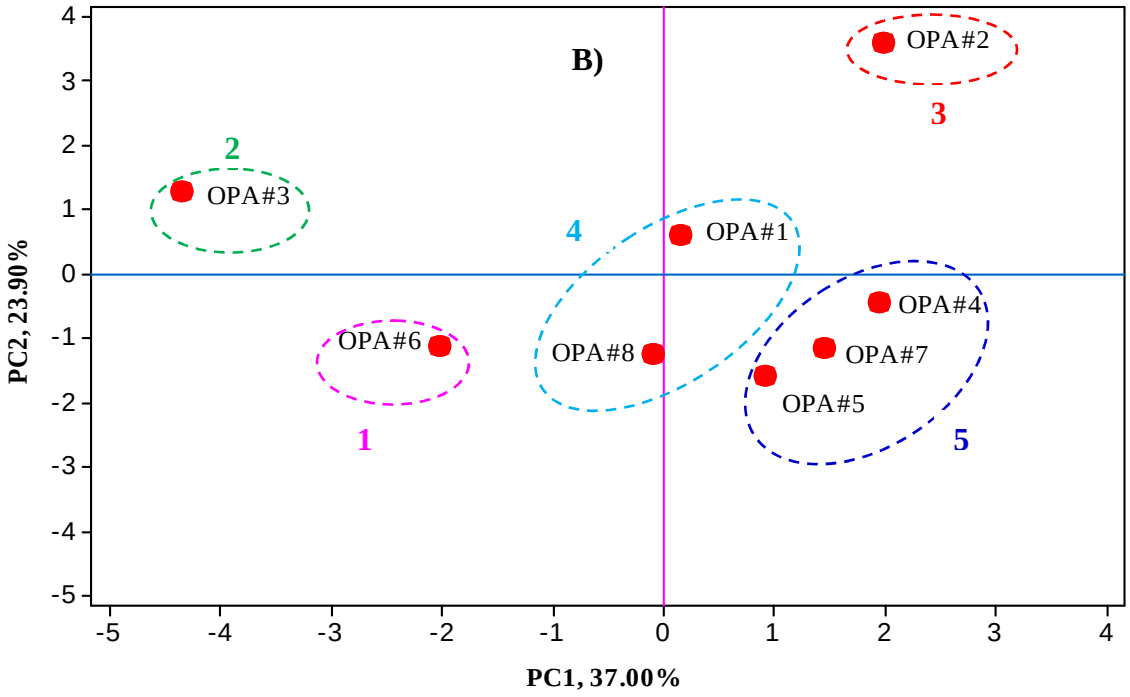
Table 3.5Eigenvector values for principal components based on the proximate and mineral compositions of pods and seeds of eight okra accessions

Proximate & mineral	**Pods**				**Seeds**			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Moisture	-0.269	-0.276	**-0.311**	0.103	0.046	**-0.450**	0.113	**0.377**
Protein	-0.009	**0.351**	**0.480**	-0.174	0.205	**-0.354**	**-0.342**	-0.074
Fat	**-0.345**	**0.186**	0.186	**-0.207**	**0.446**	0.098	-0.018	0.037
Fibre	**0.369**	-0.228	-0.081	**0.205**	**-0.362**	**-0.312**	0.042	0.080
Ash	-0.090	**0.458**	**-0.320**	-0.051	0.196	**0.216**	-0.197	**-0.343**
Carbohydrate	-0.300	**-0.309**	-0.226	-0.039	**-0.397**	**0.215**	0.206	-0.010
Gross Energy	**-0.410**	0.011	-0.253	-0.122	**0.433**	0.173	-0.019	0.004
Sodium	**-0.422**	-0.171	0.127	-0.099	**0.328**	-0.277	**0.327**	0.077
Calcium	-0.204	**0.300**	**-0.365**	0.176	-0.169	-0.236	-0.183	**-0.665**
Potassium	**-0.349**	-0.121	0.250	-0.098	-0.022	-0.149	**0.560**	-0.079
Iron	0.091	**0.416**	**-0.315**	**-0.286**	0.015	0.177	**0.515**	**-0.398**
Zinc	-0.151	**0.309**	0.087	**0.577**	**-0.307**	-0.009	-0.259	0.090
Phosphorus	**0.184**	-0.075	-0.165	-0.625	0.104	**-0.505**	0.033	**-0.325**
Eigenvalue	4.520	3.489	2.146	1.552	4.811	3.105	2.171	1.339
Percentage variation (%)	34.80	26.80	16.50	11.90	37.00	23.90	16.70	10.80
Cumulative variation (%)	34.80	61.60	78.10	90.10	37.00	60.90	77.60	87.90

Numbers in bold indicate the higher weight of each composition in each principal component factor.


A)
PC2, 26.80%
PC1, 34.80%
Ash
Fe
Protein
Ca
Zn
Fat
Energy
P
K
Na
Fiber
Moisture
Carbohydrate
B)
PC2, 23.90%
PC1, 37.0%
CHO
Fe
Ash
Energy
Fat
Zn
K
Ca
Na
Fiber
Protein
Moisture
P


A)
OPA#6
1
4
OPA#3
OPA#5
OPA#1
OPA#7
OPA#4
OPA#8
3
2
OPA#2
5
PC2, 26.80%
PC1, 34.80%


B)
OPA#2
3
2
OPA#3
4
OPA#1
OPA#4
OPA#6
OPA#8
OPA#7
OPA#5
1
5
PC2, 23.90%
PC1, 37.00%

The sample/score plot of principal component analysis for proximate and mineral analysis of pods and seeds of eight okra accessions are shown in Figure 3.6.According to this figure, five distinct groups/ clusters were identifiable for each of the pods and seeds of okra accessions and indicated by number to represent the groups. In the pods, group 1, group 2 and group 3 was represented by individual accessions OPA#6, OPA#2 and OPA#8, respectively and separated as a singleton accession from the rest in the cluster. While group 4 consists of OPA#3 and OPA#5 and group 5 contained OPA#1, OPA#4 and OPA#7 accessions. In a similar ways group 1, group 2 and group 3 of the seed of okra accessions represented by individual accessions OPA#6, OPA#3 and OPA#2, respectively. However, group 4 contained only two accessions namely, accessions OPA#1 and OPA8 and the group 5 contained three accessions, namely accessions OPA#4, OPA#5, and OPA#7.

In the pods of okra accessions, group 1 (OPA#6) was characterized by the highest composition of crude protein, crude fat, crude ash, gross energy, calcium, and zinc while group 2 (OPA#2) was characterized by the highest moisture content, utilizable carbohydrate, potassium and sodium. It also grouped the accessions with the lowest composition of protein and iron. The third clustered group (OPA#8) of the pods was characterized by high crude fibre and low in crude fat, utilizable carbohydrate, and gross energy. Group 4 (OPA#3 and OPA#5) of pods were characterized by low crude protein, crude fat and gross energy. On the other hand, the fifth group of the pods were characterized by the low crude ash and consisted of an average sodium concentration.

In the seeds of okra accessions, group 1 (OPA#6) was characterized by high crude protein, crude fat and zinc content but low in gross energy. Group 2 (OPA#2) was characterized by high crudefibre, utilizable carbohydrate and potassium and low crude protein and crude fat. Group 3 (OPA#8)of the okra seeds was characterized by high crude fat, crude ash, and gross energy and was low in moisture, phosphorus and potassium. Group 4 of the okra seeds was also characterized by high iron, potassium, and sodium and an average crude fibre and zinc. The last cluster of the okra seeds (Group 5) was characterized by high sodium and low utilizable carbohydrate. This finding revealed that significant ($P<0.05$) differences were exhibited in nutritional (proximate and mineral) compositions of the pods and seeds of okra accessions and the result could allow selecting the accessions with highest nutritional compositions.

3.4.5 Antinutritional factors

Anti-nutritional factors are chemical compounds synthesized in natural food and/or feedstuffs by the normal metabolism of species which exerts effect contrary to optimum nutrition. Anti-nutritional factors reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Ugwu & Oranye, 2006). The antinutritional composition of pods and seeds of the eight okra accessions are indicated in Table 3.6.

Phytate

Phytate content of pods and seeds of the eight okra accessions are shown in Table 3.6.The phytate content of the pod accession was highest in OPA#3 (0.87 mg/100g) and lowest in OPA#5 and OPA#4 (0.83 mg/100g) accessions, however there was no significant (P>0.05) difference among all the accessions on dry weight basis. In the seeds, phytate content ranged from 0.39 mg/100g in OPA#1 to 0.46 mg/100g in OPA#7 accession. Phytate content of seed accession, OPA#7 (0.46 mg/100g) was significantly (P<0.05) high while OPA#1 was lower (0.39 mg/100g) which was significantly different from OPA#7 but not significantly (P>0.05)differentfrom the rest of the accessions.The mean phytate content of the pods (0.85 mg/100g) was higher than that of the seeds (0.42 mg/100g) of okra accessions. The phytate content in the present study is lower than the value(mg/100g) reported by Adetuyi *et al*., (2011) (2.64-3.90) for okra and Gupta*et al*. (2005) forgreen leafy vegetables such as *Trianthema portulacastrum* (2.02), *Celosiaargentea* (2.95), *Polygala erioptera* (3.38), *Boerhavia diffusa* (4.08), *Centella hirsutus* (2.13), *Coleus aromaticus* (0.92), *Digera arvensis* (2.49), *Cocculus hirsutus* (4.40), *Commelina benghalensis* (2.38), *Amarantus tricolor* (1.95), *Gynandropsis pentaphylla* (13.06), *Cucurbita maxima* (9.23) and *Delonix elata* (5.11)

Oxalate

Table 3.6 shows oxalatecontent of pods and seeds of the eight accessions of okra used in the study. In this study, the oxalate content varied from 0.04 mg/100g to 0.53 mg/100g in the pods and in the seeds, it ranged from 0.74 mg/100g to 0.75 mg/100g. The oxalate content of pods of okra accession OPA#2 (0.53 mg/100g) was high but was not significantly (P>0.05)differentfrom accession OPA#7(0.47 mg/100g). The accession OPA#1 (0.04 mg/100g) was the lowest but was not significantly (P>0.05) differentfrom accession OPA#5 (0.06

mg/100g), OPA#3 (0.09 mg/100g) and OPA#8 (0.12 mg/100g)on dry weight basis. The oxalate value of the pods of okra accessions in this study is comparable with the finding of Adetuyi *et al.* (2011) (0.32-0.506mg/100g). In the seeds, the oxalate content was significantly ($P<0.05$) high in OPA#1, OPA#2, OPA#4, OPA#6 and OPA#8 (0.75 mg/100g) accessions and was low in OPA#3, OPA#5 and OPA#7 (0.74 mg/100g) accessions.

The mean oxalate content of the okra pods (0.22 mg/100g) was lower than the seeds (0.75 mg/100g) accessions. Okra pods have been reported to have oxalate content of 0.32-0.506 mg/100g(Adetuyi *et al.*, 2011).The oxalate content in the present study is lower than the value(mg/100g) reported bySaha *et al*. (2015)forsome green leafy vegetables such as *Basellarubra* (5.53), *Diplaziumesculentum (*1.72), *Moringaoleifera* (4.83), *Brassica juncea* (6.10), *Chenopodiumalbu* (2.81) and *Amaranthusvirdis (* 9.42). Oxalates can have a harmful effect on human nutrition and health, especially by reducing calcium absorption and aiding the formation of kidney stones. The majority of urinary stones formed in humans are calcium oxalate stones and currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50- 60 mg per day (Massey *et al*., 2001). The pods and seeds of okra accessions analyzed in this study are low compared to the recommendations for patients with calcium oxalate kidney stones. Therefore, okra pods and seeds analyzed in this study might be recommended not only for normal healthy people but also consumption for patients with a history of calcium oxalate kidney stones, assuming that about 1 kg of okra would be necessary for consumption per day.

Table 3.6 Antinutritional content (mg/100g, dwb) of the pods and seeds of the eight okra accessions.

Accessions	Pods			Seeds		
	Phytate	Oxalate	Tannin	Phytate	Oxalate	Tannin
OPA#1	0.85 ± 0.01^{a}	0.04 ± 0.04^{e}	7.61 ± 0.55^{b,c}	0.39 ± 0.01^{c}	0.75 ± 0.01^{a}	1.79 ± 0.02^{c}
OPA#2	0.85 ± 0.01^{a}	0.53 ± 0.53^{a}	6.75 ± 0.32^{c,d}	0.43 ± 0.00^{b,c}	0.75 ± 0.02^{a}	3.22 ± 0.21^{b}
OPA#3	0.87 ± 0.02^{a}	0.09 ± 0.09^{c,d}	5.75 ± 0.38^{d,e}	0.42 ± 0.02^{b,c}	0.74 ± 0.01^{b}	1.53 ± 0.11^{c,d}
OPA#4	0.83 ± 0.02^{a}	0.15 ± 0.15^{c}	8.12 ± 0.38^{b}	0.42 ± 0.01^{b,c}	0.75 ± 0.01^{a}	0.71 ± 0.03^{e}
OPA#5	0.83 ± 0.01^{a}	0.06 ± 0.06^{e}	7.48 ± 0.33^{b,c}	0.42 ± 0.01^{b,c}	0.74 ± 0.03^{b}	1.32 ± 0.25^{c,d}
OPA#6	0.85 ± 0.02^{a}	0.28 ± 0.28^{b}	9.70 ± 0.41^{a}	0.41 ± 0.02^{b,c}	0.75 ± 0.01^{a}	1.23 ± 0.19^{d}
OPA#7	0.84 ± 0.01^{a}	0.47 ± 0.47^{a}	4.93 ± 0.15^{e}	0.46 ± 0.01 ^{a}	0.74 ± 0.02^{b}	3.78 ± 0.20^{a}
OPA#8	0.86 ± 0.03^{a}	0.12 ± 0.12^{c,d}	9.90 ± 0.46^{a}	0.42 ± 0.01^{b,c}	0.75 ± 0.01^{a}	1.79 ± 0.01^{c}
Mean	0.85	0.22	7.53	0.42	0.75	1.92

Means not followed by the same superscript letters in the same column are significantly (P<0.05) different from each other. Data are expressed as mean ± standard error of replicate determinations (n=2)

Tannin

Tannin content of pods and seeds of the eight okra accessions are indicated in Table 3.6. Tannin level in pods of okra accession OPA#8 (9.90 mg/100g) was high but was not significantly (P>0.05)differentfrom accession OPA#6 (9.70 mg/100g). Although the okra accession, OPA#7 (4.93 mg/100g) had the lowest tannin content which was not significantly (P>0.05)differentfrom accession OPA#3 (5.74 mg/100g)on dry weight basis. The seeds of okra accession OPA#7 (3.78 mg/100g) was significantly (P<0.05) high while OPA#4 (0.71 mg/100g) was low on dry weight basis. The mean tannin level of the pods (7.61 mg/100g) was higher than the seeds (1.92 mg/100g). The tannin content of the pods is lower than the value (mg/100g) reported by Saha *et al*. (2015) for some green vegetables in India such as *Basella rubra* (10.40), *Diplazium esculentum (10.19*), *Moringa oleifera* (17.86), *Brassica juncea* (107.00),

Chenopodium albu (72.00) and *Amaranthus virdis*(71.66). Tannins had been reported to affect protein digestibility, adversely influencing the bioavailability of non-haemiron leading to poor iron and calcium absorption. Carbohydrate is also affected, leading to reduced energy value of a diet containing tannins, however its antinutritional/ toxicity effects depend upon their chemical structure and dosage. Therefore, the toxicity effects of the tannin may not be significant since the total acceptable tannic acid daily intake for a man is 560 mg/100g (Adeparusi, 2001). Since the tannin content of okra accessions are very low compared to its critical/ standard toxicity effect and further reduced during traditional processing, its antinutritional effect may be insignificant in both raw and processed pod and seed accessions.

3.4.6 Molar ratios and bioavailability of minerals

The molar ratios for calcium, zinc, iron, oxalate, and phytate were calculated to evaluate the effects of the elevated effect of oxalate and phytate on the bioavailability of dietary minerals. Bioavailability is the proportion of the total amount of mineral element that is potentially absorbable in a metabolically active form (Simic *et al*., 2009). It is also the ability of the body to digest and absorb the mineral in the food consumed (Norhaizan & Norfaizadatul, 2009). The calculated values of the molar ratios were also compared with the reported critical toxicity values for these ratios. The calculated Ca: Phy, Ox: Ca, Phy: Zn, Phy: Fe and [Ca] [Phy]/ [Zn] molar ratios of okra pods and seeds are shown in Table 3.7.

[Phytate] to [Calcium] molar ratios

The molar ratios of phytate to calcium (Phy: Ca) in pods and seeds of the eight okra accessions are presented in Table 3.7. The molar ratios of Phy: Ca of the pods ranged from 0.0010 to 0.0047 while in the seeds it ranged from 0.0025 to 0.0037. Phytic acids markedly decrease Ca bioavailability and the Ca: Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of [phy]: [Ca] of < 0.24 indicating good calcium bioavailability (Woldegiorgis *et al*., 2015).The Phy: Ca molar ratios of the pods and seeds in the present study were lower than the reported critical molar ratio of phytateto calcium, indicating that absorption of calcium not adversely affected by phytate in all the accessions.

[Phytate] to [Iron] molar ratios

Table 3.7 depicts the molar ratios of phytate to iron (Phy: Fe) ratios of pods and seeds of the eight accessions of okra used in the study. The Phy: Fe molar ratios of the accessions varied

from 0.0019 (OPA#5) to 0.0039 (OPA#7) in the pods while in the seeds it varied from 0.0017 (OPA#1 and OPA#8) to 0.0041 (OPA#6). Phytate begins to lose its inhibitory effect on iron absorption when phytate: iron molar ratios are less than 1.0, even ratios as low as 0.20 exert some negative effect (Hurrell *et al*., 2003). The phytate: iron molar ratios greater than 0.15 is indicative of poor iron bioavailability (Siegenberg *et al*., 1991).This result indicated that the phytate: iron molar ratios of all the accessions are less than the critical value, which implies the low impact of phytate on the bioavailability and absorption of iron.

[Phytate] to [Zinc] molar ratios

The molar ratios of phytate to zinc (Phy: Zn) in pods and seeds of the eight okra accessions are given in Table 3.7.The molar ratios of Phy: Zn among the accessions of okra varied from 0.0134 (OPA#6) to 0.0216 (OPA#5) in the pods and 0.063 (OPA#6) to 0.0106 (OPA#5) in the seeds. The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH (Bhandari & Kawabata, 2004) and the formation of the chelates depends on relative levels of both zinc and phytic acid. Hence, the phy: Zn molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone (Woldegiorgis *et al*. 2015). Foods with a molar ratio of Phy: Zn less than 10 showed adequate availability of Zinc and there will be problem encountered when the value is greater than 15. Phy: Zn molar ratios >15, is an indication of poor zinc bioavailability (Morris & Ellis, 1989). The values of pods and seeds of okra accessions were lower than the critical molar ratios of Phy: Zn, which indicates the highbioavailability of zinc.

Table 3.7 Calculatedmolar ratios of pods and seeds of the eight okra accessions

Accessions		(Phytate: Ca)[1]	(Phytate: Fe)[2]	(Phytate: Zn)[3]	(Oxalate: Ca)[4]	(Phytate*Ca: Zn)[5]
Pods	OPA#1	0.0047 ± 0.050^{a}	0.0028 ± 0.012^{c,d}	0.0204 ± 0.044^{a,b}	0.0016 ± 0.0405^{e,f}	0.0565 ± 0.014^{c}
	OPA#2	0.0019 ± 0.007^{d}	0.0034 ± 0.010^{b}	0.0182 ± 0.013^{b}	0.0087 ± 0.0775^{b}	0.1252 ± 0.013^{a}
	OPA#3	0.0017 ± 0.050^{d}	0.0023 ± 0.001^{e,f}	0.0136 ± 0.038^{c}	0.0013 ± 0.0145^{e,f}	0.1059 ± 0.001^{b}
	OPA#4	0.0036 ± 0.170^{b}	0.0030 ± 0.001^{b,c}	0.0198 ± 0.092^{a,b}	0.0049 ± 0.0695^{c}	0.0694 ± 0.025^{c}
	OPA#5	0.0010 ± 0.078^{e}	0.0019 ± 0.007^{f}	0.0216 ± 0.016^{a}	0.0010 ± 0.0255^{f}	0.1363 ± 0.079^{a}
	OPA#6	0.0017 ± 0.057^{e}	0.0022 ± 0.023^{f}	0.0134 ± 0.087^{c}	0.0040 ± 0.0220^{c,d}	0.1037 ± 0.008^{b}
	OPA#7	0.0027 ± 0.021^{c}	0.0039 ± 0.008^{a}	0.0147 ± 0.004^{c}	0.0011 ± 0.0525^{a}	0.0693 ± 0.014^{c}
	OPA#8	0.0026 ± 0.120^{c}	0.0026 ± 0.023^{d,e}	0.0195 ± 0.014^{a,b}	0.0027 ± 0.0460^{d,e}	0.0994 ± 0.066^{b}
Seeds	**OPA#1**	0.0036 ± 0.004^{a}	0.0017 ± 0.001^{c}	0.0093 ± 0.0043^{a}	0.0051 ± 0.006^{a}	0.0154 ± 0.009^{cd}
	OPA#2	0.0034 ± 0.001^{a}	0.0024 ± 0.001^{b}	0.0093 ± 0.0005^{a}	0.0045 ± 0.007^{a}	0.0175 ± 0.002^{bc}
	OPA#3	0.0030 ± 0.005^{b}	0.0024 ± 0.001^{b}	0.0067 ± 0.0048^{bc}	0.0040 ± 0.006^{ab}	0.0140 ± 0.012^{d}
	OPA#4	0.0035 ± 0.005^{a}	0.0030 ± 0.001^{b}	0.0099 ± 0.0048^{a}	0.0046 ± 0.009^{a}	0.0178 ± 0.005^{b}
	OPA#5	0.0030 ± 0.012^{b}	0.0039 ± 0.027^{a}	0.0106 ± 0.0116^{a}	0.0020 ± 0.066^{b}	0.0226 ± 0.011^{a}
	OPA#6	0.0028 ± 0.004^{b}	0.0041 ± 0.000^{a}	0.0063 ± 0.0042^{c}	0.0038 ± 0.030^{ab}	0.0141 ± 0.008^{d}
	OPA#7	0.0037 ± 0.001^{a}	0.0030 ± 0.002^{b}	0.0079 ± 0.0010^{b}	0.0045 ± 0.016^{a}	0.0150 ± 0.007^{d}
	OPA#8	0.0025 ± 0.007^{c}	0.0017 ± 0.001^{c}	0.0095 ± 0.0073^{a}	0.0033 ± 0.006^{ab}	0.0245 ± 0.014^{a}

Means not followed by the same superscript letters in each column of the pods and seeds are significantly (P<0.05) different from each other. **Notes**: [1]mg of phytate/molecular weight of phytate: mg of calcium/molecular weight of calcium; [2]mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron; [3]mg of phytate/molecular weight of phytate: mg of zink/molecular weight of

zink; [4]mg of oxalate/molecular weight of oxalate: mg of calcium /molecular weight of calcium; [5](mg of Calcium/molecular weight of Calcium) (mg of phytate/molecular weight of phytate)/ (mg of zink/molecular weight of zink).

[Oxalate] to [Calcium] molar ratios

Table 3.7 shows the molar ratios of oxalate to calcium (Ox: Ca)of pods and seeds of the eight accessions of okra used in the study. The Ox: Ca molar ratios of the accessions varied from 0.0010 (OPA#5) to 0.0087 (OPA#2) in the pods while in the seeds it varied from 0.0020 (OPA#5) to 0.0051 (OPA#1). Oxalic acid and its salts can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Bhandari & Kawabata, 2004). The importance of oxalate contents of an individual plant product in limiting total dietary calcium availability is of significance only when the ratio of Ox: Ca is greater than one (Frontela *et al*., 2009). From this result, it was observed that the Ox: Ca molar ratios of the pods and seeds of okra accessions are lower than the reported critical value (1.0), which implies that oxalate cannot have any adverse effects on bioavailability of dietary calcium in these accessions.

[Phytate][Calcium]/ [Zinc] molar ratios

The molar ratios of phytate calcium to zinc ([Ca][Phy]/ [Zn]) of the pods and seeds of the eight okra accessions are shown in Table 3.7.The ([Ca][Phy]/ [Zn] molar ratios of the pods varied from 0.0565 to 0.1363 inOPA#1 and OPA#5 accession, respectively whereas in the seeds of okra accessions it ranged from 0.0140 (OPA#3) to 0.0245 (OPA#8). The potent effect of calcium on zinc absorption in the presence of high phytate intakes has led to the suggestion that the [Phy][Ca]/[Zn] millimolar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone (Frontela *et al*., 2009). High calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca][phytate]/[Zn] millimolar ratio exceeds 0.5 mol/kg (Adetuyi *et al*., 2011). In this study, the values of okra seed accessions were lower than the critical molar ratios of [Ca][phytate]/ [Zn], which indicates the high bioavailability of zinc in all okra accessions.

3.4.7 Phytate phosphorus and non-phytate phosphorus

The percentage of phytate phosphorus to total phosphorus is very important since the phytate phosphorus cannot be utilized by human beings (Umeta*et al*., 2005).Phytate phosphorus and

non-phytate phosphorus content of okra pods and seeds are shown inTable 3.8.The phytate phosphorus content of okra pods was highest in OPA#3 (0.242mg/100g) and lowest in OPA#4 (0.233 mg/100g) but this was not significantly (P>0.05) differentfrom the rest of the accessions. In the seeds of okra accessions, the phytate phosphorus content was significantly (P<0.05) higher in OPA#7 (0.129 mg/100g) and lowest in OPA#1 (0.110 mg/100g). The nonphytate phosphorus of pod accession OPA#5 (59.487 mg/100g) was high but this was not significantly (P>0.05)differentfrom OPA#8 (58.241 mg/100g) while accession OPA#7 (25.385mg/100g) was the lowest but this also was not significantly (P>0.05)differentfrom OPA#3 (27.368 mg/100g). In the seeds of okra accessions, the nonphytate phosphorus content was significantly (P<0.05) high in OPA#8 (1497.11mg/100g) and OPA#3 (632.97 mg/100g) was the lowest.

Table 3.8 Phytate phosphorus and non-phytate phosphorus contents (mg/100g, dwb) of pods and seeds of the eight okra accessions

Accessions	Pods			Seeds		
	[1]Phytate phosphorus	[2]Non-phytate phosphorus	[3]Proportion of phosphorous as phytate (%)	[1]Phytate phosphorus	[2]Non-phytate phosphorus	[3]Proportion of phosphorous as phytate (%)
OPA#1	0.238 ± 0.003^{a}	32.782 ± 0.883^{e}	7.22 ± 0.28^{b}	0.110 ± 0.030^{c}	797.79 ± 16.59^{e}	0.438 ± 0.005^{c}
OPA#2	0.237 ± 0.001^{a}	41.933 ± 0.842^{c}	5.62 ± 0.15^{c}	0.119 ± 0.005^{b}	516.82 ± 15.19^{g}	0.231 ± 0.010^{a}
OPA#3	0.242 ± 0.004^{a}	27.368 ± 1.220^{f}	8.78 ± 0.38^{a}	0.117 ± 0.045^{bc}	632.97 ± 18.29^{f}	0.184 ± 0.005^{b}
OPA#4	0.233 ± 0.006^{a}	53.877 ± 0.001^{b}	4.30 ± 0.00^{d}	0.116 ± 0.005^{bc}	1038.48 ± 24.88^{d}	0.611 ± 0.000^{d}
OPA#5	0.233 ± 0.003^{a}	59.487 ± 1.270^{a}	3.89 ± 0.04^{d}	0.117 ± 0.020^{bc}	1220.95 ± 4.88^{c}	0.796 ± 0.005^{d}
OPA#6	0.238 ± 0.006^{a}	36.082 ± 0.053^{d}	6.57 ± 0.39^{b}	0.114 ± 0.020^{bc}	1048.09 ± 12.83^{d}	0.109 ± 0.000^{d}
OPA#7	0.235 ± 0.003^{a}	25.385 ± 1.460^{f}	9.18 ± 0.13^{a}	0.129 ± 0.010^{a}	1304.13 ± 5.83^{b}	0.999 ± 0.000^{d}
OPA#8	0.239 ± 0.007^{a}	58.241 ± 0.041^{a}	4.10 ± 0.22^{d}	0.117 ± 0.015^{bc}	1497.11 ± 25.69^{a}	0.978 ± 0.000^{e}

Means not followed by the same superscript letters in the same column are significantly ($P<0.05$) different from each other.

1 Phytate phosphorus was calculated by phytate times 28.18%.

2 Non-phytate phosphorus was the difference between phytate phosphorus and total phosphorus.

[3]Proportion of phosphorous as phytate was calculated byphytate phosphorus divided by total phosphorus.

The effect of phytate on phosphorus absorption in the presence of high phytate intakes has led to the suggestion that the proportion of phosphorus as phytate may be a better index of phosphorus bioavailability (Umeta*et al*., 2005), in which the diets with proportion of phosphorus as phytate (%) ≤ 50 % in foods are regarded as being adequate in bioavailable phosphate. The values of the proportion of phosphorus of the pods and seeds in this study were lower than the reported critical proportion of phosphorus as phytate (≤ 50 %), which implies good bioavailability of phosphorus in the okra accessions. Therefore, consumptions of okra pods may help to ameliorate prevalent mineral deficiencies caused by their limited bioavailability and may lead to better mineral status.

3.5 Conclusion

The present finding provides information on the proximate, mineral and antinutritional composition of pods and seeds of the eight okra accessions grown in Assosa Agricultural Research Center farm, Ethiopia.The pods and seeds of okra accessions were found to be a good source of crude protein, crude fat, calcium, iron, and potassium that could contribute a useful amount to the human diet and is low in antinutrient content. Particularly, the pods and seeds accession OPA#6 has a good nutritional profile with a high level of crude protein and crude fat, while in the seed accession OPA#8 was high in calcium, iron, and potassium. The proximate and mineral contents of the pods of okra accessions are also high when compared to the commonly consumed green vegetables in Ethiopia such as cabbage, Ethiopian kale, lettuce, swiss chard, carrot, tomato and celery.The principal component analysis showed a nutritional variability and five independent clusters in the pods and seeds of okra accessions and this may be useful to breeders for improvement of accessions based on the desired trait of the clusters. Hence, increasing the production and consumption of these nutrient rich okra accessions will help in new product development, food supplementation and alleviate malnutrition in the country.

Chapter 4

Phytochemical Profile and Antioxidant Activity of the Pods and Seeds of Okra (*Abelmoschus esculentus)* Accessions Grown in Benishangul Gumuz Region, Ethiopia

4.1 Abstract

The methanolic extract of pods and seeds of eight okra accessions were evaluated for their total phenolics, total flavonoids, andantioxidant activities in order to find the possible sources of natural antioxidants. The antioxidant activity was studied by using DPPH scavenging, reducing power, metal chelating and ABTS scavenging assays. The results were compared with different synthetic antioxidants. The antioxidant levels of the pods and seeds of okra accessions increased with increasing concentration of the samples and were dependent on the extract concentration. Pods and seeds of okra accessions had respective ranges of total phenol (mg GAE/g) 28.10-95.21 and 21.28-57.34 and total flavonoid (mg CE/g) 8.18-18.72 and 10.73-29.04. The study indicated that EC_{50} values (mg/ml) of pods and seeds of okra accessions had respective ranges of DPPH scavenging 2.10-10.30 and 3.1->12; reducing power 1.20-4.20 and 1.18-4.30; metal chelating 0.50-1.52 and 0.32-1.11; and ABTS scavenging 0.31-1.33 and 0.07-1.5. The antioxidant activity of both pods and seeds of okra accession OPA#6 was high in almost all assays (except ABTS scavenging activity for pods of okra accession) with lower EC_{50} values, whereas the antioxidant activity of the pod of OPA#5 and the seed of OPA#7were relatively low, but with higher EC_{50} values. The present study revealed that antioxidant activity, total phenolics, and total flavonoid levels varied widely across pods and seeds of the okra accessions and this made the pods and seeds of the okra accessions to be consideredas a source of natural antioxidants. Particularly, okra pods and seeds of the OPA#6 accession is a potentially rich source of natural antioxidants.

Key words: Okra; Pod; Seed; Phenolics; Flavonoids; Antioxidant Activity

---

4.2 Introduction

Antioxidants are substances capable of inhibiting oxidation, reducing the concentration of free radicals in the body and/or chelating metal ions, and preventing lipid peroxidation (Ozsoy *et al*., 2008). Antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects (Gülçin, 2006; Valdés *et al*., 2015). Reactive oxygen species (ROS) are essential cellular components produced in aerobic living organisms as a result of normal cellular

metabolism (Ong *et al*., 2016), which play an important role in different physiological and pathological processes. Particularly, at low to moderate concentrations, they function in physiological cell processes like in signal transduction and gene transcription (Amoussa *et al*., 2015), but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA (Birben *et al*., 2012). Oxidative stress is an imbalance between the production of reactive oxygen species and antioxidant defenses (Betteridge, 2000).

There are several evidence that show oxidative stress resulting from reactive oxygen species including free radicals such as hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot-}$), peroxyl ($ROO^{\cdot}$) and nonfree radical like hydrogen peroxide and singlet oxygen. All these species play an important role in the development of several pathological conditions including lipid peroxidation, protein oxidation, DNA damage and cellular degeneration (Hamzah *et al*., 2013). Reactive oxygen species have been implicated in the etiology (the study of the causes of a disease) of pathological conditions related to cancer, heart disease, hypertension, diabetes and neurological disorders (Sayre *et al*., 2001; Kašparová *et al*., 2005; Farías *et al*., 2014),

Several epidemiological and in vivo studies have provided evidence that antioxidant is used to protects cells from oxidative stress by scavenging free radicals, chelating catalytic metals and halting lipid per oxidation chain reactions. Antioxidants are also widely used as ingredients in dietary supplements for maintaining health and preventing diseases (Bjelakovic *et al*., 2007). Antioxidants are also used as food additives to provide protection against oxidative degradation of foods by free radicals (González-Vallinas *et al*., 2013).

Several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in the food industry due to their abilities to prevent food deterioration and to extend the shelf life of foods (Hotta *et al*., 2002). Unfortunately, the usage of synthetic antioxidants was restricted due to their side effects, such as an increase in the risk of cancer (Chandran *et al*., 2013) and liver damage in humans (Hue *et al*., 2012). Commonly used synthetic antioxidants such as β-carotene, vitamin C, and vitamin E are widely sold in food markets and have been shown to increase the risk of mortality in adults who consumed them. The exact mechanism of action is still unknown but it has been suggested that it could be due to the rigorous toxicity that they possess compared to natural antioxidants (Bjelakovic *et al*., 2007).

Therefore, the intense search for alternative rich sources of natural antioxidant is becoming increasingly important.

Plants are potential sources of natural antioxidants (Amoussa *et al*., 2015). Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because of the frequent consumption of natural antioxidants is associated with a lower risk of diseases (Temple, 2000; Thaipong *et al*., 2006). Okra (*Abelmoschus esculentus L.*) is one of the important vegetable crops originated in Ethiopia (Kumar *et al*., 2013), which is widely distributed in Africa, Asia, Southern Europe, and America (Alba *et al*., 2013). It is a multipurpose crop due to the various uses of the pods, fresh leaves, buds, flowers, stems and seeds (Gemede *et al*., 2015b). Okra has been described as a 'storehouse' of nutrients (Ahiakpa *et al*., 2013). It is also known for being high in antioxidants activity in the different parts of the plant (Shui & Peng, 2004). In addition, Arapitsas (2008)reported that okra seeds are rich in phenolic compounds, mainly composed of flavonol derivatives and oligomeric catechins, suggesting that it might possess some antioxidant properties. Okra seed isa rich source of natural phenolics (Huang *et al*., 2007; Arapitsas, 2008). According to Adetuyi & Ibrahim (2014), total phenolic content of the pulp and seeds of okra extracts were 10.75±0.02mg GAE/100g extract and 142.48±0.02 mg GAE/100g. Okra is also rich in flavonoid compounds that have antioxidant activity (Adelakun *et al*., 2009). Epidemiological studies have suggested that the consumption of foods rich in flavonoid compounds could reduce the risk of diabetes, cardiovascular diseases, obesity, hyperlipidemia, stroke and cancers (Verma *et al*., 2012).

Promoting the consumption of important traditional vegetables such as okra could provide cheap sources of antioxidants. However, Hu *et al*., (2014)reported that there islittle information on antioxidant properties of okra. In addition, even if okra is native to Ethiopia and important crop for the Berta community, it is underutilized. To date, there is no single published information on the antioxidant properties of the pods and seeds of indigenous Ethiopian okra accessions. Therefore, the aim of this study was to determine and compare the total phenolic, total flavonoid and antioxidant activities of underutilized pods and seeds of indigenous okra (*Abelmoschus esculentus)* accession grown in Benishangul Gumuz Region, Ethiopia.

4.3 Materials and Methods

4.3.1Methanolic extraction

Pods and seeds of eight okra accessions which were collected and prepared for the proximate and mineral compositions (chapter 3 under section 3.3.4) were further evaluated for their phytochemical profiles and antioxidant activity. Samples were extracted based on the procedures outlined by Woldegiorgis *et al*. (2014). About 10 gram of sample was extracted by stirring with 100 ml of methanol at 25 ^{0}C at 150 rpm for 24 hrs using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was extracted again with additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated to dryness at 40 ^{0}C using rotary evaporator (Stuart R3300), and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4 ^{o}C for further use.

4.3.2 Determination of total phenolic and flavonoid

4.3.2.1 Determination of total phenolics

Total phenolic content (TPC) was determined based on the procedures described by Ferreira *et al*. (2007), using gallic acid as a standard for the calibration curve. One milliliter of the sample was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min after which the absorbance was measured spectrophotometrically at 725 nm using a UV-VIS Spectrophotometer (Agilent Cary Corporation, 1001, Kyoto, Japan). Gallic acid was used to construct the calibration curve. The concentration of the standard solution ranged from 0.5–100 µg/ml (Absorbance= 427.63 gallic acid µg + 0.1453, R^2 = 0.999 and Absorbance= 311.77 gallic acid µg + 0.1107, R^2 = 0.993 which was conducted in different day for pods and seeds of okra accession, respectively). The total phenolic content in milligram of gallic acid equivalents per gram (mg GAE/g) of the extract was calculated by the following formula:

$$\text{Total Phenolic Content (mg GAE/g)} = \frac{c \times v}{m}$$

Where:

c: the concentration of gallic acid obtained from the calibration curve in mg/g

v: the volume of sample extract in litres

m: the weight of sample extract in grammes

4.3.2.2 Determination of total flavonoids

Total flavonoid content (TFC) was determined by a colorimetric method as described by Xu & Chang (2007) and Woldegiorgis *et al*. (2014). About 0.25 ml of the extract was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed for 5 min and the absorbance of the pink color was measured at 510 nm against the blank. For calibration curve (+)-Catechin was used with a concentration range of 10-1000 µg/ml (Absorbance = 27.05 catechin µg + 0.1453, R^2 = 0.995 and Absorbance = 24.57 catechin µg + 0.0404, R^2 = 0.994 which was conducted in different day for pod and seed accession, respectively). The total flavonoid content (milligram of (+)-catechin equivalent per gram) of the extract was calculated by the following formula:

$$\text{Total Flavonoid Content (mg/g)} = \frac{\text{c x v}}{\text{w}}$$

Where:

c: the concentration of (+)-catechin obtained from the calibration curve in mg/g

v: the volume of sample extract in litres

w: the weight of sample extract in grams

4.3.3 Determination of in vitro antioxidant activity

4.3.3.1 Determination of DPPH scavenging activity

DPPH *(2,2'-diphenyl-1 - picrylhydrazyl)* scavenging activity of the methanolic extract of the sample was determined according to the procedure of Woldegiorgis *et al*. (2014). A 0.004% solution of DPPH radical solution in methanol was prepared and then 4 ml of this solution was mixed with 1 ml of various concentrations (2–14 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically (Perkin Elmer Lamda 950 UV/Vis/NIR) by monitoring the decrease in absorbance at 517 nm. The absorption maximum was first verified by scanning freshly prepared DPPH from 200 to 800 nm using the scan mode of the spectrophotometer. Butyl hydroxytoluene (BHT) and L-ascorbic acid were used as the positive control with the same concentrations as above. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of DPPH scavenging activity percentage against extract

concentration. Inhibition of radical DPPH in percent (%) was then calculated by using the following formula:

$$\text{DPPH Scavenging Activity}(\%) = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where:

A_0: the absorbance of the control

A_1: the absorbance of the sample

4.3.3.2 Determination of ferric reducing power

Ferric reducing power was carried out according to the method established by Ferreira *et al*. (2007) and Woldegiorgis *et al*. (2014). About 1 ml of the extract at different concentrations (2–12 mg/ml), phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and potassium hexacyanoferrate solutions (1% v/ m, 2.5 ml) were mixed in a test tube and incubated for 20 min at 50 ^{o}C. Then 2.5 ml trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. The upper layer (2.5 ml) was transferred into another tube, mixed with 2.5 ml deionized water and 0.5 ml ferric chloride (0.1%) and left to react for 10 min. Finally, the absorbance of the reaction mixture was measured at 700 nm. Stronger absorbance at this wavelength indicates the higher ferric reducing power of the antioxidant. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. BHT was used as a control.

4.3.3.3 Determination of metal chelating effects

Metal chelating effects on ferrous ions was determined according to the method established by Woldegiorgis *et al*. (2014). About 2 ml of various concentrations (0.05-1.5 mg/ml) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). Total volume was adjusted to 5 ml with methanol and then, the mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. A lower absorbance indicates a higher ferrous ion chelating capacity and 2, 2-bipyridyl, disodium ethylenediaminetetraacetate (EDTA) was used as a positive control. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of ferrous ion inhibition percentage against extract concentration. The inhibition percentage of ferrozine- Fe^{2+} complex formation was then calculated by using the following formula:

$$\text{Metal Cheleting Effect } (\%) = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where:
A_0: the absorbance of the control
A_1: the absorbance of the sample

4.3.3.4 Determination of ABTS scavenging activity

The ABTS (*2, 2'-azino-bis 3-ethyl-benzothiazoline -6-sulfonic acid)* radical scavenging activities were determined using the method of Nisha *et al*. (2012). The ABTSradical cation was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 hrs until the reaction was completed and the absorbance was stable. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol to get an absorbance of 0.700 ± 0.020 at 734 nm. Then, 1 ml of various concentrations (0.2–1.8 mg/ml) of the extracts were homogenized with 1ml of ABTS solution and its absorbance was recorded at 734 nm. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured immediately. L-ascorbic acid was used as the positive control with the same concentrations as above. ABTS scavenging ability was expressed percentage (Gülçin *et al*., 2011). The inhibition percentage of ABTS scavenging activity was calculated using the following formula:

$$\text{ABTS scavenging activity}(\%) = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where:
A_0: the absorbance of the control
A_1: the absorbance of the sample

4.3.4 Statistical analysis

The Completely Randomized Design (CRD) was used with two replicates. All the statistical analyses were performed on the results obtained using SPSS version 20.0 for windows. One way analysis of variance (ANOVA) was used to evaluate the data. Means of the experiment were separated by Duncan's multiple range tests and reported as a mean ± standard error (SE). A p-value of 0.05 or less ($P \leq 0.05$) was considered as statistically significant. Graphs of effective concentration at 50% (EC_{50}) of the respective antioxidant activities were constructed by using Microsoft Excel.

4.4 Results and Discussions

4.4.1 Total phenolics

The total phenolic contents of pods and seeds of okra accessions were expressed in milligram gallic acid equivalent per gram extract (mg GAE/g) on comparison with a standard gallic acid graph. The result of total phenolic contents of pods and seeds of the eight accessions of okra is shown in Table 4.1. Total phenolic content (mg GAE/g) of the extract was between 28.10 to 95.21 for the pods and 21.28 to 57.34 for the seeds of okra accessions. Pods of okra accession OPA#6 (95.21 mg GAE/g), OPA#1 (92.38 mg GAE/g) and OPA#8 (92.26 mg GAE/g) were significantly ($P<0.05$) high in total phenolic content whereas pod accession OPA#7 was the lowest (28.10mg GAE/g).

Similar to the pod extract, the seed extracts of accession OPA#6 (57.34 mg GAE/g) was significantly ($P<0.05$)high in total phenolic content and was followed by OPA#7 (52.98 mg GAE/g), OPA#8 (49.03 mg GAE/g) and OPA#4 (44.07 mg GAE/g). The accession OPA#5 (21.28 mg GAE/g) exhibited the lowest total phenolic content on a dry weight basis. The mean total phenolic content of the pods of okra accessions (68.21 mg GAE/g) were higher than the seed accessions (40.04 mg GAE/g) (Table 4.1).The mean total phenol content (40.04 mg GAE/g) of the seeds of okra accessions is higher than the value reported for full fat okra seed flour (25.24 mg GAE/g) by Adetuyi & Komolafe (2011).In addition, the mean total phenolic content (68.21 mg GAE/g) of okra pods was higher than that of the common fruits and vegetables reported for their relatively high phenolic constituents (mg GAE/100 g) such as cranberries (52.72±2.15), apple (29.63±0.64), strawberries (16.00±0.12), pineapple (9.43±0.15), banana (9.04±0.32), lemon (8.19±0.35), orange (8.12±0.11), pear (7.06±0.16), and grape (4.96±0.26) (Wojdyło *et al*., 2007).Sreeramulu & Raghunath (2010)evaluated the antioxidant activity and phenolic content of nineteen vegetables commonly consumed in India and okra fruits ranked in third position according to their phenolic content (167.70 mg GAE/100 g) behind red cabbage and broad beans.

Table 4.1Total phenolics and flavonoids content of the methanolic extract of pods and seeds of the eight okra accessions

Accessions	Total phenols (mg GAE/g)		Total flavonoids (mg CE/g)		Flavonoid/Phenolic	
	Pods	Seeds	Pods	Seeds	Pods	Seeds
OPA#1	92.38±2.29^{a}	27.15±0.01^{e}	16.45±0.07^{c}	21.35±0.04^{d}	0.18	0.79
OPA#2	73.14±0.08^{c}	28.97±1.01^{e}	13.90±0.04^{d}	11.66±0.04^{g}	0.19	0.40
OPA#3	44.12±0.05^{d}	39.48±2.30^{d}	18.72±0.04^{a}	10.73±0.03^{h}	0.42	0.27
OPA#4	79.28±0.02^{b}	44.07±0.02^{c}	11.91±0.49^{e}	24.22±0.22^{c}	0.15	0.55
OPA#5	41.25±0.05^{d}	21.28±0.02^{f}	8.79±0.02^{f}	13.18±0.03^{f}	0.21	0.62
OPA#6	95.21±0.04^{a}	57.34±1.17^{a}	17.09±0.03^{b}	29.04±0.03^{a}	0.18	0.51
OPA#7	28.10±0.05^{e}	52.98±0.13^{b}	8.18±0.08^{g}	26.62±0.04^{b}	0.29	0.50
OPA#8	92.26±1.69^{a}	49.03±2.35^{b}	14.29±0.04^{d}	19.91±0.03^{e}	0.15	0.41
Mean	68.21	40.04	13.66	19.59	0.21	0.64

Means not followed by the same superscript letters in the same column are significantly (P<0.05) different from each other. Data are expressed as a mean ± standard error of replicate determinations (n=2).

4.4.2 Total flavonoids

In this finding, the total flavonoid content was determined as milligram of catechin equivalent per gram (mg CE/g) extract after comparison with a catechin standard graph.The total flavonoids content of pods and seeds of the eight okra accessions is shown in Table 4.1. The total flavonoid content in the pods varied from 8.18 mg CE/g to 18.72 mg CE/g while in the seeds it varied from 10.73 mg CE/g to 29.04 mg CE/g. The pod accession OPA#3 (13.90 mg CE/g) was significantly (P<0.05)high in total flavonoid content and was followed by OPA#6 (17.09 mg CE/g), OPA#1 (16.45 mg CE/g). However, the accession OPA#7 (8.18 mg CE/g) recorded the lowest.

In the seeds, the total flavonoid content of accession OPA#6 (29.04mg CE/g) was significantly (P<0.05) high while accession OPA#3 (10.73mg CE/g) was the lowest. Mean total flavonoid contents were 13.66 mg CE/g and 19.59 mg CE/g for pods and seeds of okra accessions, respectively (Table4.1). This finding revealed that the mean total phenolic content of the pods of okra accessions (68.21 mg GAE/g) is higher than the total flavonoid content (13.66 mg CE/g) of the pods of okra accessions. However, this finding disagrees with the result obtained by Ahiakpa *et al*. (2013), in which okra pods possesses high amounts of total flavonoids as well as moderate

amounts of total phenolics. The phenolic compounds are known to possess antioxidant properties and contribute to the oxidative stability of oil (Gomez-Alonso *et al*., 2002). Excellent linear correlation has been reported between the total phenolic (TP) content and antioxidant activity (Javanmardi, *et al*., 2003; Huang *et al*., 2005; Silva *et al*., 2007), which is true for this finding.

4.4.3 Ratio of total flavonoids to total phenolics

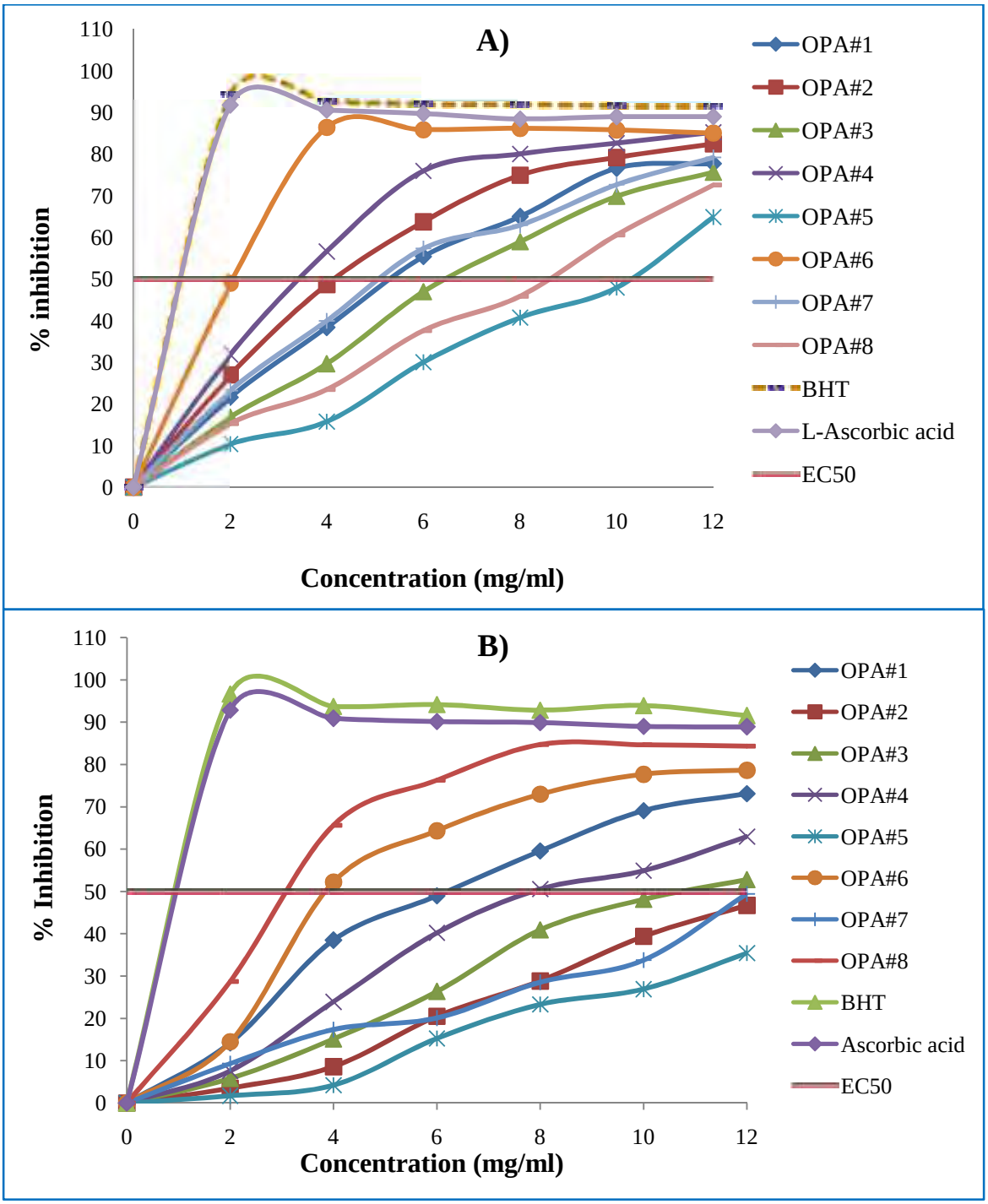
The total flavonoids to phenolics ratio were calculated from the value of total flavonoids divided by total phenolics of pods and seeds of each okra accessions. The ratio of total flavonoid to total phenolic contents of pods and seeds of the eight okra accessions are shown in Table 4.1.The percentage of the total flavonoids to phenolics ratio was between 15% (OPA#4 and OPA#5)to 42% (OPA#3) in the pods and 27% (OPA#3) to 79% (OPA#1)in the seed accessions. The results indicate that total flavonoids are one of the major contributors for the total phenolics of the pods and seeds of okra accessions. As antioxidants, flavonoids have been reported to be able to interfere with the biochemical pathways involved in the generation of reactive oxygen species (ROS), quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction (Heim*et al*., 2002). Therefore, the presence of high amount of flavonoids and their multifaceted actions make the pods and seeds extract a good candidate for exploration of antioxidants.

4.4.4 In vitro antioxidant activity

DPPH scavenging activity

The result of concentration response curves of DPPH scavenging activity of methanolic extracts of the pod and seed of eight okra extracts witha positive control are shown in Figure 4.1. The synthetic antioxidant of Butylated hydroxytoluene (BHT) and L-ascorbic acid were used as positive control using the same concentration. The percentage inhibition of DPPH scavenging activity of methanolic extracts of the pods and seeds of okra accessions were evaluated at concentrations of 2-12 mg/ml. There was an increase in DPPH radical scavenging activity with increasing concentration of the pods and seeds extract used in this study. This result agreed with the report of Motalleb*et al*. (2005), whichshowed the scavenging effects on the DPPH radical increase sharply with increasing concentration of the samples and standards.

At each concentration of the pods of okra accessions, the percentage inhibition of OPA#6 accession was the highest whereas the pod accession OPA#5 was the lowest. Specifically, at 4 mg/ml the scavenging effect of the pods of accession OPA#6 was 86.355%, whereas pods of accession OPA#5 was15.737% inhibition. The scavenging effect of synthetic antioxidant BHT and L-ascorbic acids were 92.629% and 90.538% inhibition, respectively. This is an indication that the scavenging effect of the pods of the accession OPA#6 (86.355%) is very close to the synthetic antioxidants (BHT and L-ascorbic acid) commonly used by the food industry.

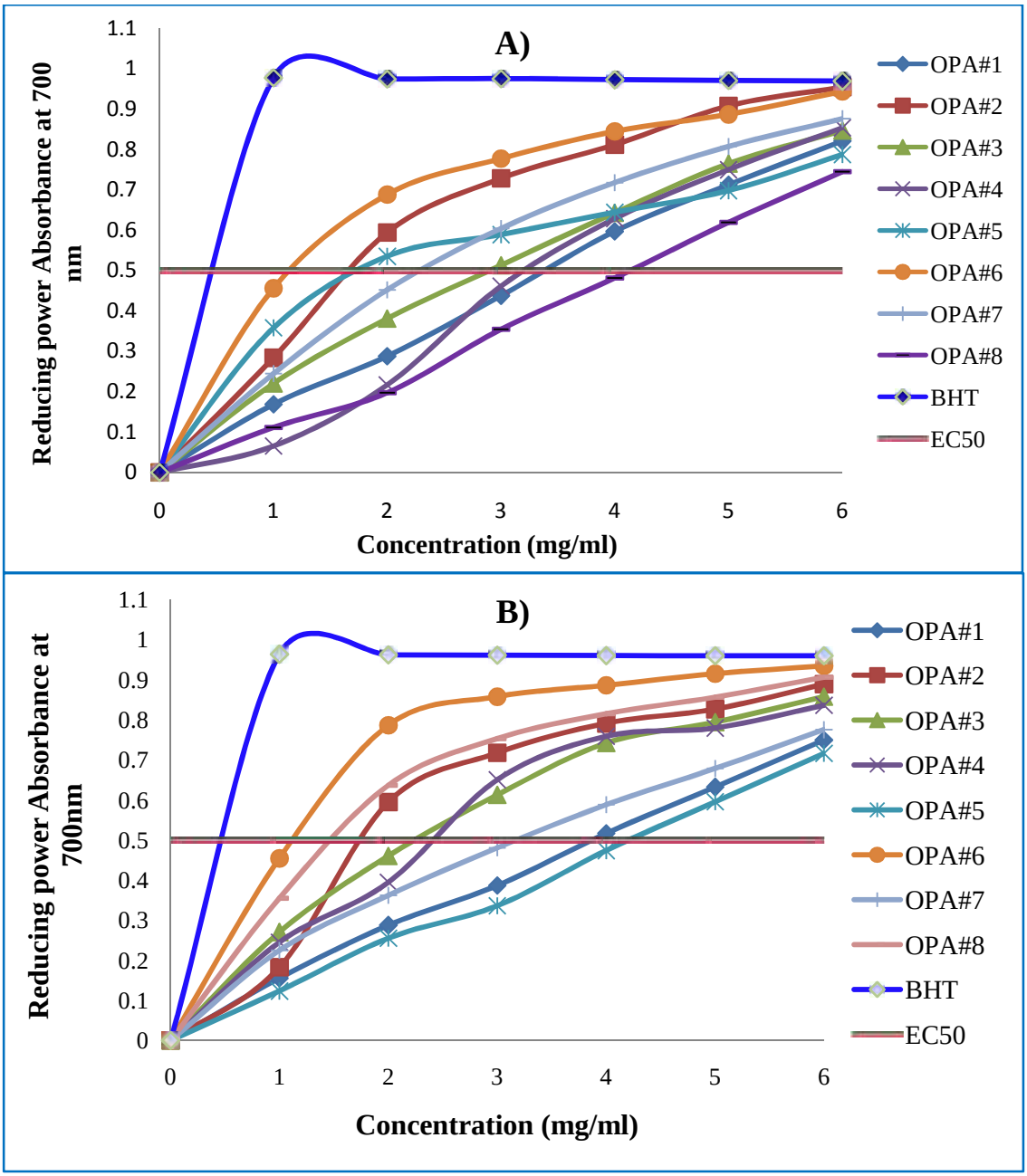

A)
% inhibition
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
BHT
L-Ascorbic acid
EC50
B)
% Inhibition
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
BHT
Ascorbic acid
EC50

The variability in the scavenging activities of the free DPPH radicals may be attributed to the variability in the flavonoid and phenolic contents of the extracts of the accessions (Kumar *et al*., 2014). The scavenging effect of the seeds of accession OPA#8 was high, with 65.64% inhibition whereas the seed accession OPA#5 was the lowest with 4.23% inhibition at 4 mg/ml concentration. The radical scavenging activity of all the seed extracts was lower than that of BHT and ascorbic acids with 93.71% and 90.86% inhibition, respectively.

Overall, the methanolic extract of the pod exhibited better scavenging effect than the seeds of okra accessions. On the other hand, even though the radical scavenging activity of pods and seeds of some accessions were low when compared to synthetic antioxidants (BHT and Ascorbic acid), coming to the point of safety, it can be prescribed as a safe antioxidant source as the synthetic antioxidant is reported to pose certain side effects. Specifically, this result indicated that the methanolic extract of okra pods of OPA#6 exhibited a better scavenging effect and can be considered as a potential natural additive to replace synthetic antioxidants.

Ferric reducing power

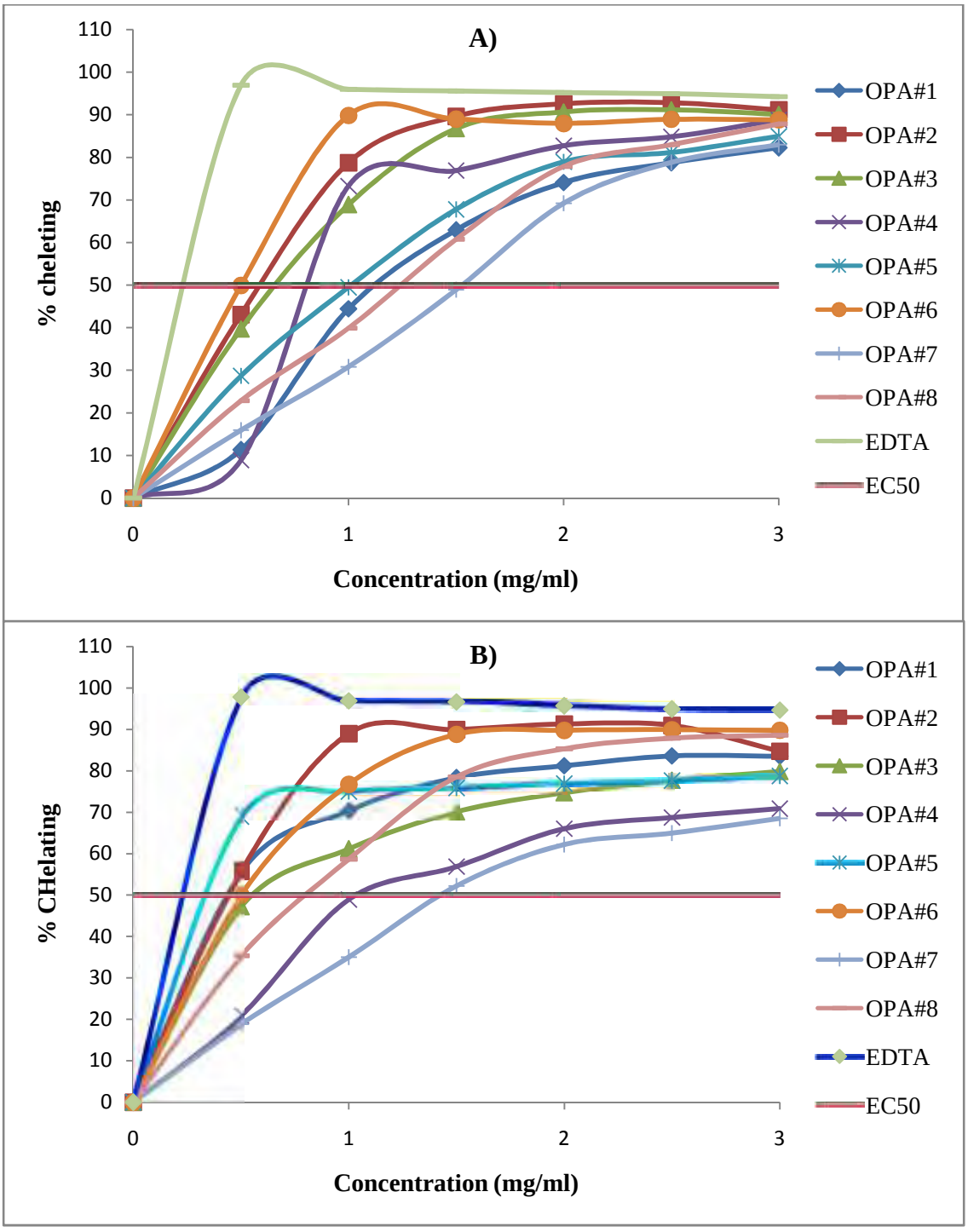
The ferric reducing power of methanolic extracts of pods and seeds of the eight okra accessions with positive control (BHT) are shown in Figure 4.2. The ferric reducing power of these extracts was observed in a concentration dependent manner. In the present study, the highest ferric reducing power was shown by pods of the accession OPA#6 with the mean absorbance of 0.84 at 4 mg/ml, which is in agreement with its higher DPPH scavenging effect of the pods of okra accession. In contrary, OPA#8 pod accession had the least ferric reducing power effect with the mean absorbance of 0.48 at 4 mg/ml. The absorbance of the ferric reducing power of synthetic antioxidant BHT was 0.97 at 4 mg/ml concentration.


A)
Reducing power Absorbance at 700 nm
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
BHT
EC50
B)
Reducing power Absorbance at 700nm
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
BHT
EC50

okra seeds and pods of OPA#6 is higher than the rest of the accessions and close to the synthetic antioxidants. The higher ferric reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential (Lee *et al*., 2007).

Metal chelating effect

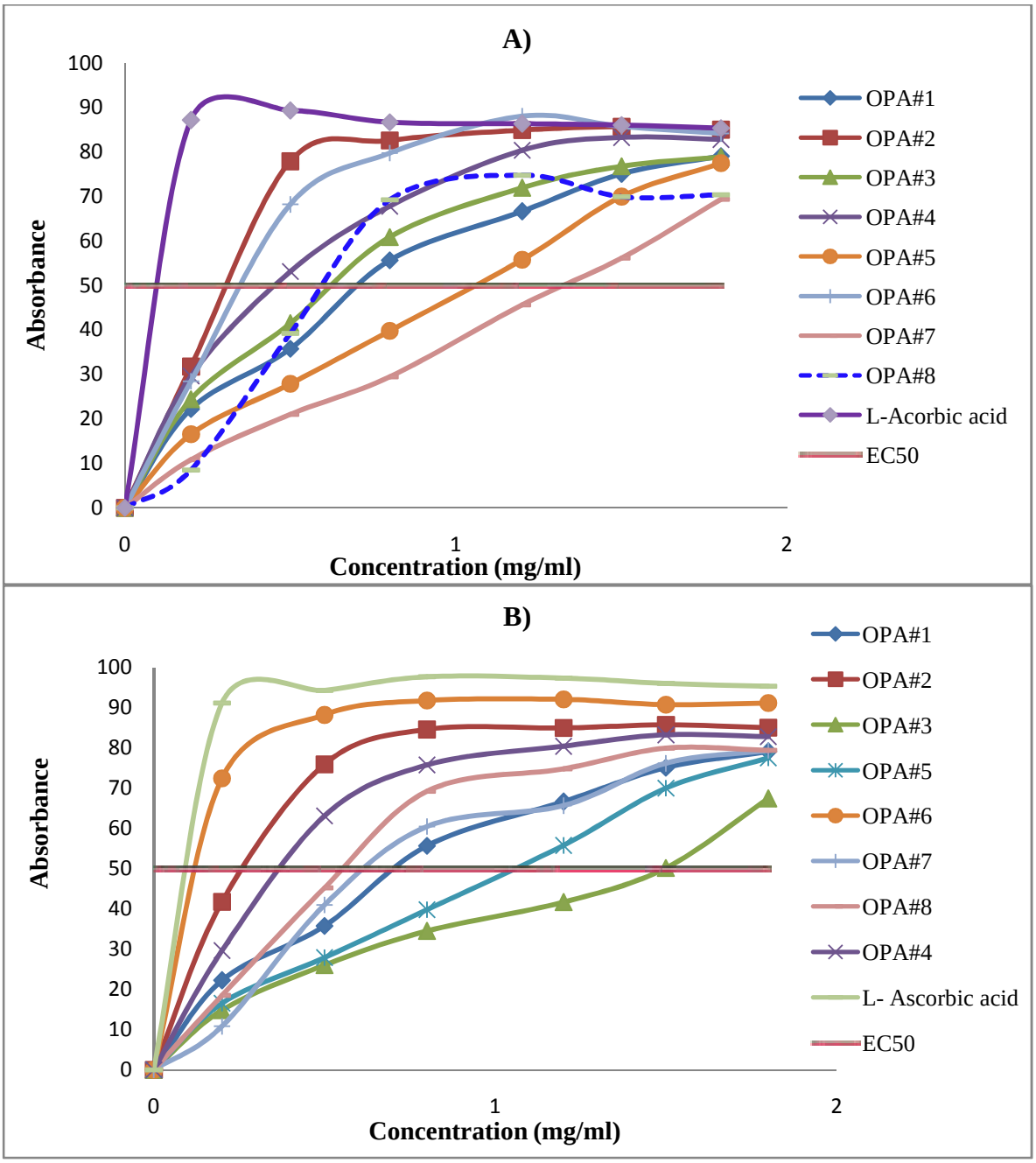
The chelating effect of pods and seeds of the eight okra accessions with the positive control (EDTA) are shown in Figure 4.3.Similar to DPPH scavenging and reducing power assay, the trend of the chelating effect of the methanolic extract increased with concentration and was high for okra pods of accession OPA#6 with 89.81% chelating at 1 mg/ml concentration. On the contrary, the pod accession OPA#7 had the least chelating effect with 30.81% at 1 mg/ml concentration. The chelating effect of synthetic antioxidant EDTA was 95.91 at 1 mg/ml concentration.


A)
% cheleting
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
EDTA
EC50
B)
% CHelating
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
EDTA
EC50

concentration. The radical scavenging activity of all the seed extracts was lower than that of EDTA with 96.87% chelating effect.

ABTS scavenging activity

The ABTS radical scavenging activity of the pod and seed of eight okra accessions with reference compounds (L-Ascorbic acid) is shown in Figure 4.4.The percentage inhibition of ABTS radical scavenging activity of methanolic extracts of the pods and seeds of okra accessions were evaluated at concentrations of 0.2-1.8 mg/ml. The result indicated that pods of accession OPA#2 were the highest with 77.91% inhibition of ABTS, whereas the pod accession OPA#5 was the lowest with 27.87% inhibition of ABTS at 0.5mg/ml concentration. The ABTS radical scavenging activity of the pod extracts of all okra accessions was lower than that of BHT and ascorbic acids with 89.31 inhibitions at 0.5mg/ml concentration. In seed accessions, OPA#6 was the highest with 88.22% inhibition effect of ABTS whereas accession OPA#3 was the lowest with 25.99% chelating effect at 0.5 mg/ml concentration. The radical scavenging activity of all the seed extracts was lower than that of ascorbic acid with 94.31% chelating effect.


A)
Absorbance
100
90
80
70
60
50
40
30
20
10
0
0
1
2
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
L-Acorbic acid
EC50
B)
Absorbance
100
90
80
70
60
50
40
30
20
10
0
0
1
2
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#5
OPA#6
OPA#7
OPA#8
OPA#4
L- Ascorbic acid
EC50

varied and ranged from 2.10-10.30 for DPPH scavenging; 1.20-4.20 for reducing power; 0.50-1.52 for chelating effect and 0.31-1.33 for ABTS scavenging activity.The result of this study revealed that okra pods of accession OPA#6 had better antioxidant properties with the lowest EC_{50} values of 2.10, 1.20, 0.50 and 0.35 mg/ml for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively. In the contrary, okra pod accession, OPA#5had the least antioxidant activity with the highest EC_{50}values of 10.30, 1.64, 1.00 and 1.08 mg/ml for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively. The synthetic antioxidant, which was used as a positive control, had a superior performance with the least EC_{50} in all the assays, except for okra pods of OPA#6.

Table 4.2Effective concentrations (EC_{50}) values (mg/ml) of pods and seeds of okra accessions

Accessions	**DPPH Scavenging (EC_{50}[a])**		**Reducing Power (EC_{50}[b])**		**Chelating Effect (EC_{50}[c])**		**ABTS scavenging (EC_{50}[d])**	
	Pods	Seeds	Pods	Seeds	Pods	Seeds	Pods	Seeds
OPA#1	5.30	6.2	3.40	4.00	1.10	0.41	0.70	0.71
OPA#2	4.10	>12	1.63	1.74	0.60	0.42	0.31	0.27
OPA#3	6.50	10.8	2.85	2.20	0.65	0.52	0.64	1.5
OPA#4	3.40	8.0	3.20	2.40	0.80	1.11	0.46	0.38
OPA#5	10.30	>12	1.64	4.30	1.00	0.32	1.08	0.07
OPA#6	2.10	3.9	1.20	1.18	0.50	0.50	0.35	0.13
OPA#7	5.10	>12	2.30	3.20	1.52	1.41	1.33	0.62
OPA#8	8.60	3.1	4.20	1.50	1.35	0.80	0.60	0.55
Mean	5.68	8.51	2.88	2.57	0.94	0.69	0.68	0.53
BHT	0.80	1.0	0.43	0.43	-	-	-	-
Ascorbic Acid	0.90	0.9	-	-	-	-	0.10	0.10
EDTA	-	-	-	-	0.22	0.22	-	-

[a]EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged.

[b]EC_{50} (mg/ml): effective concentration at which the absorbance is 0.5.

[c]EC_{50} (mg/ml): effective concentration at which 50% Fe^{2+}/ferrozine complex are inhibited.

[d]EC_{50} (mg/ml): effective concentration at which 50% ABTS radicals are scavenged.

TheEC_{50} values (mg/ml) of the seed accessions were varied and ranged from 3.1->12 for DPPH scavenging; 1.18-4.00 for reducing power; 0.32-1.41 for chelating effect, and 0.07-1.50 for ABTS scavenging. Similar to the pods, seeds of accession OPA#6 had better antioxidant

properties with low EC_{50} values of 3.9, 1.18, 0.50 and 0.13 mg/ml for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively. Okra seeds of OPA#7 had the least antioxidant activity with high EC_{50}values of >12, 3.2, 1.41 and 0.62 mg/ml for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively. Like in that of the pod accession, the synthetic antioxidant (BHT, L-Ascorbic acid and EDTA), which was used as a positive control, had a superior performance with the least EC_{50} in all the assays, except for seeds of the accession OPA#6.

The mean EC_{50} values (mg/ml) of the pods of okra accessions were 5.68, 2.55, 0.94 and 0.68 for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively, whereas the mean EC_{50} values (mg/ml) of the seeds of okra accession was 8.51, 2.57, 0.69 and 0.53 for DPPH scavenging, reducing power, chelating effect, and ABTS scavenging, respectively (Table 4.2).Over all, okra pods had better antioxidant properties for DPPH scavenging and reducing power with low EC_{50} values than the seeds of okra accessions. However, chelating effect and ABTS was higher in seed with relatively low EC_{50} values (mg/ml) than the pods of okra accessions. Comparatively, the mean EC_{50} values (mg/ml) of both pods and seeds of okra accession were far higher than the synthetic antioxidant (i.e. the synthetic antioxidant contain much higher antioxidant activity than the mean of the pods and seeds of okra accession).

4.5 Conclusions

Antioxidant compounds in food play a significant role as a health-protecting factor. In this study, pods and seeds of eight okra accessions were evaluated for their antioxidant activities, total phenolics and total flavonoids content in order to find the possible rich sources of natural antioxidants. The results showed that antioxidant activity, total phenolics and total flavonoids levels vary widely across pods and seeds of okra accessions. It has also been shown that the antioxidant levels of the pods and seeds of okra accessions increased with the increasing concentration of the samples to a certain extent. In general, the pods and seeds of indigenous Ethiopian okra accessions evaluated for the first time in this study represent a source of potential antioxidants. Particularly, both pods and seeds of okra accession OPA#6 is a significant source of natural antioxidants that could probably be used as functional food ingredients and replace synthetic antioxidants in the future. In addition, further studies are needed to identify the phenolic compounds that confer the antioxidant properties of edible okra.

Chapter 5

Physicochemical, functional and antioxidant properties of okra seedoil and pod mucilage accessions grown in Benishangul Gumuz region, Ethiopia

5.1 Abstract

Physicochemical properties of okra seed oil and the functional and antioxidant properties of the pod mucilage of the eight okra accessions were evaluatedin order to identify the possible sources mucilage and oils. The crude oil yield of the seed accessions varied significantly ($P<0.05$) and ranged from 19.25-38.19%. The physicochemical properties of the okra seed oils varied significantly ($P<0.05$) (except the saponification value) and the results werespecific gravity (0.904-0.923), refractive index (1.460-1.466), acid value (1.315-5.055), peroxide value (2.990-9.060), iodine value (100.45-132.92) and saponification value (188.97-194.78), these properties would make okra seed oil suitable for food and industrial applications. This study had shown that the mucilage contents of the pods of eight okra accessions ranged from 1.25 to 3.45 g/100g. Functional properties of the mucilage of okra pods varied significantly ($P<0.05$) and had respective ranges of bulk density of 0.58 to 0.64 g/ml; water absorption capacity of 2.45 to 4.60 ml/g; oil absorption capacity of 0.02 to 3.64 ml/g; emulsifying capacity of 42.22 to 74.45%; emulsion stability of 42.22 to 74.45%; foaming capacity of 50.51 to 62.50% and foam stability of 36.04 to 54.35%. Total phenolic and flavonoid contents of the mucilage of the okra pods ranged from 4.66 to 49.93 mg GAE/g and 8.18 to 18.72 mg CE/g, respectively.The study also revealed that the mucilage of the okra pods was found to exhibit good functional properties and can offer a great potential in various food systems. Particularly, mucilage of the okra pods from OPA#5 and OPA#7 had desirable water and oil absorption capacities, whereas the mucilage of accession OPA#1 and OPA#6 had high emulsifying and foaming properties.Overall results suggested that okra seed oil may be considered as a new candidate and valuable source of edible oil and can be utilized for industrial and nutritional purposes and need to be improved through selection and breeding.

Keywords: Okra; Seed; Pod; Functional; Antioxidant;Physicochemical; Mucilage; Oil

5.2 Introduction

Vegetable oils are important sources of nutritional oils (FAO, 2009), industrial raw materials (Ramadan *et al*., 2006) and pharmaceutical applications(Nzikou *et al*., 2010). Their usefulness in various applications aside from edible purpose depends on their yields, different compositions and their physical and chemical properties (Aluyor & Ori-Jesu, 2008). These factors rely heavily on the crop species or cultivar and upon the environmental conditions in which the crop is grown (Velasco *et al*., 2005). Indeed, no oil from a single source has been found to be suitable for all purposes because oils from different sources generally differ in their composition (Mohammed & JorfThomas, 2003) and this necessitates the search for new sources of novel oils.

In addition, at present, a few vegetable crops such as soybean, cottonseed, peanuts, rapeseed, and sunflower dominate the international edible oilseed market (Diemeleou *et al*., 2014; Sorkhe*et al*., 2016). The world consumption of the vegetable oils from soybean, palm, rapeseed, and sunflower are 31.6, 30.5, 15.5, and 8.6 million tons per year, respectively (Stevenson *et al*., 2007). However, these conventional sources of vegetable oil no longer meet the ever increasing demands of domestic and industrial sectors (Idouraine *et al*., 1996, Sorkhe*et al*., 2016). Therefore, tomeet the demand, there is a need not only to increase the production of the major oilseed crops but also to diversify the sources by exploring and increasing the production of minor and neglected crops such as okra.

Okra seeds contain about 20 to 40% oil (Benchasri, 2012; MEF, 2013). Okra has a potential for cultivation as an oilseed crop because its mature pods contain high quantity of seeds that containa considerable amount of oil which could be characterized and utilized for commercial purposes (Anwar *et al*., 2011). Okra seed oil yield is comparable to most oil seed crops but lower than palm and soybean oil (Kumar*et al*., 2010). Moreover, okra seed oil has a potential hypocholesterolemic effect. The potential for wide cultivation of okra for edible oil for cake is very high (Kumar*et al*., 2010). Although a number of studies have been reported on the characteristics of the oil and other components of okra vegetables (Camciuc *et al*., 1998a; Pham *et al*., 2003; Ndangui *et al*., 2010; Jarret *et al*., 2011; Ogungbenle & Omosola, 2015). However,

there is no published report currently available on oil yields and physicochemical properties of Ethiopian okra seed.

On the other hand, okra (*Abelmoschus esculentus*) pod containsmucilage which is thick slimy polysaccharides (Ahiakpa *et al*., 2014a; Biswal*et al*., 2014).Okra mucilage has the potential for use as food, non-food products, and medicine (Kumar *et al*., 2010; Haruna *et al*., 2016). The food applications include a whipping agent for reconstituted egg whites, an additive in the formulation of flour-based adhesives, an additive for clarifying sugarcane juice. It is also used to modify the food quality in terms of food stability, texture and appearance properties by acting as an emulsifier, thickener, gelling agent or texture modifier (Noorlaila *et al*., 2015). Okra mucilage also contributes to improved functionality, especially water binding, emulsifying and foaming properties of food products (Jideani, & Bello, 2009).

There are studies on the functional properties of mucilage from okra vegetables (Woolfe *et al*., 1977, Jideani& Bello, 2009; Adetuyi & Dada, 2014; Noorlaila *et al*., 2015). However, there is very little information reported on the antioxidant properties of okra mucilage. Despite the fact that Ethiopia is native to okra vegetable, there is no published literature on physicochemical properties of okra oil and functional and antioxidant properties of mucilage of Ethiopian okra pods. Hence, the objectives of this study were to determine physicochemical,functional and antioxidant properties of okra seed oil and pod mucilage accessions grown in Benishangul Gumuz region, Ethiopiain order to evaluate the possible sources and applications of okra oil and mucilage.

5.3 Materials and Methods

5.3.1 Extraction of okra seed oil

The soxhlet extractoin method described by AOAC (2000) was used for the extraction and determination of the percentage of the oil yields. About 50.000g of okra seed powders was extracted for 4 hrs with 300 ml of n-hexane (40-60°C) in a Soxhlet extractor. Then the solvent was distilled off at 40^{o}C under vacuum in a rotary evaporator (Model N-1Eyela, Tokyo Rikakikal Co.Ltd. Japan). The extracted oil was weighed to determine the oil content of the seed. The extracted crude oils were stored under refrigerator (4^{o}C) in air tight brown sterile glass bottles (Ejikeme *et al*., 2010)for subsequent physicochemical analyses. The percentage yield of the extracted oil was calculated by using the following formula:

$$\text{Yield of oil (\%)} = \frac{\text{Weight of oil}}{\text{Initial weight of sample}} \text{ x } 100$$

5.3.2 Physicochemical properties of okra seed oil

5.3.2.1 Determination of specific gravity

The specific gravity of the oil was determined according to AOAC (2000) official method described under sub-number 920.213. The density of the oil was determined by using density bottle. A clean and dried density bottle with a stopper was weighed. The density bottle was then filled with cold distilled water and kept in a water bath for 30 minutes at 25^0C. The weight of the bottle together with the water was taken. After weighing, the bottle was emptied and then dried in an oven for 2 minutes. The dried bottle was then filled with the oil and then weighed. The specific gravity was calculated using the following formula:

$$\text{Specific Gravity (g/cm3)} = \frac{(W_1 - W_o)}{(W_2 - W_o)}$$

Where:

W_o = Weight of empty bottle (g),

W_1 = Weight of water and bottle (g),

W_2 = Weight of oil and bottle (g)

5.3.2.2 Determination of refractive index

Refractive index of the oils was determined by a refractometer according to AOAC (2000) method 921.08. Three drops of the sample were transferred into the glass slide of the refractometer. Water at 30°C was circulated around the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index.

5.3.2.3 Determination of acid value

The acid value was determined according to AOAC (2000) official method using sub-component 940.28. The acid value was determined by adding 25 ml of diethyl ether and 25 ml of ethanol to 0.20 g of the extracted oil sample. The mixture was then titrated with 0.1N sodium hydroxide (NaOH) with phenolphthalein as an indicator. The acid value was then calculated using the following formula:

$$\text{Acid value (mgNaOH/g)} = \frac{N \times V \times 56.1}{W}$$

Where:
N = is the normality of the NaOH in moles per liter
V = is the volume of NaOH used in ml
W = is the weight of the oil sample in g
56.1 = Molecular weight of NaOH (g)

5.3.2.4Determination of peroxide value

Peroxide value was determined according to AOAC (2000) official method using sub-component 965.33. About 5.00g of oil sample was weighed into 250 ml glass-stoppered Erlenmeyer flask and 1:1 ratio of 30 ml of $CH_3COOH–CHCl_3$ was added and swirled to dissolve. 0.5 ml of saturated KI solution was also added from Mohr pipette and kept to shake for 1 min, and 30 ml distilled water was added. Titration was done by slowly shaking with 0.1M $Na_2S_2O_3$ until the yellow color is disappear. 0.5 ml of 1% of starch solution was added, and titration was continued by shaking vigorously to release all I_2 from $CHCl_3$ layer until blue color disappears. The blank determination was also conducted following the same procedure. Peroxide value (milliequivalent peroxide/kg oil or fat) was calculated using the following formula:

$$\text{Peroxide Value (mgEquiv.O 2/Kg)} = \frac{M \times (S - B)}{W} \times 1000$$

Where:
M = is the molarity of $Na_2S_2O_3$ solution,
$_S$ = the volume of titrant(ml) for sample,
V_2 = the volume of titrant for blank,
W = is the weight of the oil sample
1000 = conversion of units(g/kg)

5.3.2.5 Determination of iodine value

Iodine value was determined according to AOAC (2000) official method using sub-component 993.20. About 0.250g of the extracted oil was dissolved in 10ml of chloroform in a conical flask. 250 ml of carbon tetrachloride was added into a conical flask. 30 ml of Hanus solution was then added and the solution was shaken continuously for 30 minutes. 100 ml of distilled water was added and the iodine solution was titrated against 0.1 N sodium thiosulfate solution till yellow color formed. To form blue solution about 2-3 drops of starch solution was added and then it was titrated till the blue color is disappeared. The blank analysis was conducted following the same

procedure but without the sample. Iodine value (mgI_2/100g) was calculated using the following formula:

$$\text{Peroxide Value (mgI2/100g)} = \frac{(B - S) \times N \times 0.127}{W} \times 100$$

Where: B: is $Na_2S_2O_3$ volume for blank

S: is $Na_2S_2O_3$ volume for sample

N: is normality of $Na_2S_2O_3$

W: is weight of oil sample

5.3.2.6Determination ofsaponification value

Saponification value was determined by indicator method according to AOAC (2000) official method using sub-component 920.160. Two grams of oil sample was weighed accurately by transfer method into a 250 ml round bottom flask. Freshly prepared 0.5N alcoholic potassium hydroxide solution (25 ml) was added to the sample by means of a pipette and the mixture was gently refluxed on a water bath using an air-condenser for one hour. Then the flask was cooled, the condenser tip washed with little-distilled water and the contents were titrated with 0.5 N hydrochloric acid solution using phenolphthalein as indicator. A blank analysis was carried out simultaneously and saponification value was calculated using the following formula:

$$\text{Saponification value (mgKOH/g)} = \frac{N \times (V_2 - V_1) \times 56.1}{W}$$

Where:

N = the actual normality of HCl used

V_1 = the volume of HCl used for sample

V2 = the volume of HCl used for blank

W = is the weight of the oil sample

5.3.3 Mucilage extraction

The mucilage of the pods of okra accessions was extracted according to theprocedure described by Farooq *et al.* (2013). About 100 gram of the sliced and dried okra wasdissolved in 300 ml of distilled water. It was heated in a water bath with continuous stirring for 1 hr at 60 °C. The concentrated solution was filtered through a muslin cloth and cooled to room temperature. About 20 ml of acetone was added to the concentrated solution. The mucilage was filtered again through a muslin cloth and cooled (Appendix 5.1). The filtered mucilage was further dried to constant weight at 45°C in drying oven. Hard mucilage cake was groundinto a fine powder by mortar and pestle until it is small enough to pass through 0.425 mm sieve size. The mucilage

powder was packed in airtight polyethylene plastic bags and was stored in a desiccator until required for analysis.

5.3.4 Determination of functional properties of okra pod mucilage

5.3.4.1 Determination of bulk density

The bulk density of the mucilage powder was determined according to the method described by Gupta*et al*. (2015). About two grams of mucilage powder was placed in 10 ml test tube. The test tube was tapped several times (minimum 10 times) on the laboratory bench to compact the mucilage powder. The final bulk volume was recorded. Bulk density was calculated as the weight of mucilage powder (g) divided by its final volume (ml) using the following formula:

$$\text{Bulk density (g/ml)} = \frac{\text{Wieght of mucilage powder (g)}}{\text{Final Volume of mucilage (ml)}}$$

5.3.4.2 Determination of water and oil absorption capacity

Water and oil absorption capacities were determined according to the method described by Aremu*et al*. (2007). One gram of sample was mixed with 10 ml of distilled water or oil (specific gravity of 0.929) in a centrifuge tube and allowed to stand at room temperature for 1 hr. It was then centrifuged at 200 rpm for 30 min and the supernatant was transferred into a 10 ml graduated cylinder. Water and oil absorption capacity was calculated as ml of water or oil absorbed per gram of sample from the following equations:

$$\text{Water absorption capacity (ml)} = \text{Volume of water added} - \text{volume of water decanted}$$
$$\text{Oil absorption capacity (ml)} = \text{Volume of oil added} - \text{volume of oil decanted}$$

5.3.4.3 Determination of emulsifying properties

The emulsifying capacity and emulsion stability of the mucilage of the pods of okra accessions were determined according to the method described by Thanatcha & Pranee, (2011). About 1g of mucilage powder was dissolved in 50 ml of distilled water, and 50 ml refined oil was added into the mixture. Then the mixture was homogenized for 1 min and was centrifuged at 2000 rpm for 5 min. Finally, the height of emulsified layer was measured and compared with the height of the whole layer. The emulsifying capacity was calculated by the following equation:

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer}} \text{ X } 100$$

The emulsion stability was estimated after heating the emulsion contained in calibrated centrifuged tube at 80 ^{0}C for 3 min in a water bath. The heated emulsion was cooled for 15 min under running tap water and was centrifuged for 15 min. The emulsion stability was calculated by using the following formula:

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsion layer after heating}}{\text{Total height of mixture after heating}} \text{ x } 100$$

5.3.4.4 Determination of foaming properties

Emulsifying capacity and stability of the flour samples were determined according to Aremu *et al.* (2007). About one gram of sample was blended in kenwood major blender with 10 ml distilled water for 30 seconds at maximum speed. Refined oil was added in 10 ml portions with continued blending. A drop in consistency was considered to be the point at which oil addition was discontinued. The emulsion was then allowed to stand in a graduated cylinder, and the volume of water separated after 24 hr was recorded as emulsions stability. The emulsifying capacity was calculated by using the following formula:

$$\text{Emulsifyin g capacity (\%)} = \frac{\text{Height of emulsion layer}}{\text{Total height of mixture}} \text{ x } 100$$

The emulsion stability was estimated after heating the emulsion contained in calibrated centrifuged tube at 80^{0}C for 3 min in a water bath, cooled for 15 min under running tap water and centrifuged for 15 min. The emulsion stability was calculated by using the following formula: $\text{Emulsion stability (\%)} = \frac{\text{Height of emulsion layer after heating}}{\text{Total height of mixture after heating}} \text{ x } 100$

5.3.5 Determination of antioxidant properties of mucilage

The total phenolic, total flavonoid, DPPH scavenging and chelating effect of the processed pods and seeds of okra accessions was evaluated according to the method described in chapter 4under section 4.3.3.1; 4.3.3.2; 4.3.4.1and4.3.4.2, respectively.

5.3.6 Statistical analysis

The completely randomized design (CRD) was used with two replicates. All the statistical analyses were performed for the result obtained using SPSS version 20.0 for windows. Data were evaluated by using one-way analysis of variance (ANOVA). Means were separated by Duncan's multiple range test and the result was reported as a mean ± standard error (SE). The statistically significant difference was stated at a p-value of 0.05 or less than 0.05 ($P \leq 0.05$).Graphs of effective concentration at 50% (EC_{50}) of the respective antioxidant activities were constructed by using Microsoft excel.

5.4 Results and Discussions

5.4.1 Oil yields

The percentage of crude oil yields of eight accessions of okra seeds are shown in Figure 5.1. It can be noted that the yield of okra seed oils varied from 19.25% (OPA#3) to 38.19% (OPA#2). The accession, OPA#2 was significantly ($P<0.05$)high in crude oil content (38.19%) and was followed by OPA#7 (34.01%), OPA#4 (33.18%) and OPA#5 (32.31%). However, the accession, OPA#3 was low (19.25%) in oil yield. The mean percentage of oil yield (29.64%)of the seeds obtained in this study was higher than the value reported by Ndangui *et al*. (2010) (24.90%) for okra seeds. The variation in oil yield with other reports may be due to the differences in avariety of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used (Mohamed & Girgis, 2005).

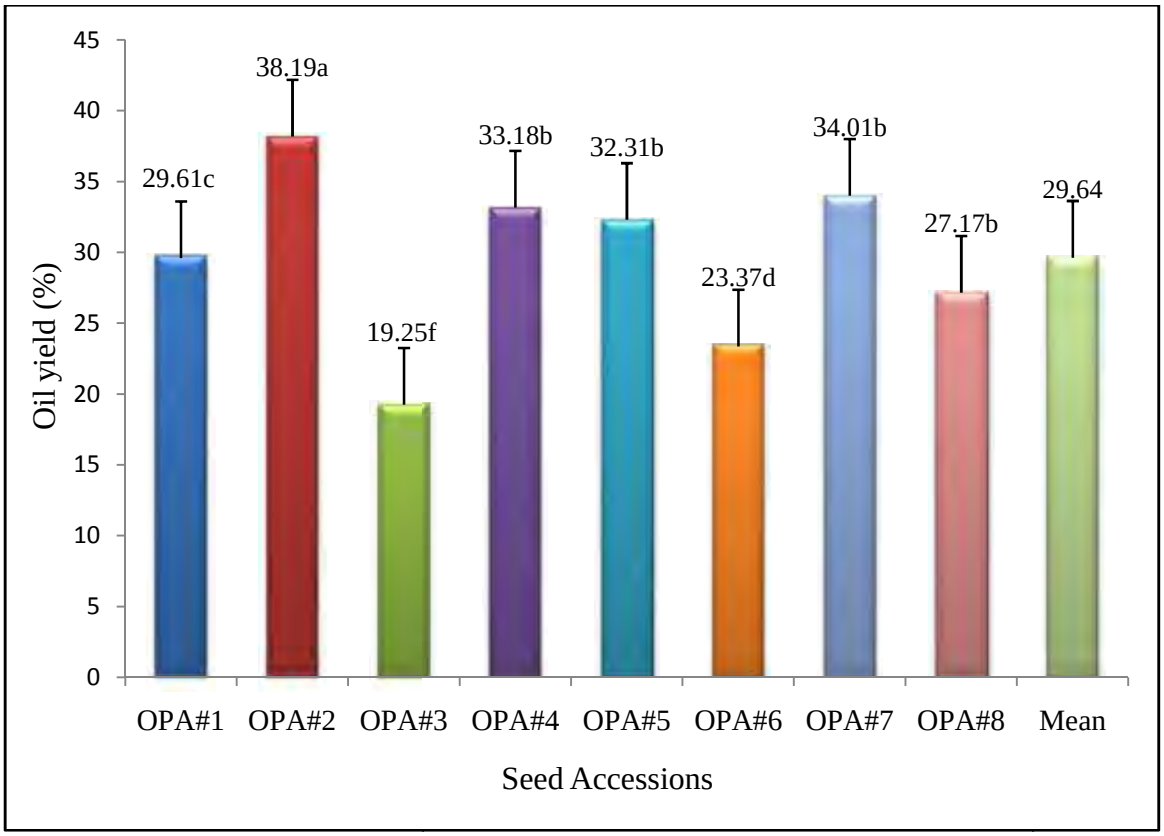


Figure 5.1 Percentage oil yields of the seeds of eight okra accessions (Values not followed by the same letters in this figure are significantly (P<0.05)different from each other).

The range of oil yield (19.25-38.19%) from okra seeds oil in the present study was found to be in the ranges of some conventional oil seed crops: cotton (15.0-24.0%), soybean (17.0-21.0 %), safflower (25.0-40.0%) and mustard (24.0-40.0%) (Knothe & Steidley, 2005) andlower than some unconventional oilseeds such as *Canariumschwenfurthii* fruits (36.1%) and B*alanites aegyptiacaalmonds* (48.3%) (Nzikou *et al*., 2006). The oil content of okra seeds (19.25-38.19%) in the present study was also found to be in the ranges of some common edible oils reported by Nichols & Sanderson (2003)for cottonseed (22-24%), safflower (30-35%), soybean (18-22%),rapeseed (40-48%), and olive (12-50%). Therefore, okra seeds could be considered as a potential source of vegetable oil for domestic and industrial purposes that would be of economic importance.

5.4.2 Physicochemical properties

Specific gravity

The physicochemical properties of okra seed oil are presented in Table 5.1. The specific gravity of okra seed oil at 25ºC ranged from 0.904 - 0.923 g/cm^3, indicating that the oils are less dense thanwater.The specific gravity of seeds of okra accession OPA#6 (0.923 g/cm^3) was high but did not significantly (P<0.05) different from OPA#1 (0.916 g/cm^3); OPA#2 (0.914 g/cm^3); OPA#4 (0.914 g/cm^3) and OPA#7 (0.917 g/cm^3). Accessions OPA#5 and OPA#3 (0.904 g/cm^3) was low but not significantly (P<0.05)differentfrom accession OPA#8 (0.913 g/cm^3) on a dry weight basis. The mean specific gravity (0.913 g/cm^3) of the present okra seed oils at 25ºC were similar to the value reported for okra oil (0.913 g/cm^3) (Ogungbenle & Omosola, 2015); cottonseed (0.917 g/cm^3); soybean (0.9175 g/cm^3); corn (0.918 g/cm^3); sunflower (0.920 g/cm^3); sesame (0.918 - 0.921 g/cm^3) and safflower oils (0.919 - 0.924 g/cm^3) (Amoo *et al*., 2008; Khanzadeh *et al*., 2012).

Refractive index

Table 5.1 shows the refractive index of the seed oil of eight accessions of okra. The refractive index of the oil of seeds of okra accessions was significantly (P<0.05) high in OPA#6 (1.466) while it was low in OPA#8 (1.460). The high refractive index of these oils seems to confirm the high number of carbon atoms in their fatty acids (Faladeet *al*., 2008). Refractive index also increases as the double bond increases and vice versa (Eromosele & Paschal, 2003; Omari *et al*., 2015).

The refractive index of oils ranged from 1.460 - 1.466, which was in close agreement with values reported at 25ºC for okra seed oils (1.463) (Ogungbenle & Omosola, 2015) and for conventional oils like soybean (1.466- 1.470) and palm kernel (1.449- 1.451) (Falade *et al*., 2008), cotton seed (1.468-1.472), safflower (1.473-1.476) and soybean (1.4728) (Khanzadeh *et al*., 2012). These values were eligible as edible oil.The mean refractive index (1.463) of okra seed oil was also in close agreement to those reported for unconventional vegetable oils by Olubaet *al.* (2011) for egusi melon seed oil (1.45) and Ardabilie*t al.* (2011) for pumpkin seeds (1.4662). This shows that these oils are less thick compared with most drying oils whose refractive indices were between 1.48 and 1.49 (Nichols & Sanderson, 2003; Akinhanmi *et al*., 2008).

Table 5.1 Physicochemical properties of seed oil of eight okra accessions

Accessions	Specific Gravity	Refractive Index	Acid Value	Peroxide Value	Iodine Value	Saponification Value
OPA#1	0.916 ± 0.02^{ab}	1.465 ± 0.04^{ab}	3.645 ± 0.15^{b}	2.990 ± 0.98c	101.40 ± 0.26c	188.97 ± 1.04a
OPA#2	0.914 ± 0.05^{ab}	1.464 ± 0.01^{ab}	5.055 ± 0.15^{a}	8.075 ± 0.01ab	126.85 ± 0.79a	194.78 ± 1.51a
OPA#3	0.904 ± 0.03^{c}	1.462 ± 0.01^{ab}	2.285 ± 0.29^{c}	2.990 ± 0.99c	100.45 ± 8.11c	189.20 ± 1.42a
OPA#4	0.914 ± 0.03^{ab}	1.460 ± 0.03^{b}	1.315 ± 0.15^{d}	9.060 ± 1.00a	116.29 ± 0.49b	190.63 ± 2.74a
OPA#5	0.904 ± 0.03^{c}	1.463 ± 0.00^{ab}	4.555 ± 0.29^{a}	6.980 ± 1.00ab	132.92 ± 0.34a	189.27 ± 1.42a
OPA#6	0.923 ± 0.02^{a}	1.466 ± 0.01^{a}	3.425 ± 0.29^{b}	3.015 ± 1.00c	128.20 ± 0.72a	193.44 ± 2.73a
OPA#7	0.917 ± 0.02^{ab}	1.464 ± 0.03^{ab}	2.450 ± 0.12^{c}	4.970 ± 1.01bc	126.50 ± 0.40a	189.16 ± 1.24a
OPA#8	0.913 ± 0.02^{bc}	1.460 ± 0.01^{b}	1.740 ± 0.29^{cd}	7.030 ± 1.00ab	113.26 ± 3.11b	192.05 ± 4.27a
Mean	0.913	1.463	3.059	5.639	118.23	190.94

Means not followed by the same superscript letters in the same column are significantly (P<0.05) different from each other. Data are expressed as amean ± standard error of replicate determinations (n=2).

Acid value

The result of the acid value (mgKOH/g) of okra oilseeds is shown in Table 5.1.The acid value varied from 1.315 (OPA#4) to 5.055mgKOH/g (OPA#2). The accession OPA#2 (5.055mgKOH/g) and OPA#5 (4.555 mgKOH/g) were significantly (P<0.05)high in acid value and was followed by OPA#1 (3.645 mgKOH/g) and OPA#6 (3.425 mgKOH/g) in that order. However, OPA#4 was a low acid value (1.315 mgKOH/g) which was not significantly (P<0.05) different from OPA#8 (1.740 mgKOH/g). The mean acid value (3.059 mgKOH/g) in the present study was comparable to the value reported for okra seed oil (3.39 mgKOH/g) (Ogungbenle & Omosola, 2015) but lower than the finding reported for benniseed oil (4.76 mgKOH/g) (Oshodi

et al., 1999);calabash seed oil (5.92 mg/KOH); lump-in-neck oil (4.59mgKOH/g) and bottle gourd seed (5.21 mgKOH/g) (Olaofe *et al*., 2012). The lower the acid values the more its acceptability for edibility purpose (Sani*et al*., 2014).

The mean acid value (3.059 mgKOH/g) of the present study was also found in the permissible limits i.e. 10 mgKOH/g of oil and found to be suitable for dietary purposes (Eka, & Chidi, 2009). The acid value of 0.00 to 3.00 mgKOH/g is recommended for oil to be used for cooking (Barkatullah *et al*., 2012). Thus, the seed oil of most of the okra accessions except OPA#2 and OPA#5 could be suitable for cooking. Ogungbenle & Omosola, (2015) reported that okra oil is used for cooking and in the formulation of pomades and margarine. Nzikou *et al*. (2006) also reported that okra seed oils could probably be good edible oils that may be stored for a long time without spoilage via oxidative rancidity. It is a common knowledge that these parameters are a measure of the level of spoilage of oil, hence it is concluded that okra seed oil are of low magnitude and a reflection of the freshness and edibility of the crude oil (Ouattara *et al*., 2015). Thus, the okra seed oil could be suitable for dietary purposes. However, the higher acid values of accession OPA#2 and OPA#5 seed oil require refining to minimize their acidity before it is been eventually used for food purposes.

Iodine value

Table 5.1 depicts the iodine value (mgI_2/100g) of the oils of seeds of okra accessions. The iodine values of the seed oils varied from 100.45 to 132.92 mgI_2/100gand this indicates thatthe iodine values in all accessions were high. The seed accessions OPA#5 (132.92 mgI_2/100g), OPA#6 (128.20 mgI_2/100g), OPA#2 (126.85 mgI_2/100g) and OPA#7 (126.50 mgI_2/100g) were significantly ($P<0.05$)high in iodine value, whereas OPA#1 (101.40 mgI_2/100g) and OPA#1 (100.45 mgI_2/100g) were lower than the rest of accessions in their iodine value.

The mean iodine value (118.23 mgI_2/100g) of the seed oils of okra accessions in this study was slightly higher than those reported for okra seed by Ogungbenle & Omosola (2015)(112.16 mgI_2/100g). If the iodine values of okra seed oils were in the range of the values reported for unsaturated fatty acid rich oils such as soybean oil (120-143), corn oil (103-128) and sunflower oil (125-136) (Khanzadeh*et al*., 2012), cotton seed (100.0- 123.0) (Aremu *et al*., 2006). However, seed oils of okra accessions had higher iodine value than those of saturated fatty acid-

rich oils such as cocoa butter (32.0-42.0) (Lge *et al*., 1994), coconut (6.0- 10.0), palm oil (50.0-55.0), palm kernel (14.0-1.0) (Aremu *et al*., 2006).

The higher the iodine value is the more unsaturated the oil (Ziyada & Elhussien, 2008). However, when the iodine value becomes too high, the stability of the oil reduces because it is more likely to undergo oxidation. Vegetable oils are classified as drying, semi-drying, and non-drying based on their iodine values (Khanzadeh*et al*., 2012).When the iodine value obtained is less than 100, it is suggesting the absence of unsaturated fatty acids and this places the oil in the non-drying groups. However, okra seed oils can be regarded as semi-drying oils and have a high degree of unsaturation. The high degree of unsaturation of semi drying oil further suggests that the oils can be used for the manufacture of cosmetics and oil paints (Peace & Aladesanmi, 2008) as well as shoe polish and varnishes (Akintayo, 2004). Ogungbenle & Omosola (2015)also reported that the iodine values of okra seed oils enable them to be employed in the manufacture of soaps, lubricants, and candles.

Peroxide value

The peroxide value (mgEquiv.O_2/Kg) of okra seed oils is presented in Table 5.1. Peroxide value of the seed oils varied from 2.990 mgEquiv.O_2/Kg (OPA#4 and OPA#6) to 9.060 mgEquiv.O_2/Kg (OPA#4). Peroxide value of oilseeds of okra accession OPA#4 (9.060 mgEquiv.O_2/Kg) was high, but was not significantly ($P<0.05$) different from accession OPA#2 (8.075 mgEquiv.O_2/Kg), OPA#8 (7.030 mgEquiv.O_2/Kg) and OPA#5 (6.980 mgEquiv.O_2/Kg). The peroxide value of OPA#1 and OPA#3 (2.990 mgEquiv.O_2/Kg), OPA#6 (3.015 mgEquiv.O_2/Kg) and OPA#7 (4.970 mgEquiv.O_2/Kg) was low.

The mean peroxide value (5.639 mgEquiv.O_2/Kg) of the oils of okra seeds in this study was lower than that reported for okra seeds (7.31 mgEquiv.O_2/Kg) (Ogungbenle & Omosola, 2015)andwascomparable to legume oils (5.63- 6.63 mgEquiv.O_2/Kg) (Olaofe *et al*., 2012), itwas higher than that of quinoa oil (2.44 mgEquiv.O_2/Kg) (Ogungbenle, 2003). Peroxide value is a measure of oxidative rancidity and deterioration of oil level that could be used as an indication of the quality and stability of fats and oils (Ekwu & Nwagu, 2004). Oxidative rancidity is the addition of oxygen across the double bonds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds. Thus, the high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage (Mohammed & Hamza, 2008). The peroxide values of

okra seed oils from all the accessions were below the maximum acceptable value of (10 mgEquiv.O_2/Kg) set by Codex Alimentarius Commission for oils (Abayeh *et al*., 1998; Codex Alimentairus Commission, 1993).

The low peroxide values obtained from the okra oils are simply an indication that the oil is less liable to rancidity at room temperature. Again, okra seed oils fall in the range of 1-10 mgEquiv.O_2/Kg stipulated for freshly prepared oils (Bwai *et al*., 2013). On the other hand, according to the Codex Alimentarius Commission, the peroxide value for unrefined olive oil may be as high as 20 meq/kg oil (Gohari Ardabili*et al*., 2011). Therefore, considering that the oil studied was unrefined and its initial quality indicators were within the reported limits, the okra seed oil can be regarded as an edible oil with good quality.

Saponification Value

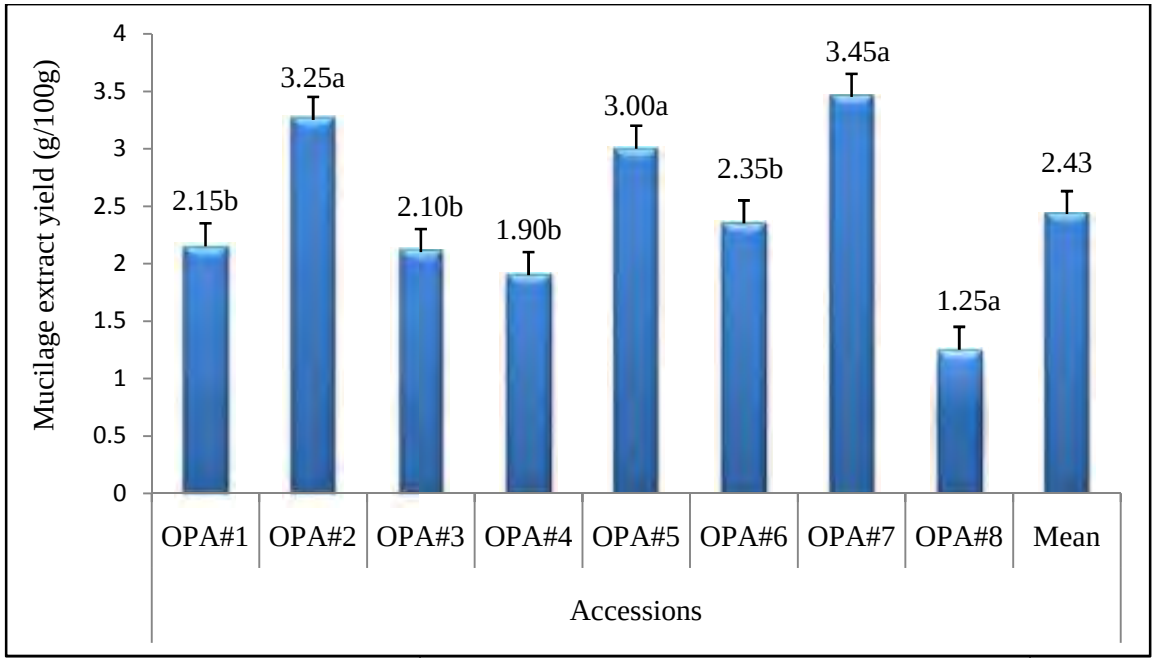
Table 5.1shows the saponification value (mgKOH/g) of eight seed oils of okra accessions. Saponification value of the oils ranged from 188.97 to 194.78 mgKOH/g, which was in agreement with the finding of Nzikou*et al*. (2006) for okra seed oils (180.3 to 191.2); however, there is no significance difference among all the accessions. The mean saponification value (190.94 mgKOH/g) of okra seed oil in this study was higher than the saponification value reported for okra seed by Ogungbenle & Omosola (2015) (182.20 mgKOH/g)andNdangui *et al*. (2010)(183.1 mgKOH/g).

The saponification value obtained during the present study was in agreement with the range reported for common oils such as soybean (189- 195mgKOH/g), peanut (187 - 196 mgKOH/g) and cotton seed oils (189- 198 mgKOH/g) (Codex Alimentairus Commission, 1993).Oils with high saponification values are desirable in the soap making industry (Akanni *et al*., 2005). This suggests the suitability of okra seed oil for industrial soap making since its saponification value falls within the range of oils currently used for the same purpose. Ogungbenle & Omosola (2015) also reported that dry okra seed oils have a potential as an ingredient in the industrial manufacture of soap and cosmetics. These properties make them as useful sources of essential fatty acids required in the body (Akanni *et al*., 2005). Therefore, the higher saponification value of the okra seed oil could be highly useful in the saponification industry and they contain high amounts of higher fatty acids.

5.4.3 Mucilage yields

The mucilage yield from pods of eight different okra accessions is presented in Figure 5.2.The mucilage yield of the pods of okra accessions was ranged from 1.25 to 3.45 g/100g. The mucilage yield of OPA#7 accession was significantly (P<0.05) high, whereas it was low in OPA#8 accession on a dry-weight basis. The variation in mucilage yield was attributed to species type, maturity at harvesting time, effect of drying, genetic factor, the season of collection and topographic variation like rain distribution, temperature, soil type, etc. (Gebresamuel & Gebre-Mariam, 2011).Moreover,Kaewmaneeet *al*. (2014)pointed out that the extraction yield of mucilage can vary as a function of environmental factors, such as the climatic condition and crop age.

The mean mucilage content (2.43 g/100g) of Ethiopian okra is higher than the value reported byAdetuyi & Dada (2014) for Nigerian okra (1.4 g/100g). The mean mucilage yield (2.43 g/100g) of Ethiopian okra is also higher than the yields of mucilage (1.10-2.55%) from leaves of rose cactus reported by Hong & Ibrahim, (2012). The mucilage yield of linseed (3.6-9.4%) reported by Fedeniuk & Biliaderis (1994)is alsohigher than the present finding. Kaewmanee *et al*. (2014) alsoreported the mucilage yield of flaxseed (*Linumusitatissimum*) cultivar that it ranges from 1.80 to 6.65%.



Values not followed by the same letters are significantly (P<0.05) different.

Figure 5.2Mucilage yields from pods of eight okra accessions

5.4.4 Functional properties

Bulk density

Table 5.2 shows the bulk density of mucilage of the pods of eight okra accessions. The bulk density of the mucilage flour ranged from 0.58 to 0.64 g/ml. Bulk density of the mucilage flour of OPA#6 accession was highest (0.64 g/ml) but this was not significantly (P>0.05) different from OPA#3, OPA#8, and OPA#4 accessions, whereas OPA#2 was the lowest (0.58 g/ml) again this was not significantly (P>0.05) different from the rest of the accessions except OPA#6. The mean bulk density (0.60 g/ml) of the mucilage in this finding was lower than the value (0.69 g/ml) reported by Farooq *et al*. (2013) for okra mucilage, which is stated as heavy in nature. The bulk density in the present finding is also lower than the value (0.68-0.69 g/ml) reported by Gebresamuel & Gebre-Mariam (2011)for cactus pears (*Opuntia spp.*) grown in northern Ethiopia.

Water absorption capacity

The result of water absorption capacity of the mucilage of the pods of eight okra accessions is given in Table 5.2. Water absorption capacity of the pod mucilage of okra accessions ranged from 2.45 to 4.60 ml/g. Water absorption capacity of OPA#8 was the highest but this was not significantly (P>0.05) different from OPA#5 and OPA#7 accessions. It was low in OPA#1 and OPA#2. The mean water absorption capacity of mucilage from pods of okra accessions in this study was lower than the water absorption capacity reported by Hong, & Ibrahim, (2012)for rose cactus mucilage (461.87%) but higher than Arabic gum (17.13%).The ability of mucilage to hold water producing gels or highly viscous solution is desirable in industrial application (Simas-Tosin*et al*., 2010).

Oil absorption capacity

The oil absorption capacity of the mucilage of the pods of eight okra accessions is presented in Table 5.2. The oil absorption capacity of the mucilage of the pods of okra accessions ranged from 2.02 to 3.64 ml/g. The oil absorption capacity of the pods of okra accession OPA#5 was the highest but this was not significantly (P>0.05) different from OPA#7. On the other hand, the oil absorption capacity of OPA#1 and OPA#6 was the lowest but this also was not significantly

(P>0.05) different from OPA#3. Oil absorption capacity is of great importance, since fat acts as flavor retainer and also increases soft texture to mouth feel of foods, especially bread and other baked foods (Aremu*et al*., 2006; Akobundu, 2009). A high oil absorption capacity is valuable in ground meat formulations, meat replacers and extenders, doughnuts, pancakes and baked foods (Amandikwa & Chinyere, 2012).

Table 5.2Functional properties of mucilage from pods of okra accessions

Accessions	BD (g/ml)	WAC (ml/g)	OAC (ml/g)	EC (%)	ES (%)	FC (%)	FS (%)
OPA#1	0.59 ± 0.02^{b}	2.70 ± 0.30^{cd}	2.02 ± 0.11^{e}	64.39 ± 3.06^{abc}	59.20 ± 2.34^{ab}	60.41 ± 3.72^{ab}	53.56 ± 0.99^{a}
OPA#2	0.58 ± 0.02^{b}	3.70 ± 0.10^{b}	2.98 ± 0.24^{bc}	42.22 ± 3.24^{e}	39.83 ± 2.54^{c}	52.80 ± 0.27^{ab}	36.04 ± 0.69^{e}
OPA#3	0.61 ± 0.01^{ab}	3.05 ± 0.15^{c}	2.38 ± 0.06^{de}	48.33 ± 6.22^{de}	42.94 ± 4.34^{c}	50.51 ± 0.50^{b}	40.40 ± 1.01^{de}
OPA#4	0.61 ± 0.02^{ab}	3.10 ± 0.10^{c}	2.52 ± 0.10^{d}	64.28 ± 2.40^{abc}	57.87 ± 0.47^{ab}	58.20 ± 0.90^{ab}	46.32 ± 0.87^{b}
OPA#5	0.59 ± 0.01^{b}	4.40 ± 0.10^{a}	3.64 ± 0.11^{a}	60.33 ± 2.64^{bc}	54.71 ± 0.86^{b}	52.39 ± 0.81^{ab}	47.09 ± 0.28^{b}
OPA#6	0.64 ± 0.01^{a}	2.45 ± 0.05^{d}	2.08 ± 0.17^{e}	74.45 ± 2.82^{a}	65.26 ± 2.93^{a}	62.50 ± 0.47^{a}	54.35 ± 2.45^{a}
OPA#7	0.58 ± 0.01^{b}	4.05 ± 0.25^{ab}	3.28 ± 0.04^{ab}	55.35 ± 2.66^{cd}	53.27 ± 3.27^{b}	54.41 ± 0.80^{ab}	45.61 ± 1.28^{bc}
OPA#8	0.61 ± 0.01^{ab}	4.60 ± 0.20^{a}	2.68 ± 0.07^{cd}	68.01 ±2.43^{ab}	56.70 ±0.45^{ab}	54.69 ± 2.61^{ab}	41.18 ± 2.64^{cd}
Mean	0.60	3.51	2.69	59.67	53.72	55.74	45.57

Means not followed by the same superscript letters in each column of the pod and seed are significantly (P<0.05) different. Data are expressed as amean ± standard error of replicate determinations (n=2). **Note:** BD-Bulk density; WAC - Water absorption capacity; OAC - Oil absorption capacity; EC - Emulsifying capacity; ES - Emulsion stability; FC - Foaming capacity; FS - Foam stability.

Emulsifying capacity

The result of the emulsifying capacity of the mucilage of the pods of okra accessions is presented in Table 5.2. The emulsifying capacity of the pod mucilage of okra accessions ranged from 42.22 to 74.45%. The emulsifying capacity of OPA#2 was high but was

not significantly (P>0.05) different from OPA#3 accession. It was low in OPA#6 but this also did not significantly (P>0.05) different OPA#8, OPA#1 and OPA#4 accessions. Protein being the surface active agents can form and stabilize the emulsion by creating electrostaticrepulsion on oil droplet surface (Kaushal *et al*. 2012). The capacity of proteins to enhance the formation and stabilization of emulsion is critical for many applications in food products like chopped and comminuted meat, cake batter, coffee whitener, milk, mayonnaise, salad dressing, and frozen dessert(Elbaloula *et al*., 2014). The mucilage of all the accessions showed relatively good emulsion capacities.

Emulsion stability

Emulsion stability the mucilage of the pods of okra accessionsisshown inTable 5.2. Emulsion stability of the pod mucilage ranged from 42.22 to 74.45%. Emulsion stability of OPA#2 was high but was not significantly (P>0.05) different from OPA#3 accession. It was low in OPA#6 but this also was not significantly (P>0.05) different from OPA#8, OPA#1 and OPA#4 accessions. All the mucilage samples showed a high emulsion stability lasting for 60 min after whipping.The mean emulsion stability of okra mucilage in the present study is lower than the value (80%) reported by Kaewmanee *et al*. (2014)for flax cultivars. The mucilage of the pods of okra accessions showed relatively good emulsion stability. Capitani *et al*., (2013)also reported thatregarding the formulation of stable emulsions, the meal with mucilage is recommended for use given the role of mucilage as a thickening agent.

Foaming capacity

Foaming capacity of mucilage of the pods of okra accessionsis presented inTable 5.2. Foaming capacity of the pod mucilage ranged from 50.51 to 62.50%. OPA#6 recorded the highest foaming capacity and OPA#3 the lowest, but there was no significant difference in the foaming capacity of all the okra accessions studied. The mean foaming capacity (55.74%) of okra mucilage in the present finding was higher than the value (20–25%) reported by Kaewmanee*et al*. (2014)for the mucilage of flax cultivars.

Foam stability

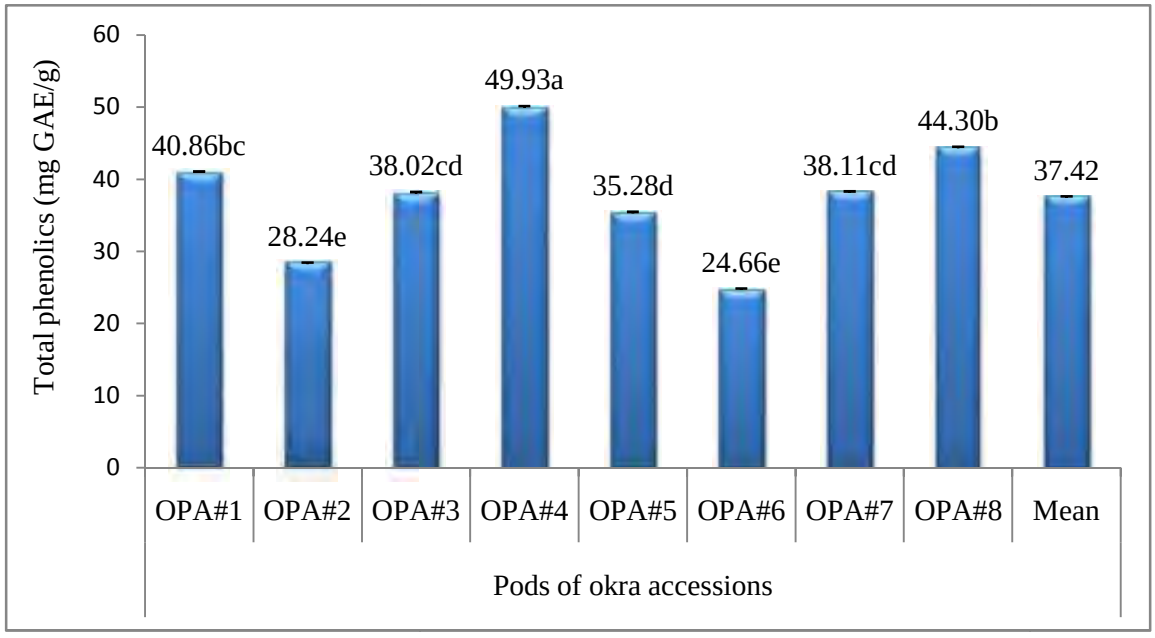
Foam stability of mucilage of the pod accessionsis shown inTable 5.2. Foaming stabilityof the pod mucilage ranged from 36.04 to 54.35%. Foaming stability of OPA#6 and OPA#1 was significantly (P<0.05) high and it was low in OPA#2 but this was not significantly (P>0.05)

different from OPA#3 accession. The ability to form stable foam is an important property in whipped toppings, frozen desserts and sponge cakes (Adelakun *et al*., 2012); thus, the mucilage of the pods of okra accessions particularly OPA#6 and OPA#1 accessions are the best candidate for the food that requires high stable foam.

5.4.5Total phenolics and flavonoids of pod mucilages

Total phenolics

The result of the total phenolic contents of the mucilage of the pods of eight okra accessions is shown inFigure 5.3. Total phenolic contents of the pods of okra mucilage ranged from 24.66 to 49.93 mg GAE/g. The total phenolic content of the pods of accession OPA#4 was significantly ($P<0.05$) high while it was low in OPA#6 and OPA#2. The mean total phenolic content of okra mucilage was 37.42%. Plants rich in phenolics are being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Saeed *et al*., 2012).

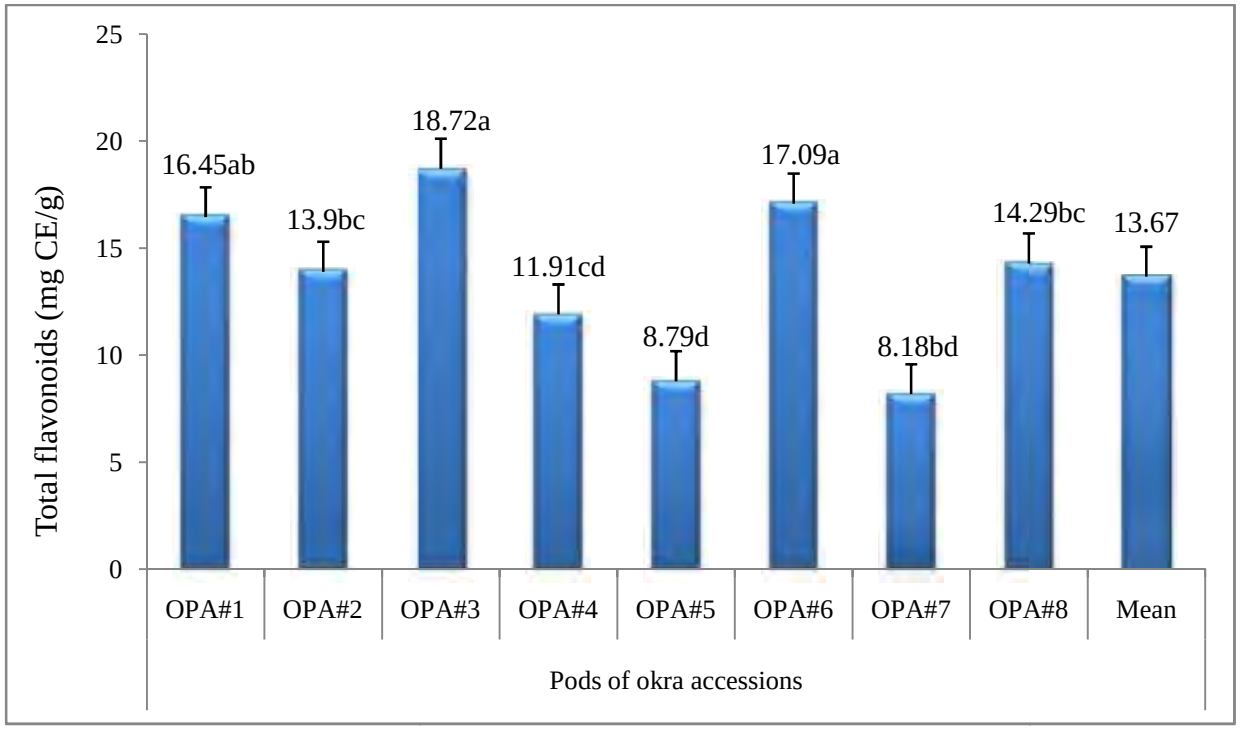


Values not followed by the same letters are significantly ($P<0.05$) different.

Figure 5.3Total phenolics (mg GAE/g) of mucilage from pods of eight okra accessions

Total flavonoids

The result of total flavonoids content of the mucilage of the pods of eight okra accessions is presented inFigure 5.4. Total flavonoid contents of the pods of eight okra mucilage ranged from 8.18 to 18.72 mg CE/g. The pods of OPA#3 accession was high but was not significantly (P>0.05) different from OPA#6 and OPA#1. It was low in OPA#7 but this also was not significantly (P>0.05) different from OPA#5 and OPA#4. The mean total flavonoid content of okra mucilage was 13.67 mg CE/g. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases (Saeed *et al*., 2012).



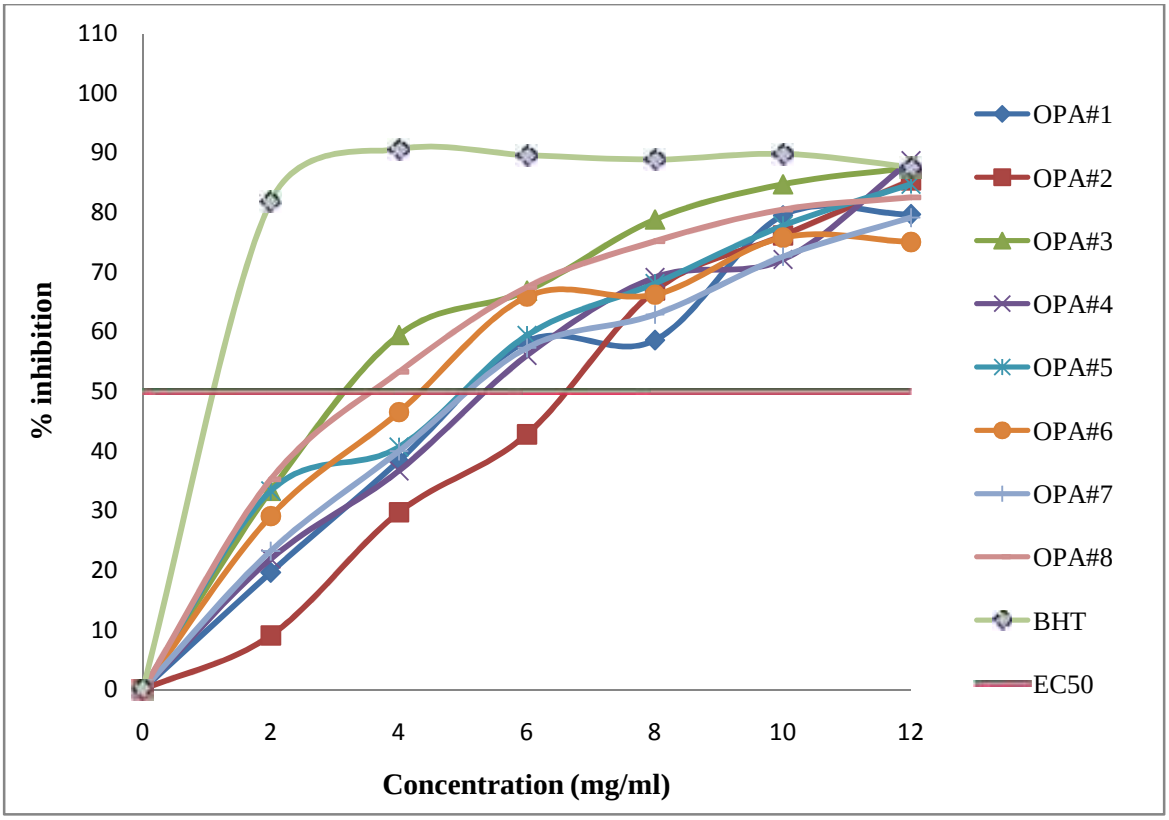
Values not followed by the same letters are significantly (P<0.05) different.

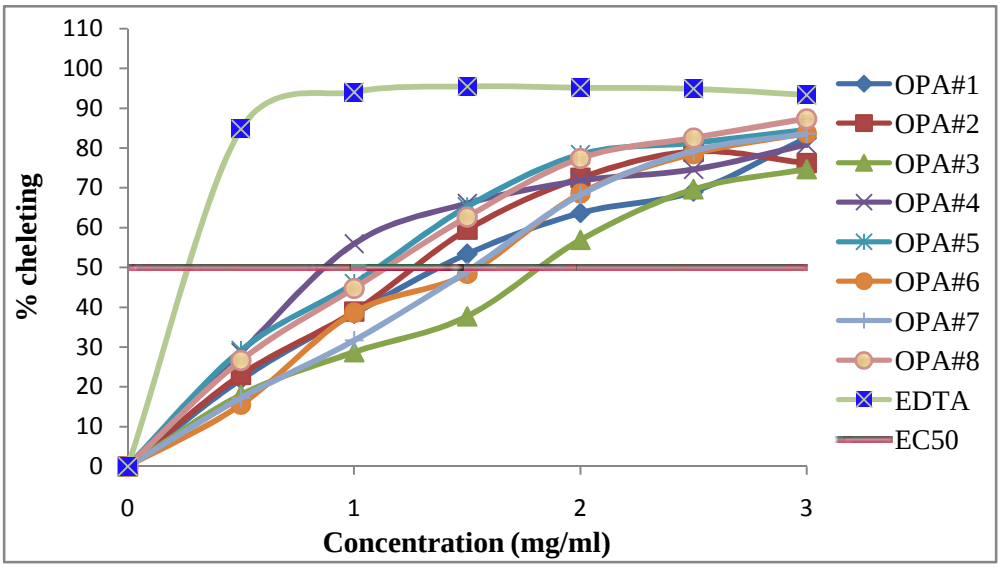
Figure 5.4Total flavonoids (mg CE/g) of mucilage from pods of eight okra accessions

5.4.6 Antioxidant activity assays

DPPH scavenging activity

The result of concentration response curves of DPPH scavenging activity of the mucilage of the pods of eight okra extracts with positive controls is shown in Figure 5.5. The synthetic antioxidant of Butylated hydroxytoluene (BHT) was used as a positive control using the same concentration. The percentage inhibition of DPPH scavenging activities of mucilage of the pods


110
100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
2
4
6
8
10
12
Concentration (mg/ml)
OPA#1
OPA#2
OPA#3
OPA#4
OPA#5
OPA#6
OPA#7
OPA#8
BHT
EC50



Accessions	DPPH Scavenging (EC_{50}[a])	Chelating Effect (EC_{50}[b])
OPA#1	5.05	1.38
OPA#2	6.60	1.27
OPA#3	3.15	1.85
OPA#4	5.28	1.10
OPA#5	5.00	1.15
OPA#6	4.35	1.60
OPA#7	5.10	1.55
OPA#8	3.55	1.20
Mean	4.76	1.39
BHT	1.10	-

EDTA	-	0.87

[a]EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged.

[b]EC_{50} (mg/ml): effective concentration at which 50% Fe^{2+}/ferrozine complex are inhibited.

The result of this study revealed that the mucilage of the pods of okra accession OPA#3 and OPA#8 had a better DPPH scavenging ability with low EC_{50} values while OPA#2 had low DPPH scavenging abilitywith high EC_{50} value. The mucilage of the pods of accession OPA#4 and OPA#5 had a relatively high metal chelating effect. The synthetic antioxidant (BHT and EDTA), which was used as a positive control, had a superior performance with the least EC_{50} in all the assays, which indicate that the pod accessions of okra mucilage had low antioxidant activities.

5.5 Conclusion

The crude oil yield of the seeds of eight okra accessions varied significantly ($P<0.05$) and ranged from 19.25-38.19%. Compared with other vegetable oils, the present study revealed that okra seeds could be considered as potential sources of edible oil specifically the seed of OPA#2 accessions. The results of the present study also showed that okra seed oils contained appreciable physicochemical characteristics which suggest that this oil has high edibility quality and could be useful for industrial applications. This studyrevealed thatthe pods of okra accessions contain a desirable amount of mucilage contents and is potential sources of natural antioxidants. The study also revealed that the mucilage of the pods of okra accessions was found to exhibit good functional properties and can offer a great potential in various food systems. Particularly, mucilage of the pods of OPA#5 and OPA#7 had desirable water and oil absorption capacities, while mucilage of accession OPA#1 and OPA6 had high emulsifying and foaming properties. In general, this study could be used as baseline data to develop okra seed oil for both domestic and industrial purposes and also for promotion and cultivation of this vegetable in a sustainable manner.In order to encourage the use of mucilage from pods of okra accessions, more research has to be conducted on its extraction optimization.

Chapter 6

Effect of Traditional Processing on Nutritional, Antioxidant and Functional Properties of the Pods and Seeds of Selected Okra (*Abelmoschus esculentus*) Grown in Benishangul Gumuz Region, Ethiopia

6.1 Abstract

The effect of traditional processing methods on nutritional, antioxidant and functional properties of pods and seeds of selected Ethiopian okra were investigated for the first time in order to explore their potential uses. The processing methods adopted were boiling and sundrying for pods and boiling, soaking, germination and roasting for seeds of okra. Boiling caused a significant ($P<0.05$) decrease in the crude protein, crude fat, crude ash, zinc and total flavonoid content of the pods by 14.22, 30.92, 23.54, 10.94 & 2019%, respectively and increased the total phenolic content of the pods by 4.80%, while it significantly ($P<0.05$) decrease the crude protein, crude fat, zinc and total flavonoid contents of the seeds by 9.29, 25.91, 26.48 and 7.30%, respectively. Sun drying significantly ($P<0.05$) decreased the crude protein and crude fat contents of the pods by 7.53 and 34.94%, respectively and increased the crude fibre and ash content of the pods by 27.90 and 20.44%, respectively. Soaking also significantly ($P<0.05$) decreased the crude protein, crudefibre, zinc and total phenolic content of the seeds by 3.68, 21.22, 9.03 and 21.03%, respectively. Germination significantly ($P<0.05$) increased the crude protein, crudefibre, total phenolic and total flavonoid content by 3.68, 16.25, 17.32 and 15.12%, respectively and it decreased the crude fat and zinc content of seeds by 17.65 and 34.89%, respectively. Roasting caused a significant ($P<0.05$) decrease in the crude protein, crude fat and crude fibre content by 23.97, 31.49 and 50.86%, respectively and it increased the ash, total phenolic and total flavonoid content by 15.34, 14.89 and 14.12%, respectively. All processing methods resulted in increase in the water and oil absorption capacity and decrease in the bulk density, emulsion capacity and stability and foaming capacity and stability. In general, germination and roasting increasedthe total phenolic and flavonoid content and enhanced DPPH scavenging and chelating properties of okra accession. The flour of raw and processed pods and seeds of okra was found to exhibit good functional properties and can offer a great potential in various food systems.

Keywords:Okra; Pods; Seeds; Processing methods; Nutritional; Antioxidant; functional

6.2 Introduction

Okra (*Abelmoschus esculentus*) is an important vegetable crop grown in tropical and sub-tropical parts of the world (Saifullah & Rabbani, 2009). Okra is a multipurpose crop due to its various uses of the fresh fruits (pods), seeds, and leaves (Yonas*et al*., 2014). Okra is a major traditional food vegetable for Berta community (one of the ethnic groups in Benishangul Gumuz region, Ethiopia) and could be consumed as both fruit and vegetable (Dandena, 2010).

Most vegetables including okra are commonly cooked before being consumed (Adetuyi& Stella, 2012). In addition, like other fruits and vegetables, okra is perishable and seasonal plants foods which is subjected to postharvest losses. Therefore, traditionally, the pods of okra are sundried in order to extend their shelf life for consumers (Tsado, 2015).This is true in that in Benishangul Gumuz, the community always sell both raw and sundried okra pods in the Assosa market (Appendix 6.1).

Immature okra fruits (pods), which are consumed as vegetables, can be used in salads, soups, and stews, fresh or dried, fried or boiled (Ndunguru & Rajabu, 2004). The seeds of mature okra fruits are reported to be roasted, ground and used as a coffee substitute in Turkey (Calisir *et al*., 2005). Roasting is reported to improve flavor (Akingbala *et al*., 2003). Traditionally, some forms of processing methods such as boiling, sundrying, soaking, germination, and roasting are applied to okra before consumption. Such processing methods can have both detrimental and beneficial effect on the nutritional, antioxidant and functional properties of food. Presumed purpose of such processing is to make okra more palatable and digestible. Even though such traditional processing methods may result in improvements of some nutritional values of okra, nutrients and antioxidants may be lost (Oghbaei & Prakash, 2016).

On the other hand, an acquisition of an understanding of the functional properties of food and its processing effect may demonstrate its further utilization and potential uses in the food system. However, there is no published information available on the effect of traditional processing methods on nutritional, antioxidant and functional properties of Ethiopian indigenous okra pod and seed. Therefore, it is imperative to investigate the effect of traditional processing methods on nutritional, antioxidant and functional properties of pods and seeds of selected okra accession grown in Benishangul Gumuz region, Ethiopia.

6.3 Materials and Methods

6.3.1 Selection of accession

In the third and fifth chapters, it was observed that pods and seeds of the okra accession OPA#6 contained relatively high proximate, mineral and antioxidant contents as compared to the rest okra accessions. Based on this information, the pods and seeds of OPA#6 were selected and further evaluated for the effect of traditional processing methods on nutritional, antioxidant and functional properties. Pods in this chapter refer to the pods of OPA#6 and the seeds refer to OPA#6 of the seed accession. The selected pod and seed was shown in Figure 6.2.

6.3.2 Sample preparation

The traditional processing methods utilized in this study are shown in Figure 6.1.

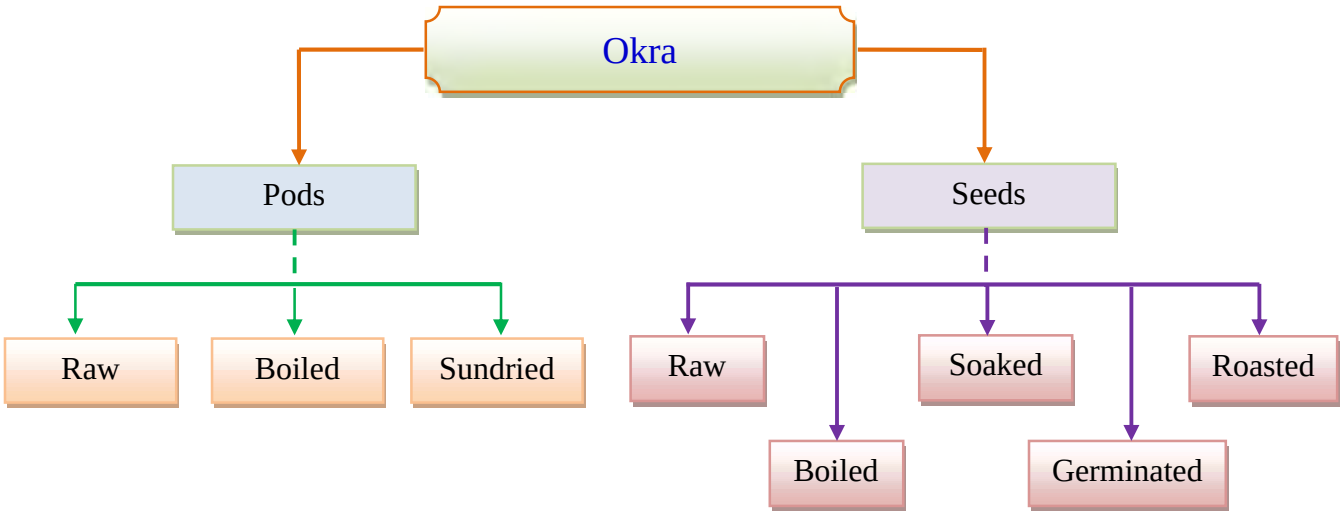


Figure 6.1 Edible parts of okra and type of traditional processing methods

6.3.2.1 Raw okra

The pods and seeds of raw (unprocessed) okra were collected and prepared according to the detailed procedure described in chapter 3under section 3.3.4. The pods and seeds of raw okra are shown in Figure 6.2.

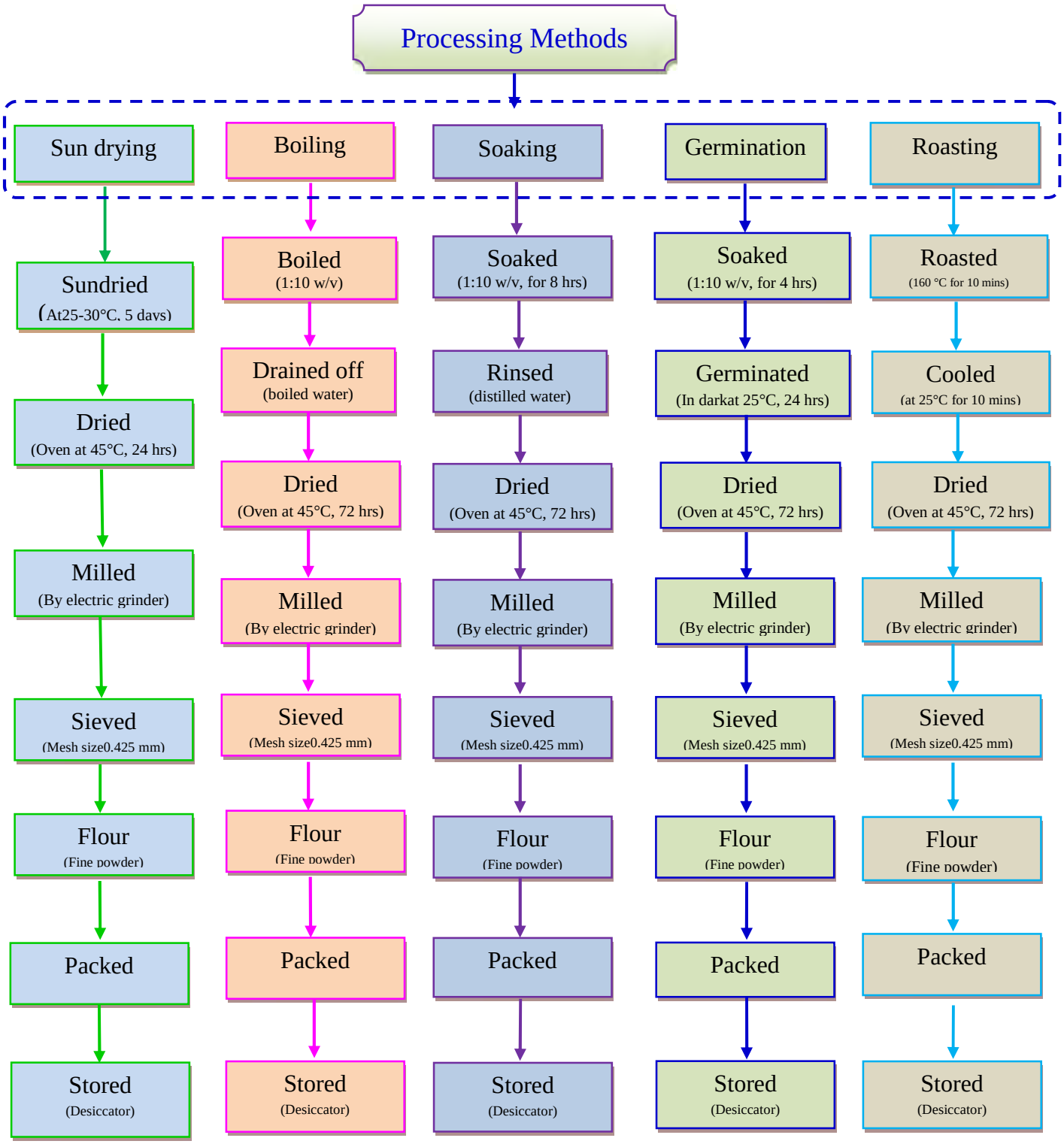


Figure 6.8Preparation of okra flour by traditional processing methods

6.3.3 Determination of proximate and mineral compositions

The proximate and mineral composition of processed pods and seeds of the selected okra accession was conducted according to the procedure described in chapter 3 under section 3.3.5and3.3.6,respectively.

6.3.4 Determination of antioxidant properties

The total phenolic, total flavonoid, DPPH scavenging and metal chelating of the processed pods and seeds of okra accessions determined according to the method described in chapter 5section 5.3.3.1; 5.3.3.2; 5.3.4.1 and 5.3.4.2, respectively.

6.3.5 Determination of functional properties

Determination of bulk density

The bulk density of the raw and processed flour was determined according to the method described by Butt & Batool (2010). About ten grams of the sample was put into 100 ml graduated cylinder and the cylinder was tapped several times (minimum 10 times) on the laboratory bench for the sample to settle, become compact and to eliminate air pockets. Bulk density was calculated as weight of flour sample (g) divided by its volume (ml) using the following formula:

$$\text{Bulk density (g/ml)} = \frac{\text{Wieght of sample (g)}}{\text{Volume of sample (ml)}}$$

Determination of water and oil absorption capacity

Water and oil absorption capacity of mucilage powder was determined according to the procedure described in chapter 5section 5.3.4.2.

Determination of emulsifying properties

Foaming properties including foaming capacity and stability of mucilage powder were determined according to the procedure described in chapter 5 section 5.3.4.4.

Determination of foaming properties

Foaming properties including foaming capacity and stability of the flour samples were determined according to Aremu *et al.* (2007). One gram of the sample was dispersed in 50 ml of distilled water. The resulting solution was vigorously whipped for 30 min in a kenwood blender

and poured into a 100 ml graduated cylinder. The volume before and after whipping was recorded and the foaming capacity was calculated as percentage volume increase. Foaming stability was determined as the volume of foam that was remained after 8 hours and was expressed as a percentage of the initial volume**.** The foaming capacity and stability were calculated by using the following equation:

$$\text{Foaming capacity (\%)} = \frac{V2 - V1}{V1} \times 100$$ Where $V1$: Volume of initial solution, $V2$: Volume of final after mixing

$$\text{Foaming stability (\%)} = \frac{VR2 - V1}{V1} \times 100$$ Where $V1$: Volume of initial solution, $VR2$: Foam volume remained after 8 hours

6.3.6 Statistical analysis

The Completely Randomized Design (CRD) was used with two replicates. All the statistical analyses were performed for the result obtained using SPSS version 20.0 for windows. Data were evaluated by using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means and the result was reported as a mean ± standard error (SE). A p-value of 0.05 or less ($P \leq 0.05$) was considered as statistically significant.

6.4 Result and Discussion

6.4.1 Effect of processing on the proximate composition of the pods and seeds

Food processing may affect the nutritional quality of the food products (Chukwuma *et al*., 2016). Proximate composition of raw and processed pods and seeds of okra is shown in Table 7.1.

Moisture content

The result of the moisture content of the raw and processed pods and seeds of okra is presented in Table 6.1.The moisture content of the pods of okra was 9.69, 7.39 and 15.19 g/100g for raw, sun-dried and boiled, respectively while in the seed of okra, it was 11.30, 15.19, 11.22, 10.02 and 7.31 g/100g for raw, boiled, soaked, germinated and roasted, respectively. The moisture content of soaked seeds of okra was not significantly ($P>0.05$)differentfrom that of the raw seeds of okra.

Sun drying of the pods and roasting of the seeds significantly($P<0.05$) decreased the moisture content by23.74and35.31%,respectively. But boiling significantly ($P<0.05$) increased the moisture content of the pods and seeds by56.76and34.42%, respectively (Figure 6.9). This result

was in agreement with those reported by Adegoke *et al*. (2004) who stated that roasting decreased moisture content of peanut. Mariod *et al*. (2012)also reported thatthe roasting temperature and time are the main factors affecting the moisture content. The lower moisture content of sun dried pods is also in agreement with the results reported by Ukegbu & Okereke (2013) on African spinach and okra species. The increase in the moisture content of boiled pods and seeds of okra might be due to the water absorption capacity of fibres and other natural chemical components during heating (García-Arias*et al*., 2003; Lola, 2009; El Sohaimy, 2013).

According to Kolawole *et al*.(2011), food substances, especially vegetable crops, with high moisture content are subjected to the growth of microorganisms, moisture content higher than 15% is said to promote enzymatic reactions leading to loss of vitamins. In this study, sundried pod and roasted seed contain the least moisture contents and this will favor the preventive properties of the flour against microbial attack.

Crude protein

Crude protein content of the raw and processed okra pods and seeds is shown in Table 6.1. The crude protein contents of raw, sun-dried and boiled pods were 26.16, 22.44 and 24.19 g/100g, respectively and it was 38.09, 34.55, 37.07, 40.49 and 28.96 g/100g for raw, boiled, soaked, germinated and roasted seed samples, respectively. Crude protein contents significantly ($P<0.05$) decreased in the boiled and sun-dried pods by 14.22 and 7.53%,respectively (Figure 6.9).

Boiling, soaking, and roasting significantly ($P<0.05$) decreased the crude protein content of the okra seeds by9.29, 3.68 and 23.97%,respectively(Figure 6.9).In line with this, Jayewardenapura,(2000) stated that the reduction of crude protein during boiling may be attributed to leaching and denaturation of the protein caused by boiling. Onyango *et al*. (2004)also reported that thereduction in the protein content of maize varieties after roasting could be attributed to the denaturation and loss of protein due to the participation of amino acids in Maillard reactions as a result of heating.

Heating cause's reaction between reducing sugar aldehyde groups and a free amino group of amino acids sometimes producing insoluble brown polymers called melanoidins(Oboh, 2006).Therefore, reduction of thecrude protein content of the pod and seed in this finding might be due to protein denaturation during boiling and roasting. However, the decreasing trends of the

pods and seeds in this finding is in contrast to the finding ofOlanipekun*et al*. (2015), who reported that protein values of the flour from processed kidney bean seeds were significantly higher ($p<0.05$) than that of the raw and the author stated that the increase in the protein value of the processed kidney bean seeds may be due to break down of crude protein to amino acids during processing (Oboh, 2006). It has been earlier reported that when food is subjected to roasting, the activity of proteolytic enzymes is increased (Mbah *et al*., 2012),which hydrolyze inherent proteins to their constituent amino acids and peptides.

The germinated okra seeds were significantly ($P<0.05$) increased by 3.68% compared to the raw seeds (Figure 6.9).This increase might be due to the reduction of seed nitrates into protein or ammonium compound (Hooda & Jood 2003). The increase in protein content of germinated

seeds might be attributed to enzymatic synthesis of protein, which is in agreement with the findings of Mathur & Chaudhary (2009). The result of this study shows that the increase in the protein value of the germinated seeds could make okra a good and cheap source of dietary protein, where animal proteins are presently highly unaffordable.

Table 6.1 Effect of traditional processing methods on proximate composition (g/100g, dwb) of okra pods and seeds

Treatments		Moisture content	Crude protein	Crude fat	Crude fibre	Crude ash	Utilisable carbohydrate	Gross Energy (Kcal/100g)
Pods	**Raw**	9.69 ± 0.29^{b}	26.16 ± 0.12^{a}	2.49 ± 0.28^{a}	11.97 ± 0.83^{b}	11.30 ± 0.19^{ab}	38.41 ± 0.56^{a}	280.63 ± 4.29^{a}
	Boiling	15.19 ± 0.69^{a}	22.44 ± 0.47^{c}	1.72 ± 0.01^{b}	14.05 ± 0.41^{ab}	8.64 ± 0.97^{b}	37.97 ± 0.23^{a}	257.05 ± 2.82^{b}
	Sun drying	7.39 ± 0.41^{c}	24.19 ± 0.16^{b}	1.62 ± 0.01^{b}	15.31 ± 0.83^{a}	13.61 ± 0.92^{a}	37.90 ± 0.89^{a}	262.85 ± 2.99^{ab}
Seeds	**Raw**	11.30 ± 0.30^{b}	38.09 ± 0.31^{b}	22.77 ± 0.30^{a}	5.23 ± 0.68^{b}	3.92 ± 0.55^{b}	18.69 ± 2.14^{b}	432.09 ± 6.57^{a}
	Boiling	15.19 ± 0.69^{a}	34.55 ± 0.16^{c}	16.87 ± 0.67^{b}	6.64 ± 0.11^{ab}	3.65 ± 0.27^{b}	23.12 ± 0.24^{b}	382.49 ± 7.57^{b}
	Soaking	11.22 ± 0.07^{b}	37.07 ± 0.85^{b}	20.84 ± 1.81^{ab}	4.12 ± 0.02^{c}	5.00 ± 0.49^{ab}	21.76 ± 2.26^{b}	422.88 ± 10.64^{a}
	Germination	10.02 ± 0.62^{b}	40.49 ± 0.02^{a}	18.75 ± 1.18^{b}	6.08 ± 1.03^{a}	4.53 ± 0.01^{ab}	21.14 ± 0.76^{b}	411.25 ± 7.50^{a}
	Roasting	7.31 ± 0.49^{c}	28.96 ± 0.73^{d}	15.60 ± 2.20^{b}	2.57 ± 0.21^{c}	6.05 ± 0.61^{a}	39.52 ± 2.37^{a}	414.26 ± 7.43^{a}

Means not followed by the same superscript letters in each column of the pods and seeds are significantly ($P<0.05$) different from each other. Data are expressed as a mean ± standard error of replicate determinations (n=2).

Crude fat

Table 6.1showsthe crude fat content of raw and processed pods and seeds of okra. The crude fat contents of raw, sun-dried and boiled pod sample were 2.49, 1.72 and 1.62 g/100g, respectively and were 22.77, 16.87, 20.84, 18.75 and 15.60 g/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. The crude fat content of soaked okra seeds was not significantly ($P>0.05$) different from the raw seeds while boiled, germinated and roasted seed was significantly ($P<0.05$) decreasedby25.91, 17.65 and 31.49%,respectively when compared to the raw seeds (Figure 6.9).

the raw seeds while boiled, germinated and roasted seed was significantly (P<0.05) decreased by25.91, 17.65 and 31.49%,respectively when compared to the raw seeds (Figure 6.9).

Loss of fat during germination may be due to its consumption as an energy source in the process of germination (Pandey & Awasthi, 2015),which require energy to proceed. The effect of roasting upon the fat content of the beans is to reduce its actual weight with the shrinkage. Some of the more volatile fatty acids are driven off, and the fats break down to give a larger percentage of free fatty acids, some light esters, acrolein, and formic acid. The fat will come to the surface, through the breaking of the fat cells, with a certain alteration in the chemical nature of the fat. Reduction in fat content upon roasting may be due to loss of volatile oils on open dry heat treatment (Mathur & Chaudhary, 2009). This decreased trend is in agreement with the value reported by Kiin-Kabari & Akusu (2014), who stated that roasting decreased the oil content of the flour; this may probably be due to volatilization or melting out of the fat as earlier observed by Kiin-Kabari & Akusu (2014).

Akingbala *et al.* (2003)reported that the fat content of the okra seed was reduced significantly from 31.04% in untreated okra seed flour to 17.22% after 40 min of roasting. The decrease might be due to the fact that direct heat helps to separate out oil from the cells of nuts and oil seed and subsequent removal during milling (Mohini & Eram, 2005).However, such decreasing trend is in contrast to the finding reported by Oboh *et al.* (2010),who stated that roasting will increase significantly the crude fat content of yellow and white maize varieties;which may be associated with heat-induced break down of the bonds that exist between the fat and matrix of the maize, resulting in efficient release/mobilisation of the oil reserve in the maize grain after roasting.

The crude fat content of okra pods in this finding was also significantly(P<0.05)decreased during boiling by 30.92%and during sundrying by34.94%compared to raw pods (Figure 6.9). The decrease in the fat content of the vegetable that was subjected to boiling in a water could be attributed to the fact that some of the fats may have leached into the boiling water (Ali, *et al*, 2010).On the other hand, this decreasing trend in this finding is also in contrast with the value reported by Adeniyan *et al.* (2013),who stated that boiling significantly increased the crude fat contentof beniseed, that could be attributed to the disruption of the cell structures and membrane partitions of the seeds by heat during boiling causing the fat to melt and be easily released from the seeds.

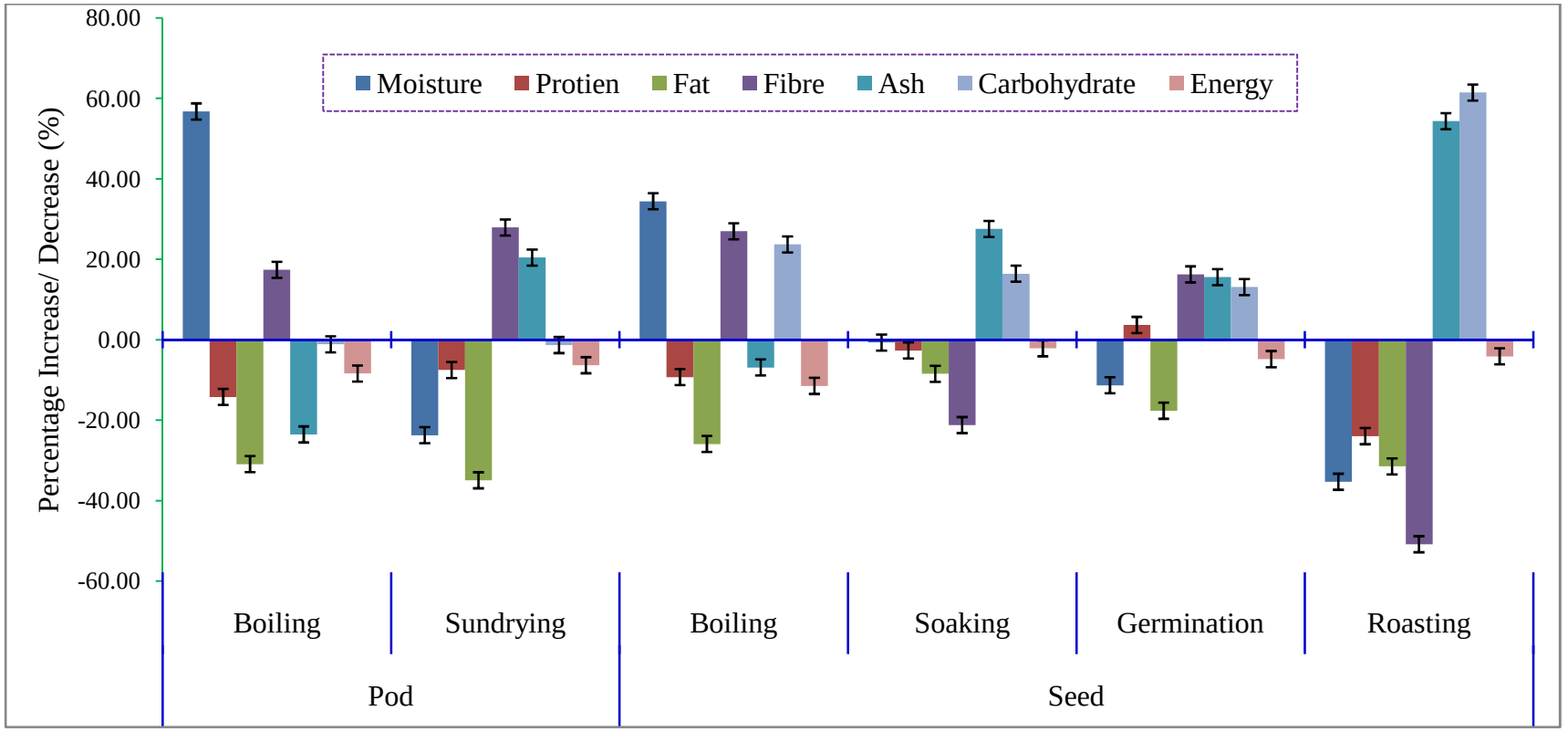
Crude fibre

Table 6.1showsthe result of crude fibre content of raw and processed okra pod and seed samples. Crude fibre contents of okra pods were 11.97, 14.05 and 15.31 g/100g for raw, sundried and boiled, respectively and were 5.23, 6.64, 4.12, 6.08 and 2.57 g/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. The crude fibre content of germinated seeds was not significantly (P>0.05) different from that of the raw okra seeds. Simiraly, crude fibre content of boiled okra pods and seeds was not significantly (P>0.05) different when compared to raw pods and seeds, respectively. However, this result was in disagreement with findings of Mepba *et al*. (2007), who reported that boiling decreased the crude fibre in some Nigerian edible vegetables due to the thermal degradation of fibre or leaching of thesolublefibre during theboiling process.

Crude fibrecontent was significantly (P<0.05) decreased in soaked seeds by21.22%andincreased in germinated seeds by16.25%compared to raw seeds (Figure 6.9).This decrease in the fibre content of soaked seeds might be attributed to enzymatic degradation of seeds during soaking(Mathur & Chaudhary 2009). The increase in crude fibre content upon germination might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination(Pandey & Awasthi, 2015).Crude fibre content was also significantly (P<0.05) increased by 27.90% during sundrying of the pods anddecreased in roasted seeds by50.86%compared to raw okra pods and seeds, respectively(Figure 6.9).

Reduction in crude fibre content after roasting probably might be due to retrogradation of starch during roasting(Mathur & Chaudhary, 2009). Obohe*t al*. (2010) also reported that the structural alteration in the cell wall structure as a result of increased roasting temperature may lead to breakage of weak bonds between polysaccharide chains and glycosidic linkages in thefibre . A decreased association between fibre molecules and ⁄ or depolymerisation of the fibre results in solubilisation, hence, the observed decrease in crude fibre after roasting. In contrast to this finding, Akingbala *et al*. (2003) reported that the fibre content of okra seeds pretreated by roasting had been reported to increase compared with the untreated one. The thick hull of the matured okra seeds consists mainly of fibre.

The release of fat during roasting makes the cotyledon sticky and the separation of cotyledon from the hull more difficult thereby contributing to the increase in fibre content (Adelakun *et al*., 2009). Crude fibre is an indication of the roughage/bulkiness of the sample and its presence in


80.00
60.00
40.00
20.00
0.00
-20.00
-40.00
-60.00
Percentage Increase/ Decrease (%)
Moisture
Protien
Fat
Fibre
Ash
Carbohydrate
Energy
Boiling
Sundrying
Boiling
Soaking
Germination
Roasting
Pod
Seed

Crude Ash

The result of crude ash contents of raw and processed okra pods and seeds is given in Table 6.1.Crude ash content of the raw, sun-dried and boiled pods were 11.30, 8.64 and 13.61 g/100g, respectively and were 3.92, 3.65, 5.00, 4.53 and 6.05 g/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Crude ash content of the pods was decreased during boiling by 23.54% and increased during sundrying by 20.44% but this was not significantly ($P>0.05$) different from that of raw okra pods(Figure 6.9).

Crude ash contents of boiled, soaked and germinated seed was alsonot significantly ($P>0.05$) different from raw okra seeds. However, the crude ash content of roasted seeds was significantly ($P<0.05$) increased by 54.34% when compared to raw okra seeds (Figure 6.9). The reduction of crude ash content may be due to the leaching of minerals into the cooking/boiling water (D'souza, 2013)andwater absorption during the boiling (Lewu *etal*., 2009).

Utilizable carbohydrate

Table 6.1 showsthe result of utilizable carbohydrate contents of raw and processed pods and seeds of okra. The utilizable carbohydrate contents were 38.41, 37.97 and 37.90 g/100g for raw, sun-dried and boiled okra pods, respectively and were 18.69, 23.12, 21.76, 21.14 and 39.52 g/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Utilizable carbohydrate contents of boiled and sundried okra pods and boiled, soaked and germinated okra seeds were not significantly ($P>0.05$) different from raw okra pods and seeds, respectively. However, the utilizable carbohydrate content of roasted seeds was significantly ($P<0.05$)increased by 61.45% (Figure 6.9). Since utilizable carbohydrate content is calculated by difference, decreased moisture, fibre, protein and ash contents of the seeds after roasting will ultimately affect the value of carbohydrate content.

In addition, the observed decrease in utilizable carbohydrate contents of the germinated seeds could be attributed to their utilization in the sprouting process as energy sources. The increase in respiration rate during germination brings about the release of energy from the breakdown of carbon compounds. Germination changes the stored insoluble nutrients in the cotyledons to soluble nutrients through the hydrolysis of macromolecules (Enujiugha *et al*., 2003).

Gross energy

The result of the gross energy content of raw and processed okra pods and seeds is presented in Table 6.1. Gross energy contents of the raw, sun-dried and boiled pods were 280.63, 257.05 and 262.85 g/100g, respectively and were 432.09, 382.49, 422.88, 411.25 and 414.26 g/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Gross energy contents of sun-dried pods and soaked germinated and roasted okra seeds were not significantly ($P>0.05$) different from that of raw okra pods and seeds, respectively. However, the gross energy contents of boiled okra pods and seeds were significantly ($P<0.05$)increased by 8.40 and 11.48%, respectively (Figure 6.9).

6.4.2 Effect of processing on the mineral content of the pod and seed

The mineral analysis is essential to guarantee the quality of any food product (Mariod *et al*., 2012). The result of mineral contents of the raw and processed okra pods and seeds is presented in Table 6.2.

Calcium

The result of the calcium content of raw and processed okra pods and seeds is given in Table 6.2.Calcium contents of the raw, sun-dried and boiled of the pod were311.35, 305.70 and 307.80 mg/100g, respectively and were 89.89, 85.90, 89.42, 91.64and 89.80mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Calcium contents of all the processed pods and seeds was not significantly ($P>0.05$) different from that of raw okra pods and seeds, respectively (Table 6.2). Calcium is the major component of bone and assists in teeth development. Calcium concentrations are also necessary for blood coagulation and for the integrity of intracellular cement substances (Okaka & Okaka, 2001).

Table 6.2 Effect of traditional processing on mineral composition (mg/100g, dwb) of okra pods and seeds

Edible part	Treatments	Calcium	Iron	Zinc	Phosphorous	Potassium	Sodium
Pod	Raw	311.35± 0.27^{a}	31.90± 1.12^{a}	6.31± 0.19^{a}	36.32± 0.24^{a}	263.12± 1.10^{a}	6.06± 0.57^{a}
	Boiling	305.70 ±0.55^{a}	25.43±1.36^{a}	5.62± 0.02^{b}	31.89± 0.21^{a}	257.91± 1.50^{a}	1.68± 0.56^{b}
	Sun drying	307.80 ±2.29^{a}	28.85± 1.40^{a}	6.30± 0.05^{a}	32.72± 0.96^{a}	261.71± 1.00^{a}	5.01± 0.56^{a}
Seed	Raw	89.89± 0.45^{ab}	8.33± 0.18^{a}	6.42± 0.20^{a}	1048.21± 1.28^{a}	90.00± 1.15^{a}	17.40± 0.62^{a}
	Boiling	85.90 ± 2.17^{b}	7.66± 0.20^{a}	4.72± 0.00^{c}	1041.03± 4.37^{a}	87.52± 1.42^{a}	16.11± 0.57^{a}
	Soaking	89.42± 0.62^{ab}	7.39± 0.13^{a}	5.84± 0.09^{b}	1049.64± 3.25^{a}	83.59± 1.80^{a}	15.41± 0.57^{a}
	Germination	91.64± 0.29^{a}	7.13± 0.99a	4.18± 0.11^{d}	1048.42± 0.54^{a}	82.67± 1.89^{a}	14.88± 0.53^{a}
	Roasting	89.80±0.45^{ab}	8.72± 0.21^{a}	6.20± 0.00^{ab}	1037.65± 1.08^{a}	86.38± 3.14^{a}	15.23± 1.73^{a}

Means not followed by the same superscript letters in each column of the pod and seed are significantly (P<0.05) different from each other. Data are expressed as a mean ± standard error of replicate determinations (n=2).

Iron

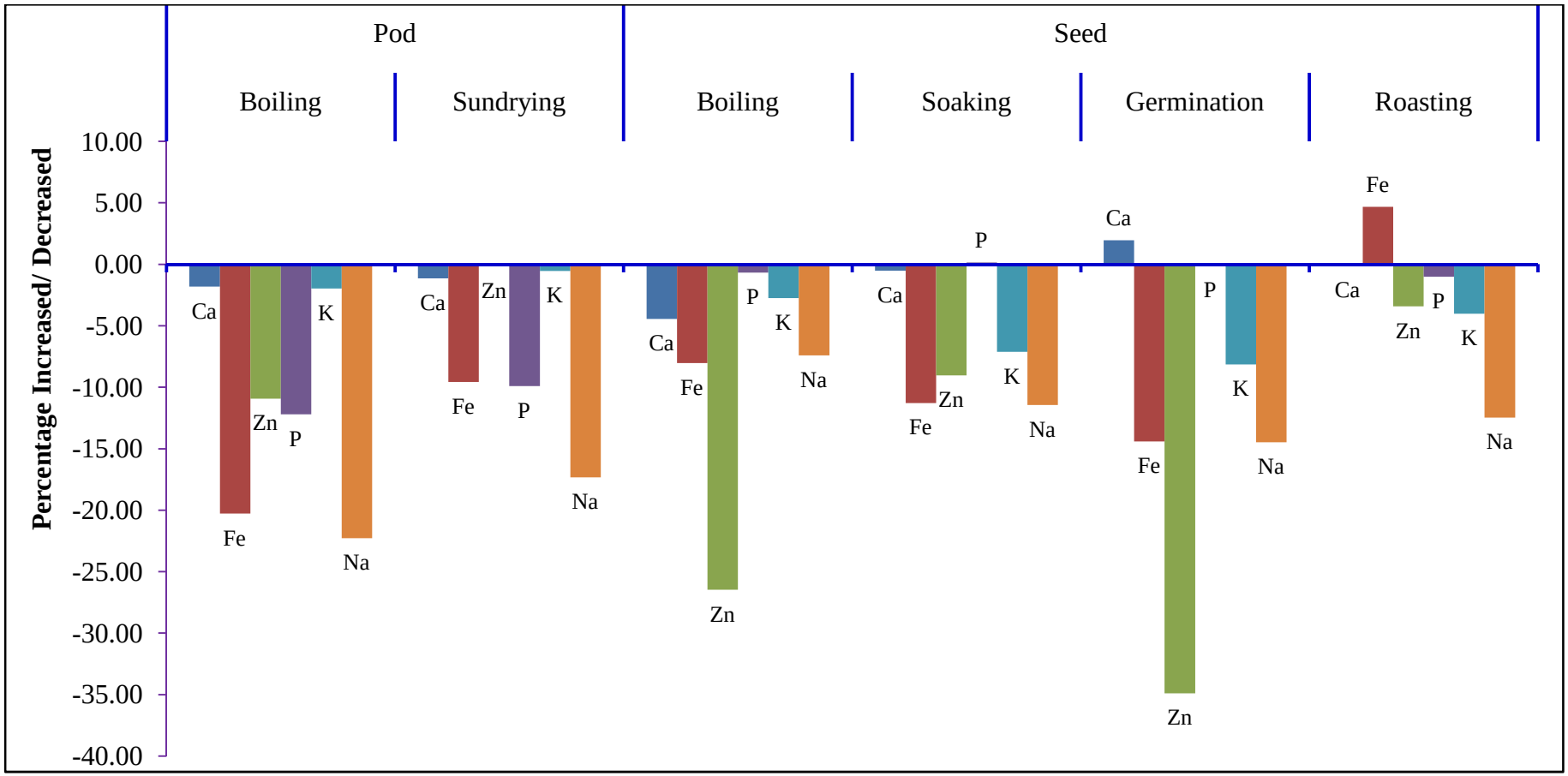
Table 6.2shows the result of iron contents of raw and processed okra pods and seeds.The iron content of the raw, sun-dried and boiled pods were 31.90, 25.43 and 28.85mg/100g, respectively and were 8.33, 7.66, 7.39, 7.13and 8.72mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Similar to the calcium content, the iron contents of all the processed okra pods and seeds was not significantly (P>0.05) different from raw okra pods and seeds, respectively (Table 6.2). Minerals are not destroyed by exposure to heat(Amarowicz *et al*., 2009). Iron is involved in many vital functions in the human body in carrying of oxygen as a critical component of the hemoprotein, hemoglobin, myoglobin, and cytochromes and it is also a co-factor for some enzymes (Walker *et al*., 2007).

Zinc

The result of zinc contents of raw and processed okra pods and seeds is presented in Table 6.2.The zinc contents of the raw, sun-dried and boiled pods were 6.31, 5.62 and 6.30mg/100g, respectively and were 6.42, 4.72, 5.84, 4.18and 6.20mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Zinc content of the boiled pods was significantly ($P<0.05$) decreased by 10.94% as compared to raw okra pods. On the other hand boiling, soaking and germination of okra seeds significantly ($P<0.05$) decreased the zinc content of okra seeds by 26.48, 9.03 and 34.89%, respectively (Figure 6.10). The loss of zinc during boiling is may be due to less leaching of the zinc into the boiling water.

Phosphorus

The result of phosphorus content of the raw and processed okra pods and seeds is given in Table 6.2.Phosphorus contents of the raw, sun-dried and boiled pods were 36.32, 31.89 and 32.72 mg/100g, respectively and were 1048.21, 1041.03, 1049.64, 1058.42and 1037.65mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Phosphorus contents of all the processed pods and seeds were not significantly ($P>0.05$) different from that of the raw okra pods and seeds, respectively (Table 6.2).


Pod
Seed
Boiling
Sundrying
Boiling
Soaking
Germination
Roasting
Percentage Increased/ Decreased
10.00
5.00
0.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
-35.00
-40.00
Ca
Fe
Zn
P
K
Na

86.38mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Similar to phosphorus, potassium contents of all the processed pods and seeds were not significantly (P>0.05) different from that of the raw okra pods and seeds, respectively (Table 6.2).

Sodium

The result of the sodium content of the raw and processed okra pods and seeds is presented in Table 6.2. Sodium contents of the raw, sun-dried and boiled pod were 6.06, 1.68 and 5.01mg/100g, respectively and were 17.40, 16.11, 15.41, 14.88and 15.23mg/100g for raw, boiled, soaked, germinated and roasted seeds, respectively. Sodium contents of all the processed pods and seeds were not significantly (p>0.05) different from the raw pods and seeds, respectively. However, boiling significantly (p<0.05) different from that of decreased the sodium contents of okra seeds by 22.28% (Figure 6.10).

6.4.3 Effect of processing on mineral ratios of the pod and seed

The mineral ratios are often more important than individual mineral levels themselves because they are useful in determining nutritional interrelationships and also provide information regarding the many possible factors that may be represented by a disruption of their relationships such as disease states, physiological and developmental factors, the effects of diets etc. (Watts, 2010). The understanding of mineral ratios is extremely exciting and much more revealing than analyzing mineral levels alone. The mineral ratios of the raw and processed seeds and pods of okra are shown in Table 6.3.

Sodium to potassium ratio

The sodium to potassium (Na/K) ratios of the raw, sun-dried and boiled okra pods were 0.023, 0.006and 0.019, respectively and were 0.194, 0.184, 0.185, 0.180and 0.186 for raw, boiled, soaked, germinated and roasted seeds, respectively (Table 6.3). Hypertension occurs when cellular Na/K ratios become too high, as a consequence of high sodium and low potassium diet (Afolabi *et al*., 2015). A high ratio of sodium to potassium may also contribute to bone loss because sodium increases calcium excretion. For prevention of high blood pressure, Na/K ratio of less than one is suggested (Zia-Ul-Haq *et al*., 2014).Ijarotimi *et al*. (2013)also reported that the Na/K ratio of less than one recommended for diets, particularly for hypertensive patients. Therefore, the observed Na/K molar ratio of all the raw and processed pods and seeds of okra flour in this study may be suitable for people who have the risk of high blood pressure.

Calcium: phosphorous ratio

The calcium: phosphorus (Ca/P) ratios of the raw, sun-dried and boiled pods were 8.575, 9.587 and 9.479, respectively and were 0.086, 0.083, 0.085, 0.087 and 0.087 for raw, boiled, soaked, germinated and roasted seeds, respectively (Table 6.3). Low Ca/P ratio leads to loss of Ca in the urine more than the normal amount, so Ca concentration in bones is reduced. Food is considered poor, if Ca/P ratio is less than 0.5 and good if it is above one (Zia-Ul-Haq *et al*., 2014). High levels of calcium are required during growth, gravidity, and lactation of animals (Zia-Ul-Haq*et al*., 2013). In addition, the diets with a high value of Ca/P ratio are considered good, particularly for growing children who require a high intake of calcium and phosphorus for bone and teeth formation (Ijarotimi *et al*., 2013).Therefore, the high Ca/P ratio of the raw and processed pods of okra observed in this study is of nutritional benefit, particularly for children and the aged who need higher intakes of calcium and phosphorus for bone formation and maintenance and will serve as a fine source of calcium for the formation of bones. However, since the Ca: P ratios of the raw and processed seeds of okra are lower than 0.5, the seed flours would have to be supplemented with calcium to avoid mineral and osmotic imbalance.

Table 6.3Mineral ratios of the raw and processed okra pods and seeds

Edible parts	Treatments	Mineral ratios			
		Na:K	Ca:P	Ca:K	Fe:Zn
Pods	Raw	0.023	8.575	1.183	5.231
	Boiling	0.006	9.587	1.185	4.529
	Sun drying	0.019	9.479	1.176	4.580
Seeds	Raw	0.194	0.086	0.999	1.297
	Boiling	0.184	0.083	0.982	1.623
	Soaking	0.185	0.085	1.070	1.266
	Germination	0.180	0.087	1.109	1.701
	Roasting	0.186	0.087	1.041	1.406
Critical value		< 0.6	> 0.5	< 4	> 2

Calcium: potassium ratio

The result of calcium: potassium (Ca/K) ratios of the raw and processed pods and seeds of okra are shown in Table 6.3. The Ca/K ratios of the raw, sun-dried and boiled pod were 1.183, 1.185 and 1.176, respectively and were 0.999, 0.982, 0.1.070, 1.109 and 1.041 for raw, boiled, soaked, germinated and roasted seeds, respectively. Calcium is affected by several hormones and is

considered to be under parasypathic control (Watts, 2010). The elevation of the Ca/K ratio can be an indication of reduced thyroid expression. The opposite, a low Ca/K ratio would indicate an elevation of thyroid expression. This ratio would also be associated with the adrenal activity.The ideal ratio ofCa/K is 4:1 (Watts, 2010). Higher Ca/K levels in foods are required for favorable calcium absorption in the intestine for bone formation (Jacob *et al*., 2015). The present study indicated that the Ca/K ratios of the raw and processed pods and seeds of okra are low and areconsidered good for thyroid activity, however, for bone formation it should be consumed with calcium rich foods for favorable calcium absorption.

Iron: zinc molar ratio

The result of iron: zinc (Fe/Zn) ratio of the raw and processed okra pods and seeds is shown in Table 6.3.The Fe/Zn ratios of the raw, sun-dried and boiled pods were 5.231, 4.529 and 4.580, respectively and were 1.297, 1.623, 1.266, 1.701 and 1.406 for raw, boiled, soaked, germinated and roasted seeds, respectively (Table 6.3).Pérès*et al*. (2001) reported that iron did not impair zinc absorption up to an iron:zinc ratio of 2:1; then a dose-dependent effect was observed up to a ratio of 5:1; when the ratio was increased from 5:1 to 10:1, no further inhibition of zinc occurred. It was noticed that the presence of iron in the raw and processed pods cannot impair zinc absorption. However, the iron in the raw and processed seeds of okra can inhibit the absorption of zinc. Hence a conscious dietary adjustment in the intake of both minerals is essential in order to prevent the inhibition of Zn absorption.

6.4.4 Effect of processing on total phenolic and flavonoid content of the pods and seeds

Total phenolics

Theresult of the total phenolic contents of the raw and processed pods and seeds of okra is presented in Table 6.4.The total phenolic contents of the raw, sun-dried and boiled pods were 95.21, 99.78 and 92.35 mg GAE/g, respectively and were 57.34, 57.99, 45.28, 67.27 and 65.88 mg GAE/g for raw, boiled, soaked, germinated and roasted seeds, respectively. Total phenolic content of the boiled pod was significantly ($P<0.05$) increased by 4.80% compared to the raw pod. This increase in total phenol could be attributed to the fact that some phytochemicals which are insoluble at room temperature get solubilised and extracted at increased temperature (Adeniyan *et al*., 2013). Thus, the consumption of boiled okra pods could be advantageous for it may lower cellular aging process in the human body(Amic, 2003). Since there was no significant

difference between the sun-dried pods and the raw pods, the consumer can use the sundried pods throughout the year without significant reduction in the phenolic content.

In the same trend, the total phenolic contents of germinated and roasted seeds were significantly ($P<0.05$) increased by 17.32 and 14.89%, respectively. However, the total phenolic content of soaked seeds was significantly ($P<0.05$) decreased by 21.03% compared to raw seeds (Figure 6.12).Pandey & Awasthi (2015)reported the same trend of increase in the total phenolic content of roasted sesame seeds.The heat-induced increase in total phenolics has also been reported in roasted common kidney, pinto beans (Siddhuraju & Beckar, 2001)and barley (Gallegos-Infante *et al*., 2010).

The reason for the increase in the total phenolic content during roasting could be due to the formation of heat induced and extractable phenolics (Manzocco *et al*., 2000). Boateng *et al*. (2008) explained that disruption of the cell wall through heating or by the breakdown of insoluble phenolic compounds as a function of thermal treatments could lead to better extractability of the phenolic compounds in dry beans. Thermal processing may also release more bound phenolic acids due to the breakdown of cellular constituents (Dewanto *et al*., 2002). Furthermore, the bound phenolics with larger molecular weight might have been liberated into simple free forms by heat treatment leading to enhanced over all total phenolic content of the samples.

Table 6.4 Effect of traditional processing on total phenolicss (mg GAE/g) and flavonoids(mg CE/g) content of pods and seeds of okra

Edible part	Treatments	Total Phenolics	Total Flavonoids	Flavonoid to Phenolic
Pods	**Raw**	95.21 ± 0.79^{b}	17.09 ± 1.78^{a}	0.18 ± 0.02^{a}
	Boiling	99.78 ± 0.86^{a}	13.64 ± 0.15^{b}	0.14 ± 0.00^{a}
	Sun drying	92.35 ± 0.06^{b}	14.28 ± 1.37^{a}	0.16 ± 0.02^{a}
Seeds	**Raw**	57.34 ± 0.94^{b}	29.04 ± 0.03^{b}	0.51 ± 0.01^{b}
	Boiling	57.99 ± 1.42^{b}	26.92 ± 0.04^{c}	0.47 ± 0.02^{c}
	Soaking	45.28 ± 0.02^{c}	29.56 ± 0.54^{b}	0.65 ± 0.01^{a}
	Germination	67.27 ± 1.39^{a}	33.43 ± 0.28^{a}	$0.50 \pm 0.01b^{c}$
	Roasting	65.88 ± 0.10^{a}	33.14 ± 0.02^{a}	$0.50 \pm 0.00b^{c}$

Means not followed by the same superscript letters in each column of the pod and seed are significantly ($p<0.05$) different. Data are expressed as mean ± standard error of replicate determinations (n=2)

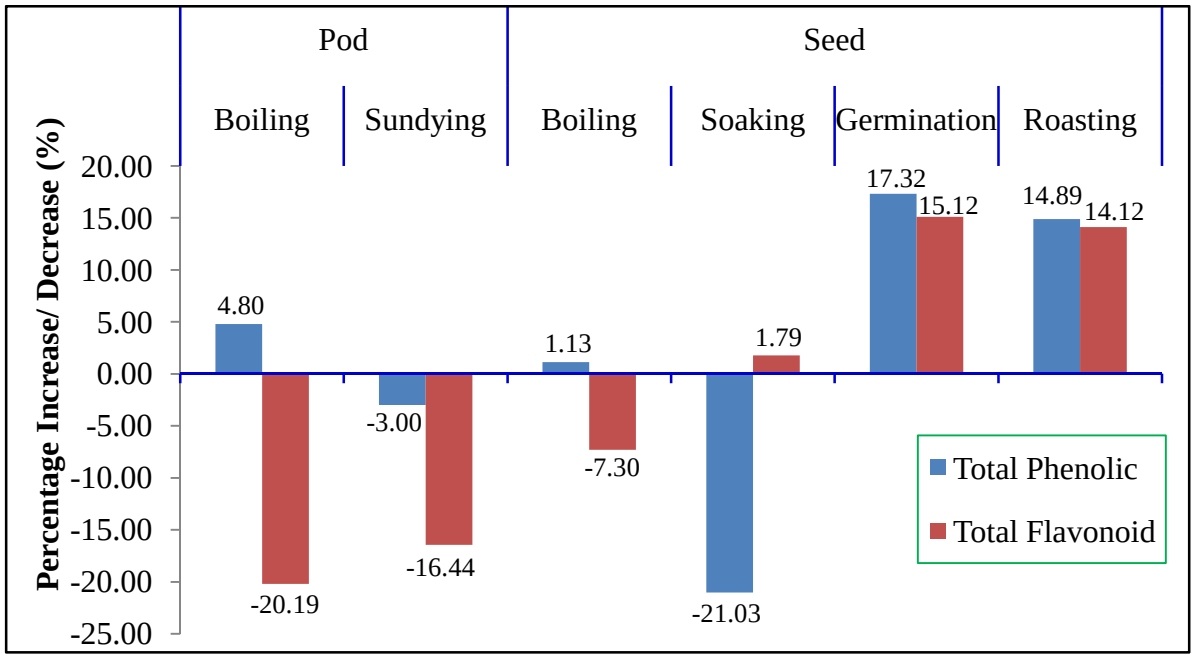
Several studies also reported that heat treatment is effective in increasing the total phenolic content in different foods such as dry beans (Boateng *et al*., 2008), carob powder (Win *et al*., 2011), vegetables (Sultana*et al*., 2008), and grape seeds (Kim*et al*., 2006). Also, roasting induces the Maillard reaction with the resultant production of many compounds which have an antioxidant activity (Manzocco *et al*., 2000). In addition, the increase in total phenolics of in this study may also be linked to the development of Maillard reaction products that are reported to be formed during the roasting process. Yu *et al*. (2005) investigated that Maillard reaction products might lead to increase in the amounts of total phenolics or phenolic-like complexes.

The increase in total phenolic content during germination of okra seeds might be due to mainly endogenous enzymes activation and the complex biochemical metabolism of seeds during the process (Duenas *et al*., 2009). An increase of total phenols after germination was also reported by Khattak *et al*. (2007) for chickpea, Duenas *et al*., (2009)for lupines and Tain *et al*., (2010) for oat. Duenas *et al*. (2009) reported that germination caused significant changes in the phenolic composition (increasing) mainly due to endogenous enzymes activation and the complex biochemical metabolism of seeds during this process.

Total flavonoids

The result of total flavonoid content of the raw and processed okra pods and seeds is shown in Table 6.4. Total flavonoid content of the raw, sun-dried and boiled pod were 17.09, 13.64 and 14.28 mg CE/g, respectively and were 29.04, 26.92, 29.65, 33.43 and 33.14 mg CE/g for raw, boiled, soaked, germinated and roasted seeds, respectively. The total flavonoid contents of the sun-dried pods and soaked seeds was not significantly ($P>0.05$) different from the raw pods and seeds, respectively. However, the total flavonoid contents of the boiled pods and seeds were significantly ($P<0.05$) decreased by 20.19 and 7.30% when compared to the raw pods and seeds, respectively (Figure 7.11). In contrast, the total flavonoid contents of the germinated and roasted seeds were significantly increased by 15.12 and 14.12%, respectively, when compared to the raw seeds (Figure 6.11).

The increase in the total flavonoid content could result from the release of bound polyphenols or from maillard reaction products forming during roasting, which then exhibited scavenging activity on the reactive oxygen species (Thidarat *et al*., 2016).The heat-induced increase in flavonoid content has also been associated with deactivation of endogenous oxidative enzymes,


Pod
Seed
Boiling
Sundying
Boiling
Soaking
Germination
Roasting
Percentage Increase/ Decrease (%)
20.00
15.00
10.00
5.00
0.00
-5.00
-10.00
-15.00
-20.00
-25.00
4.80
-20.19
-3.00
-16.44
1.13
-7.30
1.79
-21.03
17.32
15.12
14.89
14.12
Total Phenolic
Total Flavonoid

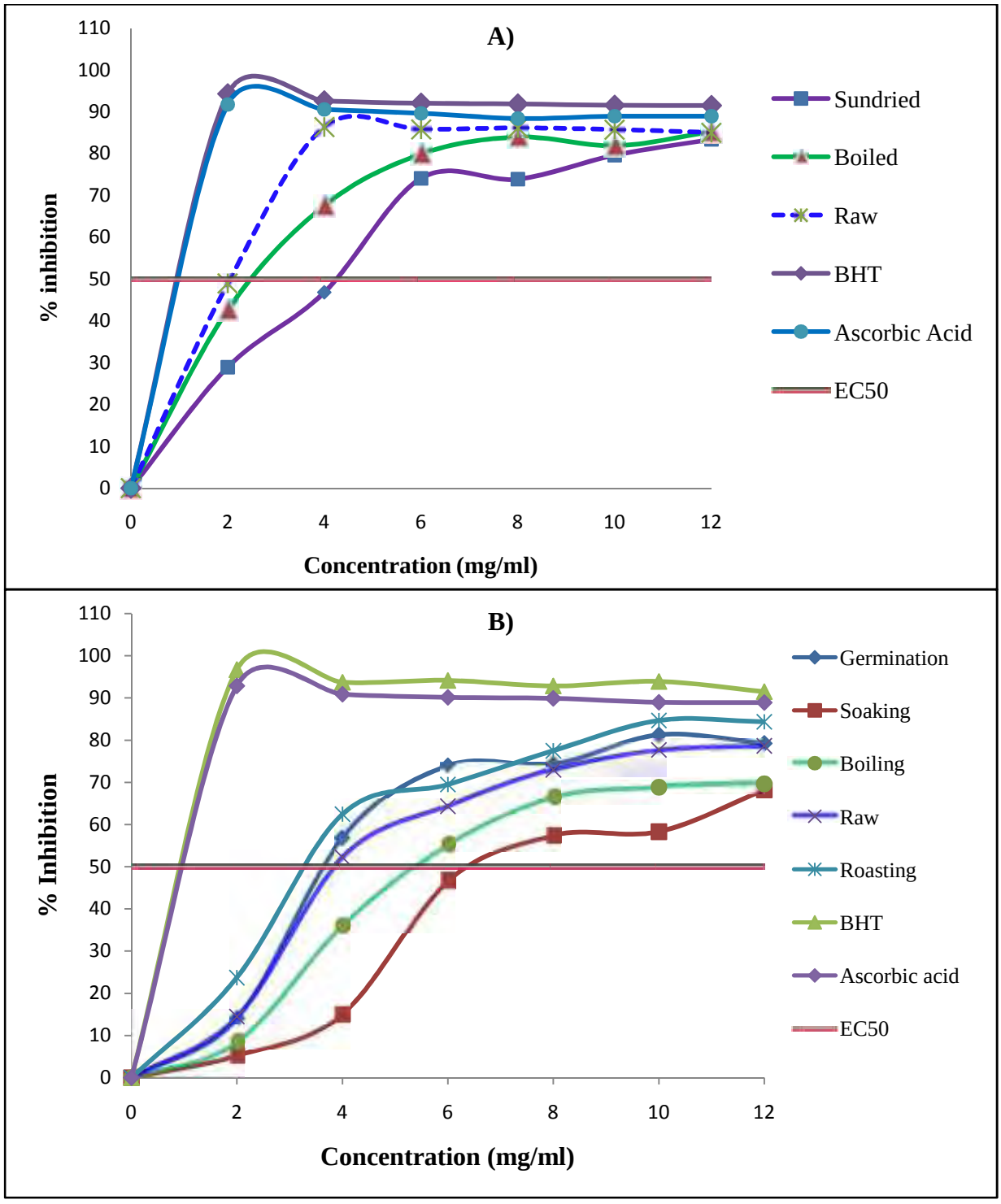
flavonoid content. However, the ratios of flavonoid to phenolic contents of the boiled, germinated and roasted seeds were significantly ($P<0.05$) reduced when compared to raw seeds.

6.4.5 Effect of processing on antioxidant activity of the pod and seed

DPPH scavenging activity

The result of concentration response curves of DPPH radical scavenging activity of the processed okra pods and seeds are shown in Figure 6.12. In addition to the raw okra pods and seeds, the synthetic antioxidants (Butylated hydroxytoluene (BHT) and L-ascorbic acid) were also used as the positive control using the same concentrations. The percentage inhibition of DPPH radical scavenging activity of the processed pods and seeds were evaluated at concentrations of 2-12 mg/ml. The present study revealed that there was an increase in DPPH radical scavenging activity with increasing concentration of the processed pod and seed extracts.

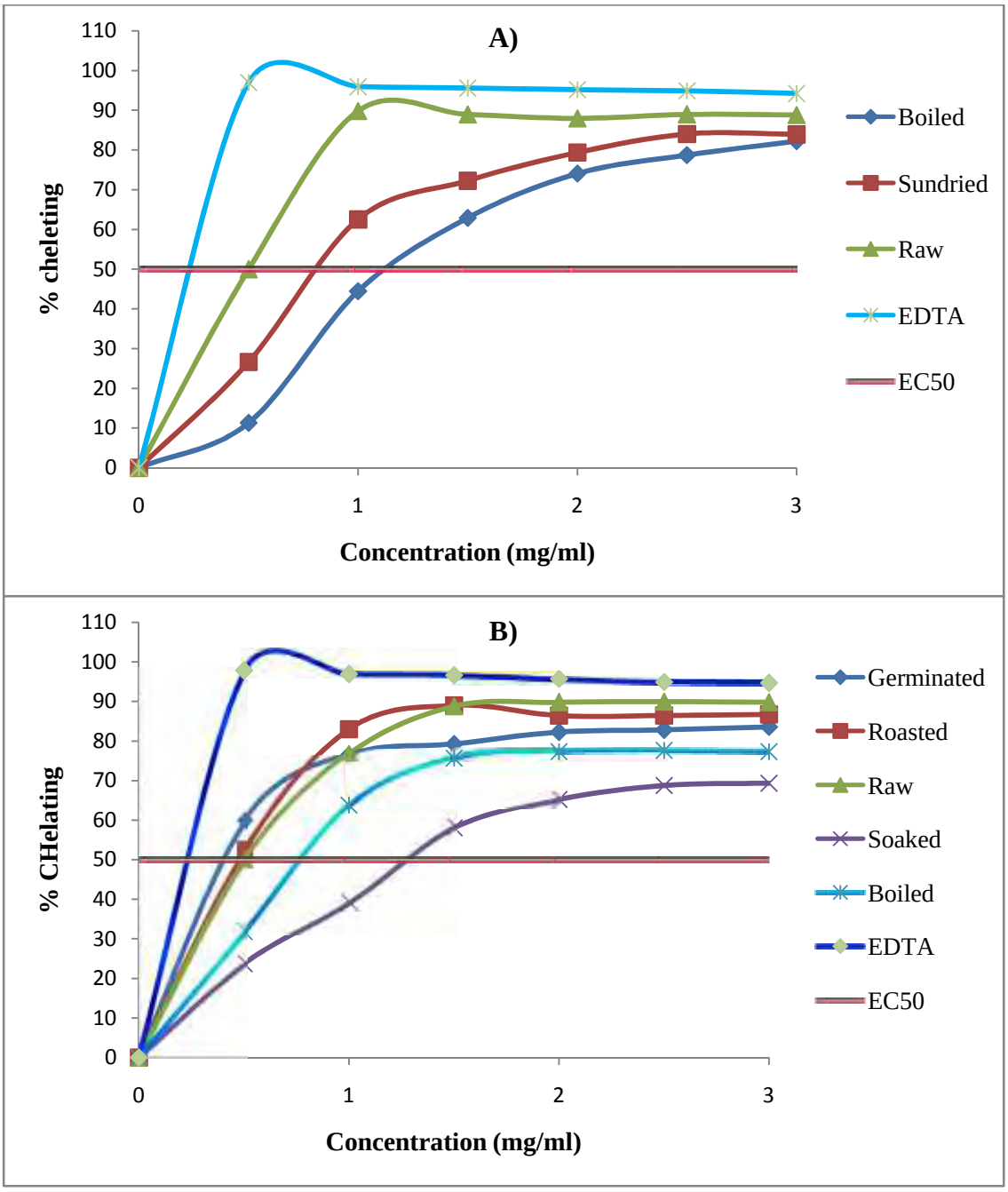
The DPPH scavenging activity of the boiled and sundried pod extracts was lower than that of the raw pods at each evaluated concentration level (Figure 6.12). In a similar trend, the DPPH scavenging activity of the boiled and soaked seed extractswere lower than the raw okra seeds at each concentration measured. However, the DPPH scavenging activities of the germinated and roasted seeds were relatively higher than that of raw seeds at each concentration (Figure 6.12). In the present study, the increase in the radical-scavenging activity of the roasted seed extract is in agreement with the previous studies on apricot kernels (Şensoy*et al*., 2006) and okra seed flour (Adelakun*et al*., 2009) and this might be attributed to their better ability to release some bound antioxidant phenolic compounds to act as free radical scavengers, from the cell matrix upon roasting (Dewanto *et al*., 2002).


A)
% inhibition
Concentration (mg/ml)
Sundried
Boiled
Raw
BHT
Ascorbic Acid
EC50
B)
% Inhibition
Concentration (mg/ml)
Germination
Soaking
Boiling
Raw
Roasting
BHT
Ascorbic acid
EC50

scavenging properties of processed foods (Dewanto *et al*., 2002). The study revealed that the extracts of roasted and germinated seeds exhibit proton-donating ability and could serve as free radical inhibitors or scavengers. Thus, the roasted okra seeds can be incorporated into the human diets like a coffee substitute and can serve as free radical inhibitors or scavengers.

Metal chelating effect

The metal chelating effect of the processed pods and seeds of okra is shown in Figure 6.13. The value of chelating effect of the processed pods and seeds was compared with the value of raw pods and seeds, respectively. In addition, it was compared with synthetic antioxidants (EDTA) as a positive control using the same concentration. The percentage of the chelating effect of the processed pods and seeds were evaluated at concentrations of 0.5 to 3 mg/ml. Similar to the DPPH scavenging activity, it was observed that there was an increase in chelating effect with increasing concentration of the processed pods and seeds extract. At each concentration, the chelating effects of the boiled and sun-dried pods were lower than the raw okra pods and synthetic antioxidant (Figure 6.13).


A)
% cheleting
Concentration (mg/ml)
Boiled
Sundried
Raw
EDTA
EC50
B)
% CHelating
Concentration (mg/ml)
Germinated
Roasted
Raw
Soaked
Boiled
EDTA
EC50

chelation after thermal processing may be attributed to alteration of phenolic structure and/or degradation of phenolic compounds to different Maillard reaction products like melanoid which could also act as antioxidants.

Effect of processing on effective concentration values of the pods and seeds

The effective concentration (EC_{50}) of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the antioxidant concentration by 50%, which is obtained by interpolation from a linear regression analysis (Liu *et al*., 2009). A lower EC_{50} indicates a higher antioxidant activity of a compound (Do *et al*., 2014). Phenolics were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity (Liu *et al*., 2009). The effective concentrationvalues for DDPH scavenging and metal chelating effects of methanolic extract of the raw and processed okra pods and seeds with positive controls are shown in Table 6.5. The effective concentrations (EC_{50}) values for DDPH scavenging of the raw, sundried and boiled pods were 2.10, 2.45 and 4.25mg/ml, respectively and were 3.9, 5.35, 6.35, 3.45 and 3.25 mg/ml for raw, boiled, soaked, germinated and roasted seeds, respectively. The result of this study revealed that boiled okra pods and germinated and roasted seeds had better antioxidant properties with lower EC_{50} values of 2.45, 3,45 and 3.25 mg/ml, respectively compared to raw pods (2.10) and seed (3.9) (Table 6.5).

Table 6.5 Effect of processing on effective concentration (EC_{50}) values (mg/ml) of the raw and processed okra pods and seeds

Edible part	Treatments	DPPH Scavenging (EC_{50}[a])	Chelating Effect (EC_{50}[b])
Pod	**Raw**	2.10	0.50
	Boiling	2.45	1.10
	Sun drying	4.25	0.80
Seed	**Raw**	3.9	0.50
	Boiling	5.35	0.78
	Soaking	6.35	1.30
	Germination	3.45	0.40
	Roasting	3.25	0.47
Control	**BHT**	0.80	-
	Ascorbic Acid	0.90	-
	EDTA	-	0.22

[a]EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged.

[b]EC_{50} (mg/ml): effective concentration at which 50% Fe^{2+}/ferrozine complex are inhibited.

The EC_{50} values for the metal chelating effect of the raw, sundried and boiled pods were 0.50, 1.10 and 0.80mg/ml, respectively and were 0.50, 0.78, 1.30, 0.40 and 0.47 mg/ml for raw, boiled, soaked, germinated and roasted seed, respectively (Table 6.5). In this study, it was observed that the EC_{50} values of the germinated and roasted seeds were lower than the raw seeds; which implies that germination and roasting exhibit superior performance and better antioxidant activities. Thus, the roasted seeds of okra can be incorporated into the human diet as a coffee substitute as it could play a preventive role against the dangerous superoxide radical, which is produced continuously during body metabolism (Finkel & Holbrook, 2000).However, the rest of processing methods of the pods and seeds exhibited low antioxidant activities compared to the EC_{50} values of the raw pods and seeds.

6.4.6 Effect of processing on the functional properties of the pods and seeds

Food processing may affect the functional properties and nutritional quality of the food products (Chukwuma *et al*.,2016).The result of functional properties of the flour of raw and processed okra pods and seeds is shown inTable 6.6.

Bulk density

Bulk densities of the raw and processed okra pods and seeds are shown in Table 6.6.The bulk densities of the flour of raw pods and seeds were 0.57 g/ml and 0.70 g/ml, respectively. This indicates that the bulk density of the raw seed was higher than the raw okra pods. This may be related to the fact that the flour of raw seeds contains high protein than the flour of raw pods.

The obtained bulk density value for the flour of raw okra pods (0.57 g/ml) was relatively similar to those from African breadfruit kernel (0.54 g/ml). However, it was lower than that of tigernut (0.62 g/ml) and wheat flour (0.71 g/ml) (Oladele& Aina, 2007). These results were also lower than those of raw and fermented wheat flours, with respective values of 0.80 and 0.86 g/ml (Steve, 2011).Indeed, the lower bulk density implies less quantity of the food samples which could be packaged in a constant volume, ensuring an economical packaging (Osundahunsi & Aworh, 2002). Thus, the flour of okra pods could be a good candidate for complementary food formulation for infant and young children.

On the other hand, the bulk density of the flour of raw okra seeds (0.70 g/ml) in this study was comparable with the value reported by Bryant *et al*. (1988) for the whole okra seed (0.68

g/ml)and higher than the value reported by Adelakun *et al*. (2017)for five okra seed varieties (0.12- 0.216 g/ml). The products with high bulk density are known to exhibit better packaging properties than those with low bulk density.Arinola *et al*. (2016)reported that high bulk density is desirable in that it offers greater packaging advantage as greater quantity may be packaged within a constant volume. A higher bulk density is desirable for a greater ease of dispersibility and a reduction of paste thickness and therefore, the high bulk density of the raw okra seeds indicates that they would serve as a good thickener in food products.

The bulk densities of the flour of boiled and sundriedpods were 0.47 and 0.40 g/ml, respectively and were 0.55, 0.65, 0.65 and 0.48 g/ml for the flour of boiled, soaked, germinated and roasted seeds, respectively.Bulk densities of the flour of boiled and sundried pods were significantly ($P<0.05$) decreased by 17.54 and 29.82%, respectively when compared to the raw pods. The bulk densities of the flour of boiled and roasted seeds were also significantly ($P<0.05$) decreased by 21.43 and 31.43%, respectively compared to the raw pods (Figure 6.14). The trends of this result was in agreement with the finding of Enujiugha *et al*. (2003); who stated that bulk density is reduced due to heat application.Drying also decreases the bulk density of flour. The high bulk density of flour indicates that they would serve as good thickeners in food products. In contrast, low bulk density would be an advantage in the formulation of complementary foods. Therefore, the high bulk density of the flour of seeds suggests their suitability to be used as thickener in food products. However, the exhibition of low bulk density value by the flour of okra pods could be an advantage in the formulation of complementary foods where high nutrient density and low bulk density is desired.

Table 6.6 Functional properties of the flour of raw and processed okra pods and seeds

Treatments		BD (g/ml)	WAC (ml/g)	OAC (ml/g)	EC (%)	ES (%)	FC (%)	FS (%)
Pods	**Raw**	0.57 ± 0.02^{a}	2.05 ± 0.15^{b}	1.70 ± 0.10^{c}	25.02 ± 0.31^{a}	31.80 ± 2.22^{a}	53.49 ± 1.80^{a}	33.59 ± 2.48^{a}
	Boiling	0.47 ± 0.01^{b}	3.80 ± 0.10^{a}	3.15 ± 0.05^{a}	21.62 ± 0.74^{b}	30.26 ± 1.32^{a}	52.05 ± 0.00^{a}	31.18 ± 0.72^{a}
	Sun drying	0.40 ± 0.02^{b}	4.45 ± 0.25^{a}	2.55 ± 0.05^{b}	22.45 ± 0.23^{b}	31.21 ± 0.79^{a}	51.30 ± 1.30^{a}	31.65 ± 1.23^{a}
Seeds	**Raw**	0.70 ± 0.01^{a}	2.55 ± 0.15^{b}	1.95 ± 0.05^{c}	45.08 ± 1.60^{a}	42.21 ± 0.39^{a}	61.51 ± 0.84^{a}	27.13 ± 2.13^{a}
	Boiling	0.55 ± 0.02^{b}	3.80 ± 0.10^{a}	2.95 ± 0.35^{b}	38.02 ± 0.77^{c}	40.46 ± 2.36^{a}	$57.83 \pm 1.00b^{c}$	21.54 ± 1.54^{b}
	Soaking	0.65 ± 0.02^{a}	3.05 ± 0.15^{b}	2.35 ± 0.05^{bc}	41.53 ± 0.79^{b}	41.96 ± 0.59^{a}	58.04 ± 1.06^{bc}	24.77 ± 0.73^{ab}
	Germination	0.65 ± 0.01^{a}	4.35 ± 0.05^{a}	2.40 ± 0.10^{bc}	43.43 ± 0.03^{ab}	41.52 ± 0.45^{a}	60.13 ± 0.70^{ab}	25.96 ± 0.96^{ab}
	Roasting	0.48 ± 0.03^{c}	4.30 ± 0.20^{a}	3.75 ± 0.15^{a}	36.08 ± 0.29^{c}	38.15 ± 0.91^{a}	54.74 ± 0.77^{c}	22.43 ± 0.21^{ab}

Means not followed by the same superscript letters in each column of the pods and seeds are significantly ($P<0.05$)different. Data are expressed as a mean ± standard error of replicate determinations (n=2). **Note** BD-Bulk density; WAC - Water absorption capacity; OAC - Oil absorption capacity; EC - Emulsifying capacity; ES - Emulsion stability; FC - Foaming capacity; FS - Foam stability.

Water absorption capacity

Table 6.6 shows the result of water absorption capacity of the flour of raw and processed okra pods and seeds. Water absorption capacities of the flour of raw pods and seeds of okra were 2.05 ml/g and 2.55 ml/g, respectively. The water absorption capacity of the raw okra seeds (2.55g/ml) was lower than the value reported by Adelakun *et al*. (2017)for five okra seed varieties (1.93- 3.23 g/cm).From this result, it was observed that the water absorption capacity of the raw pods of okra (2.05ml/g) was lower than the seeds (2.55 ml/g). This indicates that the protein content of raw seeds of okra is higher than that of the flour of okra pods since the amount of protein in the sample is the most determinant factor of the water absorption capacity.

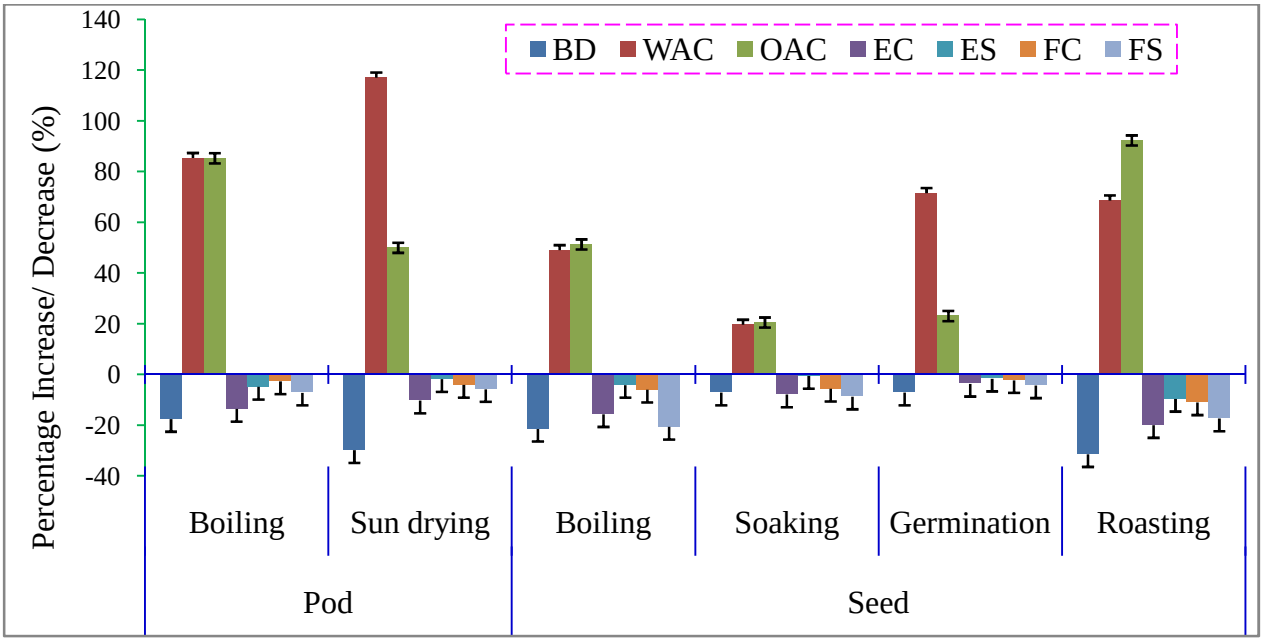
Water absorption capacities of the flour of boiled and sundried pods were 3.80 and 4.45 ml/g, respectively and were 3.80, 3.05, 4.35 and 4.30 ml/g for the flour of boiled, soaked, germinated and roasted seeds, respectively. Water absorption capacity of the soaked okra seeds was not significantly ($P>0.05$) different from that of the flour of raw okra seeds. However, the water absorption capacity of the flour of boiled, germinated and roasted seeds was significantly ($P<0.05$) increased by 49.02%, 71.52%, and 68.63%. Thewater absorption capacities of the flour of boiled and sundried pods were also significantly ($P<0.05$) increased by 85.37% and 117.07%, respectively (Figure 6.14).

The increase in water absorption capacity during germination may be due to increased levels of protein and quality of protein, which enhance interactions with water (Chauhan & Sing, 2013).The increase in water absorption capacity during roasting could be attributed to increased level of damaged starch which was induced by gelatinization of starch during roasting (Sharma & Gujral, 2013). The formation of a porous structure which imbibes and holds water by capillary action is also a reason for the increase in water absorption capacity (Sharma *et al*., 2016). Abbey & Ibeh (1988)also reported that the increase in the water absorption capacity has always been associated with an increase in the amylose leaching and solubility and loss of starch crystalline structure.

High water absorption capacity of the flours suggests that they can be used in the formulation of some foods such as sausage, dough, processed cheese and bakery products (Butt & Batool, 2010). Water absorption capacity of 1.25 ml/g and above is an indication of good bakery property (Giami & Alu, 1994).Therefore, the flour of all the raw and processed okra pods and seeds analyzed in this work would be a good functional ingredient in bakery products. This study suggests that the boiled, germinated and roasted seeds also boiled and sundried pods of okra flour can serve as a thickener in the food system.

Oil absorption capacity

The result of oil absorption capacity of the flour of raw and processed okra pods and seeds is presented inTable 6.6.The oil absorption capacities of the flour of raw okra pods and seeds were 1.70 and 1.95 ml/g, respectively. The oil absorption capacity of the raw okra seeds (1.95ml/g) in this study was in the range of the value reported by Adelakun *et al*. (2017)for five okra seed varieties (1.80- 2.94 ml/g).A high oil absorption capacity is valuable in ground meat


BD
WAC
OAC
EC
ES
FC
FS
Percentage Increase/ Decrease (%)
140
120
100
80
60
40
20
0
-20
-40
Boiling
Sun drying
Boiling
Soaking
Germination
Roasting
Pod
Seed

flours in the present study are potentially useful in structural interaction in food especially in flavor retention, improvement of palatability and extension of shelf life particularly in the bakery or meet products where oil absorption is desired (Aremu *et al*., 2007).

Emulsifying capacity

Table 6.6showsthe result of the emulsifying capacity of the flour of raw and processed okra pods and seeds. The emulsifying capacities of the flour of raw okra pods and seeds were 25.02 and 45.08%, respectively. The emulsifying capacity of the raw okra seeds (45.08%) in this study was higher than the value reported by Adelakun *et al*. (2017)for five okra seed varieties (2.92-3.44%).Emulsifying capacities of the flour of boiled and sundried pods were 21.62 and 22.45%, respectively while and were 38.02, 41.53, 43.43 and 36.08% for the flour of boiled, soaked, germinated and roasted seeds, respectively. Emulsifying capacities of the flour of boiled and sundried pods were significantly ($P<0.05$) decreased by 13.59 and 10.27%, respectively compared to the flour of raw pods. The emulsifying capacities of the flour of boiled, soaked and roasted seeds were also significantly ($P<0.05$) decreased by 15.66, 7.87 and 19.96 %, respectively compared to raw seed flour (Figure 6.14). Reduction in emulsion capacities of the samples may be probably influenced by their respective oil contents (Ihemeje *et al*., 2015). Another possible cause of the reduction could be the thermal denaturation of the protein caused by heating (Chandra*et al*., 2015).The flour of all the raw and processed pods and seeds showed relatively good emulsion capacities.

Emulsion stability

Table 6.6showsthe emulsion stability of the flour of raw and processed okra pods and seeds. The result of emulsion stability of the flour of okra pods and seeds were31.80 and 42.21%, respectively. The emulsionstabilityof the raw okra seeds (42.21%) in this work was higher than the value reported by Adelakun *et al*. (2017)for five okra seed varieties (1.33- 1.81%).Emulsion stability of the flour of boiled and sundried pods was 30.26 and 31.21%, respectively and it was 40.46, 41.96, 41.52 and 38.15% for the flour of boiled, soaked, germinated and roasted seeds, respectively (Table 6.14).Emulsion stability of the flour of all the processed pods and seeds of okra was not significantly ($P>0.05$) different from that of the flour of raw pods and seeds of okra, respectively. Emulsion stability can be greatly increased when highly cohesive films are formed by the absorption of rigid globular protein molecules that are more resistant to mechanical

deformation (Chandra *et al*., 2015). Increasing emulsion stability and fat binding during processing are primary functional properties of protein foods such as comminuted meat products, salad dressing, frozen desserts, and mayonnaise. In line with this, the raw and processed pods and seeds of okra showed relatively good emulsion stability.

Foaming capacity

Table 6.6shows the result of the foaming capacities of the flour of raw and processed okra pods and seeds. The result of foaming capacities of the flour of raw okra pods and seeds were 53.49 and 61.51%, respectively. The foaming capacityof the raw okra seeds (61.51%) in this work was higher than the value reported by Adelakun *et al*. (2017)for five okra seed varieties (73-6.44%).Flours with high foaming ability could form large air bubbles surrounded by thinner less flexible protein film. This air bubbles might be easier to collapse and consequently lower the foam stability (Jitngarmkusol *et al*., 2008).Foaming capacities of the flour of boiled and sundriedpods were 52.05 and 51.30%, respectively and were 57.83, 58.04, 60.13 and 54.74% for the flour of boiled, soaked, germinated and roasted seeds, respectively. Foaming capacities of the flour of boiled and sundried pods was not significantly ($P>0.05$) different from that of the raw pods. Foaming capacities of the flour of boiled, soaked and roasted seeds were significantly ($P<0.05$) decreased by 8.28, 8.70 and 17.32% (Figure 6.14). The decrease observed in foaming capacity might have been as a result of denaturation of protein molecules during boiling, soaking and roasting processes. The effect of heat processing on foam capacity and stability of winged bean flour has been reported (Ihemeje*et al*., 2015).

Kouakou*et al*. (2013) also reported that native protein provides higher foam capacity than denatured protein. Since proteins are heat labile, the reduced foaming capacity and stability of heat processed flours can be explained on the basis of protein denaturation, hence the flour of raw seeds gave a higher foam capacity than the processed one. The reduction in foaming capacity could be as a result of a fat reduction in processed seeds and this suggests its utilization as a supplement in infant feed formulations where aeration is not necessary.

Foam stability

The result of foam stability of the flour of raw and processed pods and seeds of okra is given in Table 6.6.The foam stability of the flour of raw okra pods and seeds were 33.59 and 27.13%, respectively. The foam stability of the raw okra seeds (27.13%) in this study was higher than the

value reported by Adelakun *et al*. (2017)for five okra seed varieties (3.78- 4.50%).Foam stability of the flour of boiled and sundried pods was 31.18 and 31.65%, respectively and it was 21.54, 24.77, 25.96 and 22.43% for the flour of boiled, soaked, germinated and roasted seeds, respectively. The foaming stability of the flour of boiled and sundried okra pods was not significantly ($P>0.05$) different from that of raw pods flour. In addition, the foaming stability of the flour of soaked and germinated seeds also was not significantly ($P>0.05$) different from that of the raw seeds. However, the foam stability of the flour of boiled and roasted seeds was significantly ($P<0.05$) decreased by 20.60 and 17.32% compared to the raw seeds (Figure 6.14).Foam stability has been suggested to be related to the amount of native protein which is considerably low in heat treated proteins (Wani *et al*., 2016). A similar effect of heat processing on foam capacity and stability of cowpea flour has been reported by Giami (2003).The ability to form stable foam is an important property in whipped toppings, frozen desserts and sponge cakes (Adelakun *et al*., 2012); thus, the flour of roasted okra seeds cannot be used in these formulations.

6.5 Conclusion

The study revealed that the various traditional food processing techniques significantly affect nutritional, antioxidant and functional properties of pods and seeds of okra. Particularly, germination of the seeds of okra significantly increased the content of crude protein, crude fibre and utilizable carbohydrate and roasting also increased crude ash and utilizable carbohydrate. Sundrying significantly increased crude fibre and crude ash content of pods of okra. The study showed that the mineral content of the processed pods was not significantly different from raw pods, except for zinc and sodium, which were reduced during boiling. The mineral content of all the processed seeds of okra was not significantly different from raw seeds, except for zinc which was reduced during boiling, soaking, and germination. The results of this finding also suggested that germination and roasting is a good way to increase total phenolic and flavonoid content and enhance DPPH scavenging and chelating effect. All the processing methods resulted in an increase in the water and oil absorption capacitiesand decrease in the bulk density, emulsion capacity and stability and foaming capacity and stability. In general, the functional properties of the flour of raw and processed pods and seeds of okra revealed their uniqueness to each parameter measured and these results may probably assist in determining the behavior and application of these flours in various food formulations.

Chapter 7
General Conclusions and Perspectives

7.1 General Conclusions

Okra is one of the indigenous vegetable crops in Ethiopia. However, lack of scientific information on nutritional quality and phytochemical properties of indigenous Ethiopian underutilized okra vegetable is a major constraint in its utilization and commercialization. In line with this, understanding the nutritional quality, phytochemical and oil characteristics of Ethiopian indigenous okra vegetable is important for its utilization and diversification of monotonous eating habit of the diet in the country. Therefore, this comprehensive study had evaluated the nutritional quality, phytochemical and oil characteristics of indigenous okra accession grown in Ethiopia for the first time.

The effect of different traditional processing on nutritional, antioxidant and functional properties of the pods and seeds of selected okra accessions was also evaluated. The pods and seeds of eight okra accessions, namely OPA#1, OPA#2, OPA#3, OPA#4, OPA#5, OPA#6, OPA#7, and OPA#8 were collected during 2014 main okra harvesting season. The analyses undertaken were proximate composition, mineral anti-nutritional, phytochemical profiles, physicochemical properties andfunctional properties.

The results of the study revealed that the pods and seeds of okra accessions were found to be a good source of crude protein, crude fat, calcium, iron, and potassium that could contribute a useful amount to the human diet and is low in antinutrient content with high mineral bioavailability. Particularly, pods and seeds of OPA#6 accession contained a significantlyhigh amount of crude protein, ash, crude fat, calcium, iron, and zinc, while the seeds of accession OPA#8 was high in calcium, iron and potassium.Moreover, principal component analysis had shown a nutritional variability and five independent clusters in the pods and seeds of okra accessions and this may be useful to breeders for improvement of accessions based on the desired trait of the clusters.The proximate and mineral contents of the pods of okra accessions are also high when compared to the commonly consumed green vegetables in Ethiopia.

The present study also showed that the total phenolics and total flavonoids levels varied widely across pods and seeds of okra accessions. It has also been shown that the antioxidant levels of the pods and seeds of okra accessions increased with the increasing concentration of the samples. The pods and seeds of indigenous Ethiopian okra accessions evaluated for the first time in this study represent a source of potential antioxidants.Particularly, both pods and seeds of okra accession OPA#6 is a significant source of natural antioxidants that could probably be used as functional food ingredients and replace synthetic antioxidants in the future.

The present study also revealed that okra seeds could be considered as potential sources of edible oil specifically the seed of OPA#2 accessions with appreciable physicochemical characteristics which suggest that this oil has high edible quality and could be useful for industrial applications. The results suggest that okra seed oil may be considered as a new candidate and valuable source of edible oil and can be utilizedfor industrial and nutritional purposes.The study also showed that okra pod mucilage contains potential sources of natural antioxidantsand high in mucilage yields. The study also revealed that the mucilage of the pods of okra accessions was found to exhibit good functional properties and can offer a great potential in various food systems. Particularly, mucilage of the pods of OPA#5 and OPA#7 had desirable water and oil absorption capacities, while mucilage of accession OPA#1 and OPA6 had high emulsifying and foaming properties.

The study also revealed that the various traditional food processing techniques significantly affect nutritional, antioxidant and functional properties of pods and seeds of okra. Particularly, germination and roasting increased total phenolic and flavonoid content and enhance DPPH scavenging and chelating effect activities. All processing methods resulted in an increase in the water and oil absorption capacities and a decrease in the bulk density, emulsion capacity and stability and foaming capacity and stability. The flour of raw and processed pods and seeds of okra was found to exhibit good functional properties and can offer a great potential in various food systems.

Generally, the results of this research indicated that indigenous Ethiopian okra containsan essential nutrients and phytochemicals. Their physicochemical characteristics suggest that the oil has high edible quality and could be utilized for industrial and nutritional purposes. The mucilage of the pods of okra accessions was also found to exhibit good functional properties and can offer a great potential in various food systems. Hence, increasing the cultivation, promotion,

and consumption of underutilized indigenous Ethiopian okra in the country couldhelp to mitigate food insecurity and alleviate malnutrition in the country.

7.2 Perspectives

This comprehensive study provides valuable information regarding nutritional quality, phytochemical and oil characteristics of the pods and seeds of eight indigenous Ethiopian okra vegetable. The following perspectives and recommendations are suggested based on the result of the findings and for further research in the future.

- Increasein the cultivation and consumption of okra need to be encouraged in the country, because of its high essential nutrient, phytochemical, oil, mucilage contents and low antinutritional factors.
- Germination and roasting are recommended for okra preparation at a household level not only due to minimal processing losses in some nutrient compositions but also in terms of increasing total phenolic and flavonoid content and enhanced antioxidant activities.
- Future work needs to focus on selection, improvement, and multiplication of accessions with good nutritional and phytochemical potential.
- The effect of processing periods, optimal processing temperature, and storage times on the nutritional, antioxidant and functional properties of okra should be studied.
- The effect of seasonal variability and maturity time on the nutritional, antioxidant and functional properties of the pods and seeds of okra accessions should be studied.
- Further study should evaluate the bioavailability of minerals by using techniques such as in vitro, in vivo, isotope labeling and efficacy studies.
- Future work on Ethiopian okra needs to befocused on the investigation of other untapped nutritional (vitamins, amino acids, fatty acids, magnesium, selenium, cupper etc); anti-nutritional factors (trypsin inhibitors, saponin, gossypol etc.); oil contents (phytosterol, gossypol etc) and phytochemicals (phenolic and flavonoid compounds).
- Studies should be undertaken for new product development using okra vegetables.
- Further study should focus on other edible parts of okra such as okra leaves, flowers etc.
- Similar to the present study, characterizations on the nutritional and phytochemical properties of other indigenous and underutilized Ethiopian food crops should be encouraged in order to diversify diet from the monotonous eating habit of the community.

References

Abayeh, O. J., Aina, E. A., & Okounghae, C. O. (1998). Oil content and oil quality characteristics of some Nigerian oil seeds. *Journal Pure Applied Sci*ence, 1(1), 17-23.

Abbey, B. W., & Ibeh, G. O. (1988). Functional properties of raw and heat processed cowpea (*Vigna unguiculata, Walp*) flour. *Journal of Food Science*, *53*(6), 1775-1777.

Aberoumand, A. (2010). A comparative study of nutrients and mineral molar ratios of some plant foods with recommended dietary allowances. *Advance Journal of Food Science and Technology*, *2*(2), 104-108.

Abeykoon, A. M. K. C. K., Fonseka, R. M., Paththinige, S., & Weerasinghe, K. W. L. K. (2010). Fertilizer Requirement for Densely Planted Okra (*Abelmoschus esculentus L.*). *Tropical Agricultural Research*, *21*(3).

Abiodun, O. A., & Adeleke, R. O. (2010). Comparative studies on nutritional composition of four melon seeds varieties. *Pakistan Journal of Nutrition*, *9*(9), 905-908.

Abiodun, O. A., Adegbite, J. A., & Omolola, A. O. (2012). Chemical and physicochemical properties of moringa flours and oil. *Global Journal of Science Frontier Research*, *12*(5), 2249-4626.

Adamma, E. P., Sallau Mohammed, S., Israel, O. K., & Yusuf, Z. S. (2014). Proximate and Anti-nutritional Constituents of *Abelmoschus esculentus* Grown in Fadaman Kubanni, Zaria, Kaduna State, Nigeria. *Journal of Scientific Research & Reports* 3(15): 2015-2027.

Adebiyi, A. P., Adeyemi, I. A., & Olorunda, A. O. (2002). Effects of processing conditions and packaging material on the quality attributes of dry-roasted peanuts. *Journal of the Science of Food and Agriculture*, *82*(13), 1465-1471.

Adegoke, G. O., Falade, K. O., & Babalola, O. C. (2004). Control of lipid oxidarion and fungal spoilage of roasted peanut (*Arachis hypogea*) using the spice *Aframomum danielli*. *Journal of food agriculture and environment*, *2*, 128-131.

Adejuyitan, J.A. Otunola, E.T. Akande, E.A. Bolarinwa, I.F. Oladokun, F.M. (2009). Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from a market in Ogbomoso, Nigeria. *African Journal of Food Science*, *3*(2), 051-055.

Adelakun, O. E., Ade-Omowaye, B. I. O., Adeyemi, I. A., & Van de Venter, M. (2012). Mineral composition and the functional attributes of Nigerian okra seed (*Abelmoschus esculentus - Moench*) flour. *Food research international*, *47*(2), 348-352.

Adelakun, O. E., Oyelade, O. J., Ade-Omowaye, B. I. O., Adeyemi, I. A., & Van de Venter, M. (2009). Chemical composition and the antioxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus Moench*) Flour. *Food and Chemical Toxicology*, *47*(6), 1123-1126.

Adelakun, O. E., Olanipekun, B. F., Akingbaso, O., & Adhikar, B. M. (2017). Effect of Fermentation and Variety on Quality Attributes of Okra Seed (*Abelmoschus esculentus (L) Moench*) Flour. Donnish *Journal of Food Science and Technology*, 3(1)

Adeniyan, O. O., Ibukun, E. O., Ogunbolude, Y., & Eseigbe, M. I. (2013). Effect of boiling on the nutritional composition and antioxidant properties of beniseed (*Sesamum indicum L.*). Food *Science and Quality Management*, 11, 123-132.

Adeoti, O. A., Elutilo, O. O., Babalola, J. O., Jimoh, K. O., Azeez, L. A., & Rafiu, K. A. (2013). Proximate, Mineral. Amino Acid and Fatty Acid Compositions of Maize Tuwo-Cirina Forda Flour Blends. *Greener Journal of Biological Sciences*, *3*(4), 165-171.

Adeparusi, E. O. (2001). Effect of processing on the nutrients and anti-nutrients of lima bean (*Phaseolus lunatus L.*) flour. *Molecular Nutrition & Food Research*, *45*(2), 94-96.

Adetuyi, F. O., & Dada, I. B. O. (2014). Nutritional, phytoconstituent and antioxidant potential of mucilage extract of Okra (*Abelmoschus esculentus*), water leaf (*Talinum triangulare*) and Jews mallow (Corchorus olitorius). *International Food Research Journal*, *21*(6).

Adetuyi, F. O., & Ibrahim, T. A. (2014). Effect of Fermentation Time on the Phenolic, Flavonoid and Vitamin C Contents and Antioxidant Activities of Okra (*Abelmoschus esculentus*) Seeds. *Nigerian Food Journal*, *32*(2), 128-137.

Adetuyi, F. O., & Komolafe, E. A. (2011). Effect of the Addition of Okra Seed (*Abelmoschus esculentus*) Flour on the Antioxidant Properties of Plantain Musa paradisiaca Flour. *Annual Review & Research in Biology 1 (4): 143, 152*.

Adetuyi, F. O., Osagie, A. U., & Adekunle, A. T. (2008). Antioxidant degradation in six indigenous okra *Abelmoschus esculentus (L) Moench* varieties during storage in Nigeria. *Journal of Food Technology*, *6*(5), 227-230.

Adetuyi, F. O., Osagie, A. U., & Adekunle, A. T. (2011). Nutrient, antinutrient, mineral and zinc bioavailability of okra *Abelmoschus esculentus (L) Moench* Variety. *American Journal of Food and Nutrition*, *1*(2), 49-54.

Adetuyi, O. F., & Stella, B. O. (2012). Effects of different traditional cooking methods on nutrients and mineral bioavailability of okra (*Abelmoschus esculentus*). Journal of Agricultural Research and Development, 11(1), 51-59.

Adeyeye, E. I. (2002). Determination of the chemical composition of the nutritionally valuable parts of male and female common West African fresh water crab *Sudananautes africanus africanus*. *International Journal of Food Sciences and Nutrition*, *53*(3), 189-196.

Adeyeye, E. I. (2006). Amino acids composition offermented African locust bean (*Parkia biglobosa*). *Journal of Applied and Environmental Sciences December*, *2*(2), 154-158.

Adeyeye, E. I., & Aye, P. A. (2005). Chemical composition and the effect of salts on the food properties of Triticum durum whole meal flour. *Pakistan Journal of Nutrition*, *4*(3), 187-196.

Adeyeye, E. I., Arogundade, L. A., Akintayo, E. T., Aisida, O. A., & Alao, P. A. (2000). Calcium, zinc and phytate interrelationships in some foods of major consumption in Nigeria. *Food chemistry*, *71*(4), 435-441.

Adeyeye, E. I., Orisakeye, O. T., & Oyarekua, M. A. (2012). Composition, mineral safety index, calcium, zinc and phytate interrelationships in four fast-foods consumed in Nigeria. *Bulletin of the Chemical Society of Ethiopia*, *26*(1), 233-241.

Afolabi, W. A., Oyawoye, O. O., Sanni, S. A., Onabanjo, O. O., & Obanla, O. O. (2015). Portion Size, Micronutrient content and Nutritional Quality of Fast Foods Sold in Ogun State, Nigeria. *International Journal of Health and Nutrition*, *6*(1), 1-8.

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*, *196*, 67-76.

Ahiakpa, J. K., Amoatey, H. M., Amenorpe, G., Apatey, J., Ayeh, E. A., & Agbemavor, W. S. K. (2014a). Mucilage Content of 21 accessions of Okra (*Abelmoschus spp L.*). *Scientia Agriculturae*, *2*(2), 96-101.

Ahiakpa, J. K., Quartey, E. K., Amenorpe, G., Klu, G. Y. P., Agbemavor, W. S. K., & Amoatey, H. M. (2014b). Essential Mineral Elements Profile of 22 Accessions of Okra (*Abelmoschus spp (L.)*) From Eight Regions of Ghana. *Journal of Agricultural Science*, *6*(5), 18.

Ahiakpa, J.K., Quartey, E.K., Amoatey, H.M., Klu, G.Y.P., Achel, D.G., Achoribo, E. & Agbenyegah, S. (2013). Total flavonoid, phenolic contents and antioxidant scavenging activity in 25 accessions of Okra (*Abelmoschus spp L.*). *African Journal of Food Science and Technology*, *4*(5), 129-135.

Ahmad T, Abraham F, Mohammedsaid H, Welday N and Gebrehiwet S (2015). Seasonal incidence of insects and diseases on okra (*Abelmoschus esculentus*) crop in zoba Anseba. *Int. Res. J. Biological Sci*. 4(1), 71-74.

Aires, A., Fernandes, C., Carvalho, R., Bennett, R. N., Saavedra, M. J., & Rosa, E. A. (2009). Seasonal effects on bioactive compounds and antioxidant capacity of six economically important Brassica vegetables. *Molecules*, *16*(8), 6816-6832.

Aja, P. M., Offor, C. E., & Orji, O. U. (2015). Proximate and a Nutrient Compositions of *Parkia biglobosa* Fruits in Abakaliki, Ebonyi state, Nigeria. *International Journal of Current Microbiology* and *Applied* Sciences, 4(2), 394-398.

Akanbi, W. B., Togun, A. O., Adediran, J. A., & Ilupeju, E. A. O. (2010). Growth, dry matter and fruit yields components of okra under organic and inorganic sources of nutrients. *American-Eurasian Journal of Sustainable Agriculture*, *4*(1), 1-13.

Akanni, M. S., Adekunle, A. S., & Oluyemi, E. A. (2005). Physicochemical properties of some non-conventional oilseeds. *Journal of Food Technology*, 3(2), 177-181.

Akingbala, J. O., Akinwande, B. A., & Uzo-Peters, P. I. (2003). Effects of color and flavor changes on acceptability of ogi supplemented with okra seed meals. *Plant Foods for Human Nutrition*, *58*(3), 1-9.

Akinhanmi, T. F., Atasie, V. N., & Akintokun, P. O. (2008). Chemical composition and physicochemical properties of cashew nut (*Anacardium occidentale*) oil and cashew nut shell liquid. *Journal of Agricultural, Food and Environmental Sciences*, 2(1), 1-10.

Akintayo, E. T. (2004). Characteristics and composition of *Parkia biglobbossa and Jatropha curcas* oils and cakes. *Bioresource technology*, *92*(3), 307-310.

Akintoye, H. A., Adebayo, A. G., & Aina, O. O. (2011). Growth and yield response of okra intercropped with live mulches. *Asian Journal of Agricultural Research*, *5*(2), 146-153.

Akinyele, B. O., & Temikotan, T. (2007). Effect of variation in soil texture on the vegetative and pod characteristics of okra (*Abelmoschus esculentus (L.) Moench*). *International Journal of Agricultural Research*, *2*(2), 165-169.

Akobundu, S. U. E. (2009). Quality characteristics of cookies from composite flours of watermelon seed, cassava and wheat. *Pakistan Journal of Nutrition*, *8*(7), 1097-1102.

Akubor, P. I. (2007). Chemical, functional and cookie baking properties of soybean/maize flour blends. *Journal of food science and technology-mysore*, *44*(6), 619-622.

Aladele, S. E., Ariyo, O. J., & de Lapena, R. (2008). Genetic relationships among West African okra (Abelmoschus caillei) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. *African Journal of Biotechnology*, *7*(10), 1426.

Alba, K., Ritzoulis, C., Georgiadis, N., & Kontogiorgos, V. (2013). Okra extracts as emulsifiers for acidic emulsions. *Food research international*, *54*(2), 1730-1737.

Aletor, V. A., & Adeogun, O. A. (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food chemistry*, *53*(4), 375-379.

Ali, A., Fadimatou, B., Tchiegang, C., Saidou, C., & Adji, M. B. (2010). Physico-chemical and functional properties of btchi or hypocotyle axes of Borassus aethiopum Mart. *African Journal of Food Science*, *4*(10), 635-641.

Alinnor, IJ. and Oze, R. (2011). Chemical evaluation of the nutritive value of *Pentaclethra macrophylla benth* (African Oil Bean) Seeds. *Pakistan Journal of Nutrition*, *10*(4), 355-359.

Aluyor, E. O., & Ori-Jesu, M. (2008). The use of antioxidants in vegetable oils–A review. *African Journal of Biotechnology*, *7*(25).

Aman, R., Schieber, A., & Carle, R. (2005). Effects of heating and illumination on trans− cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *Journal of Agricultural and Food Chemistry*, *53*(24), 9512-9518.

Amandikwa, C., & Chinyere, E. (2012). Proximate and functional properties of open air, solar and oven dried cocoyam flour. *International Journal of Agriculture and Rural Development*, *15*, 988-994.

Amarowicz, R., Carle, R., Dongowski, G., Durazzo, A., Galensa, R., Kammerer, *et al*., (2009). Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. *Molecular nutrition & food research*, *53*(S2), S151-S183.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food chemistry*, *84*(4), 551-562.

Amić, D., Davidović-Amić, D., Bešlo, D., & Trinajstić, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica chemica acta*, *76*(1), 55-61.

Aminigo, E. R., & Akingbala, J. O. (2004). Nutritive composition and sensory properties of ogi fortified with okra seed meal. *Journal of Applied Science and Envirnmental*, 8 (2) 23 - 28.

Amon, A. S., Soro, R. Y., Assemand, E. F., Dué, E. A., & Kouamé, L. P. (2014). Effect of boiling time on chemical composition and physico-functional properties of flours from taro (*Colocasia esculenta cv fouê*) corm grown in Côte d'Ivoire. *Journal of food science and technology*, *51*(5), 855-864.

Amoo, I. A., Emenike, A. E., & Akpambang, V. O. E. (2008). Compositional evaluation of Annona cherimoya (*Custard Apple*) fruit. *Trends in applied sciences research*, 3(2), 216-220.

Amoussa, A. M. O., Sanni, A., & Lagnika, L. (2015). Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of Acacia ataxacantha. *Journal of Pharmacognosy and Phytochemistry*, *4*(2), 172-178.

András, C. D., Simándi, B., Örsi, F., Lambrou, C., Missopolinou-Tatala, D., Panayiotou, *et al*., (2005). Supercritical carbon dioxide extraction of okra (*Hibiscus esculentus L*) seeds. *Journal of the Science of Food and Agriculture*, 85(8), 1415-1419.

Anonymous (2002). Annu. Rep., Horticulture Crop Statistics, Karnataka, State at a Glance, p.29.

Anonymous (1973). Tannic acid gain. Food Cosmetol Toxicol. In: Toxicants Naturally occurring in foods. National Academy of Sciences. Third Edition. pp 112.

Ansari, N. M., Houlihan, L., Hussain, B., & Pieroni, A. (2005). Antioxidant activity of five vegetables traditionally consumed by south-Asian migrants in Bradford, Yorkshire, UK. *Phytotherapy Research*, *19*(10), 907-911.

Antia, S., Akpan, E. J., Okon, P. A., & Umoren, I. U. (2006). Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pakistan Journal of Nutrition*, 5: 166-168.

Anwar, F., Rashid, U., Ashraf, M., & Nadeem, M. (2010). Okra (*Hibiscus esculentus*) seed oil for biodiesel production. *Applied Energy*, *87*(3), 779-785.

Anwar, F., Rashid, U., Mahmood, Z., Iqbal, T., & Sherazi, T. H. (2011). Inter-varietal variation in the composition of okra (*Hibiscus esculentus*.) seed oil. *Pakistan Journal of Botany*, 43(1), 271-280.

AOAC, (1984). Official Methods of Analysis Association of Official Analytical Chemists.*4th Edition.* Washington, DC.

AOAC, (2000). Association of Official Analytical Chemists. Official methods of Analysis (Vol. II 17th edition) of AOAC International. Washington, DC, USA. Official methods.

Appiah, F., Asibuo, J. Y., & Kumah, P. (2011). Physicochemical and functional properties of bean flours of three cowpea (*Vigna unguiculata L. Walp*) varieties in Ghana. *African Journal of Food Science*, *5*(2), 100-104.

Arapitsas, P. (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. *Food Chemistry*, *110*(4), 1041-1045.

Ardabili, A., Farhoosh, R., & Haddad Khodaparast, M. H. (2011). Chemical composition and physicochemical properties of pumpkin seeds (*Cucurbita pepo subsp. pepo var. Styriaka*) grown in Iran. *Journal of Agricultural Science and Technology*, 13, 1053-1063.

Aremu, M. O., Olaofe, O., & Akintayo, E. T. (2007). Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. *Journal of Food Technology*, *5*(2), 109-113.

Aremu, M. O., Olonisakin, A., Atolaye, B. O., & Ogbu, C. F. (2006). Some nutritional and functional studies of Prosopis africana. *Electron. Journal of Environmental and Agriculture and Food Chemistry*,*5*(6), 1640-1648.

Arinathan, V., Mohan, V. R., & John De Britto, A. (2003). Chemical composition of certain tribal pulses in South India. *International journal of food sciences and nutrition*, *54*(3), 209-217.

Arinola, S. O., Ogunbusola, E. M., & Adebayo, S. F. (2016). Effect of Drying Methods on the Chemical, Pasting and Functional Properties of Unripe Plantain (*Musa paradisiaca*) Flour. *British Journal of Applied Science & Technology* 14(3): 1-7.

Ariponnammal, S. (2012). A novel method of using refractive index as a tool for finding the adultration of oils. *Research Journal of Recent Sciences*, 1(7): 77-79.

Atasie, V. N., Akinhanmi, T. F., & Ojiodu, C. C. (2009). Proximate analysis and physico-chemical properties of groundnut (*Arachis hypogaea L.*). *Pakistan Journal of Nutrition*, *8*(2), 194-197.

Attigah, A. S., Asiedu, E. K., Agyarko, K., & Dapaah, H. K. (2013). Growth and yield of okra (Abelmoschus esculentus L.) as affected by organic and inorganic fertilizers. *ARPN J Agric Biol Sci*, *8*(12), 766-770.

Awobajo, F. O., Olatunji-Bello, I. I., Obilade, T. T., & Odugbemi, T. O. (2010). Knowledge of the Nutritional and medicinal use of some vegetables among a cross section of market women in two major food markets in Lagos State, South West Nigeria. *Pakistan Journal of Nutrition*, *9*(3), 216-221.

Babatunde, R. O., Omotesho, O. A., & Sholotan, O. S. (2007). Socio-economic characteristics and food security status of farming households in Kwara State, North-Central Nigeria. *Pakistan Journal of Nutrition*, *6*(1), 49-58.

Bamishaiye, E. I., Olayemi, F. F., Awagu, E. F., & Bamshaiye, O. M. (2011). Proximate and phytochemical composition of Moringa oleifera leaves at three stages of maturation. *Advance Journal of Food Science and Technology*, *3*(4), 233-237.

Bankole, S. A., Osho, A., Joda, A. O., & Enikuomehin, O. A. (2005). Effect of drying method on the quality and storability of ‘egusi\'melon seeds (*Colocynthis citrullus L.*). *African Journal of Biotechnology*, *4*(8), 799-803.

Barkatullah, M. I., & Abdur, R. Inyat-Ur-Rahman (2012). Physicochemical characterization of essential and fixed oils of Skimmia laureola and Zanthoxylum armatum. *Middle-East Journal of Medicinal Plants Research*, 1(3), 51-58.

Baye, K. (2014). Teff: nutrient composition and health benefits. *Intl Food Policy Res Inst.* (Vol. 67).

Bayer, C., & Kubitzki K., (2003). The families and genera of vascular plants. In: Malvaceae, (Ed.) K. Kubitzki, pp 225-311.

Bazzano, L.A., He, J., Ogden, L.G., Loria, C.M. & Whelton, P.K. (2003). Dietary intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I epidemiologic follow-up study. *Archives of Internal Medicine*, 163, 1897–1904.

Bello, M. O., Falade, O. S., Adewusi, S. R. A., & Olawore, N. O. (2008). Studies on the chemical compositions and anti nutrients of some lesser known Nigeria fruits. *African Journal of Biotechnology*, *7*(21), 397-409.

Benchasri, S. (2012). Okra (Abelmoschus esculentus (L.) Moench) as a valuable vegetable of the world. *Ratarstvo i povrtarstvo*, *49*(1), 105-112.

Betteridge, D. J. (2000). What is oxidative stress?. *Metabolism*, *49*(2), 3-8.

BGRIO. (2010). Invest in Benshanguel Gumuz Regional State. Benshanguel Gumuz Region Investment Office (BGRIO), Assosa, Ethiopia.

Bhandari, M. R., & Kawabata, J. (2004). Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (*Dioscorea spp.*) tubers of Nepal. *Food chemistry*, *85*(2), 281-287.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, *5*(1), 9-19.

Biswal, B., Karna, N., & Patel, R. (2014). Okra mucilage act as a potential binder for the preparation of tablet formulation. *Der Pharmacia Lettre*, *6*(3), 31-39.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *Jama*, *297*(8), 842-857.

Blessing, A. C., Ifeanyi, U. M., & Chijioke, O. B. (2011). Nutritional evaluation of some Nigerian pumpkins (*Cucurbita spp.*). *Fruit*, *Vegetable* and *Cereal Science* and *Biotechnology*,*5*(2), 64-71.

Boateng, J., Verghese, M., Walker, L. T., & Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (*Phaseolus spp. L.*). *LWT-Food Science and Technology*, *41*(9), 1541-1547.

Bozokalfa, K. M., Eşiyok, D., & Yağmur, B. (2011). Use of multivariate analysis in mineral accumulation of rocket (*Eruca sativa*) accessions. *Genetika*, *43*(3), 437-448.

Bryant, L. A., Montecalvo, J., Morey, K. S., & Loy, B. (1988). Processing, functional, and nutritional properties of okra seed products. *Journal of Food Science*, *53*(3), 810-816.

Butt, M. S., & Batool, R. (2010). Nutritional and functional properties of some promising legumes protein isolates. *Pakistan Journal of Nutrition*, *9*(4), 373-379.

Bwai, M. D., Adedirin, O., Akanji, F. T., Muhammad, K. J., Idoko, O., & Useh, M. U. (2013). Physicochemical Properties, Fatty Acids Profiles and Antioxidant Properties of Seed Oil of Breadfruit(Treculia africana). International Journal of Research in Pharmacy and Science, 3(3), 44-54.

Çalışır, S., Özcan, M., Hacıseferoğulları, H., & Yıldız, M. U. (2005). A study on some physico-chemical properties of Turkey okra (Hibiscus esculenta L.) seeds. *Journal of Food Engineering*, *68*(1), 73-78.

Camciuc, M., Bessière, J. M., Vilarem, G., & Gaset, A. (1998a). Volatile components in okra seed coat. *Phytochemistry*, *48*(2), 311-315.

Camciuc, M., Deplagne, M., Vilarem, G., & Gaset, A. (1998b). Okra—*Abelmoschus esculentus L.(Moench.*) a crop with economic potential for set aside acreage in France. *Industrial Crops and Products*, 7(2), 257-264.

Capitani, M. I., Tomás, M. C., & Nolasco, S. M. (2013). Effect of mucilage extraction on the functional properties of Chia meals. *INTECH Open Access Publisher.http://dx.doi.org/10.5772/53171*.

Cert, A., Moreda, W., & Pérez-Camino, M. C. (2000). Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A*, *881*(1), 131-148.

Chahal, G.S. and Gosal, S.S. (2002). Principles and Procedures of Plant Breeding: Biotechnology and Conventional Approaches. *Alpha Science International, United Kingdom,* ISBN: 9781842650363, Pages: 604.

Chandra, S., Singh, S., & Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of food science and technology*, *52*(6), 3681-3688.

Chandran, R., Nivedhini, V., & Parimelazhagan, T. (2013). Nutritional composition and antioxidant properties of Cucumis dipsaceus ehrenb. ex spach leaf. *The Scientific World Journal*, *3*.

Chauhan, A., & Singh, S. (2013). Influence of germination on physico–chemical properties of amaranth (*Amaranthus Spp.*) flour. *International Journal of Agriculture, Food Science and Technology*, *4*, 215-220.

Chen, G., Wang, H., Zhang, X., & Yang, S. T. (2014). Nutraceuticals and functional foods in the management of hyperlipidemia. *Critical reviews in food science and nutrition*, *54*(9), 1180-1201.

Chitsa, H., Mtaita, T., & Tabarira, J. (2014). Nutrient content of water spinach (Ipomoea aquatica) under different harvesting stages and preservation methods in Zimbabwe. *International Journal of Biological and Chemical Sciences*, *8*(3), 854-861.

Chukwuma, O. E., Taiwo, O. O., & Boniface, U. V. (2016). Effect of the Traditional Cooking Methods (Boiling and Roasting) on the Nutritional Profile of Quality Protein Maize. *Journal of Food and Nutrition Sciences*, *4*(2), 34-40.

ChutichudetBenjawan, P. C., & Kaewsit, S. (2007). Effects of green manures on growth, yield and quality of green okra (Abelmoschus esculentus L.) Har Lium Cultivar. *Pakistan Journal of Biological Sciences*, *10*(7), 1028-1035.

Codex Alimentairus Commission. (1993). Graisses et huiles vegetables, division 11. *Version abregee FAO/WHO Codex stan*, 20-1981.

D'souza, M. R. (2013). Effect of traditional processing methods on nutritional quality of field bean. *Advanced Bioresearch*, *4*(3), 29-33.

Dandena, G. (2010). Shifting to alternative food source: potential to overcome ethiopias' malnutrition and poverty problems. emilie coudel, hubert devautour, christophe-toussaint soulard, bernard hubert. montpellier, france. *Cirad-Inra-SupAgro*, *10*.

Dastmalchi, K., Dorman, H. D., Koşar, M., & Hiltunen, R. (2007). Chemical composition and in vitro antioxidant evaluation of a water-soluble Moldavian balm (*Dracocephalum moldavica L.*) extract. *LWT-Food Science and Technology*, *40*(2), 239-248.

Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary in edible seaweed products. *Food Chemistry*, *103*(3), 891-899.

Deeplata, S & Rao, DV. (2013).Study of Metabolites of Okra (Abelmoschus esculentus) After Infection of Pest. *International Journal of phermacetical science and research.*, 21(2): 347-350.

Demissie, T., Ali, A., & Zerfu, D. (2009). Availability and consumption of fruits and vegetables in nine regions of Ethiopia with special emphasis to vitamin A deficiency. *Ethiopian Journal of health development*, *23*(3).

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of agricultural and food chemistry*, *50*(10), 3010-3014.

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

Dias, J. S., & Ryder, E. (2011). World vegetable industry: production, breeding, trends. *Horticultural Reviews*, *38*, 299.

Diemeleou, C. A., Zoue, L. T., & Niamke, S. L. (2014). Basella alba seeds as a novel source of non-conventional oil with beneficial qualities. *Romanian Biotechnological Letters*, 19(1), 8966-8978.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, *80*(12), 1744-1756.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of food and drug analysis*, *22*(3), 296-302.

Duenas, M., Hernandez, T., Estrella, I., & Fernandez, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus angustifolius L.). *Food Chemistry*, *117*(4), 599-607.

Ebrahimzadeh, M. A., Pourmorad, F., & Hafezi, S. (2008). Antioxidant activities of Iranian corn silk. *Turkish Journal of biology*, *32*(1), 43-49.

Edem, CA., Dosunmu, MI. & Bassey, FI. (2009). Determination of Proximate Composition, Ascorbic Acid & Heavy Metal Content of African Walnut (*Tetracarpidium conophorum*). *Pakistani Journal Nutrition*, 8(3): 225-226.

Edema, M. O., Sanni, L. O., & Sanni, A. I. (2005). Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. *African Journal of Biotechnology*, *4*(9).

Edidiong, A. E. & Ubong, M. E. (2013). Analysis of Citrullus lanatus seed oil obtained from southern Nigeria. *Elixir organic chem*. 54:12700- 12703.

Edwards, C. A., & Parrett, A. M. (2003). Dietary in infancy and childhood. *Proceedings of the Nutrition Society*, *62*(01), 17-23.

Effiong, G. S., Ibia, T. O., & Udofia, U. S. (2009). Nutritive and energy values of some wild fruit spices in south eastern Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(10), 917-923.

EHNRI, (1997). Food composition table for use in Ethiopia. Part III. Ethiopian Health and Nutrition Research Institute. Addis Ababa; Ethiopia..

Ejikeme, P. M., Obasi, L. N., & Egbuonu, C. C. (2010). Physico-chemical and toxicological studies on *Afzelia africana* seed and oil. *African Journal of Biotechnology*, 9(13), 1959-1963.

Eka, B. E., & Chidi, E. A. (2009). Physico-chemical characterisation of Butternut (*Jugulans Cinerea*) oil. *Global Journal of Pure and Applied Sciences*, 15(3), 340.

Ekumankama, I. O. (2008). Nutrient composition of indigenous vegetables (*Pterocarpus soyanxii*, *Pterocarpus santalinoides and Gnetum africanum*). *Nig. J. Nutr. Sci*, *29*, 195-200.

Ekwu, F. C., & Nwagu, A. (2004). Effect of processing on the quality of cashew nut oils. *Journal of Science and Agriculture, Food Technology an Environment*, 4, 105-110.

El Sohaimy, S. A. (2013). The effect of cooking on the chemical Composition of Artichoke (*Cynara scolymus L.*). *African Journal of Food Science and Technology*, *3*, 182-187.

Elbaloula, M. F., Runqiang, Y., Qianghui, G. and Zhenxin G. (2014). Major nutrient compositions and functional properties of sorghum flour at 0–3 days of grain germination. *International journal of food sciences and nutrition* 65,(1): 48-52.

Elinge, C. M., Muhammad, A., Atiku, F. A., Itodo A. U., Peni, I. J., Sanni, O. M. *et al*. (2012). Proximate, Mineral and Anti-nutritional Composition of Pumpkin (*Cucurbita pepo L.*) Seeds Extracts. *International Journal of Plant Research*. 2(5):146- 150.

Emana, B., Afari-Sefa, V., Dinssa, F. F., Ayana, A., Balemi, T., & Temesgen, M. (2015). Characterization and Assessment of Vegetable Production and Marketing Systems in the Humid Tropics of Ethiopia. *Quarterly Journal of International Agriculture*, *54*(2), 163-187.

Enujiugha, V. N., Badejo, A. A., Iyiola, S. O., & Oluwamukomi, M. O. (2003). Effect of germination on the nutritional and functional properties of African oil bean (*Pentaclethra macrophylla Benth*) seed flour. *Journal of Food Agriculture and Environment*, *1*, 72-75.

Eromosele, C. O., & Paschal, N. H. (2003). Characterization and viscosity parameters of seed oils from wild plants. *Bioresource Technology*, 86(2), 203-205.

Eshun, G., Amankwah, E. A., & Barimah, J. (2013). Nutrients content and lipid characterization of seed pastes of four selected peanut (*Arachis hypogaea*) varieties from Ghana. *African Journal of Food Science*, *7*(10), 375-381.

Etong, D. I., Ayeni, K. E. Ajayi O. O. & Oladimeji M. O. (2013). Physicochemical properties and Nutritional values of Melina fruit (*Gmelina arboreal*) and mango (*Mangifera indica*) seed. *International Journal of Consumer Studies,* 6(1):56-62.

Falade, K. O., & Omojola, B. S. (2010). Effect of processing methods on physical, chemical, rheological, and sensory properties of okra (Abelmoschus esculentus). *Food and Bioprocess Technology*, *3*(3), 387-394.

Falade, O. S., Adekunle, A. S., Aderogba, M. A., Atanda, S. O., Harwood, C., & Adewusi, S. R. (2008). Physicochemical properties, total phenol and tocopherol of some Acacia seed oils. *Journal of the Science of Food and Agriculture*, 88(2), 263-268.

Fanos, T., & Derbew, B., (2015). A Review on Production Status and Consumption Pattern of Vegetable in Ethiopia. *Journal of Biology, Agriculture and Healthcare*,5(21), 214-136.

FAO, (2009), The State of Food and Agriculture, FAO, Rome, Italy.

FAO, W. I., WFP, & IFAD. (2012). The State of Food Insecurity in the World 2012: Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. *FAO, Rome. doi*, *10*.

Farias, G. A., Guzmán-Martínez, L., Delgado, C., & Maccioni, R. B. (2014). Nutraceuticals: a novel concept in prevention and treatment of Alzheimer's disease and related disorders. *Journal of Alzheimer's Disease*, *42*(2), 357-367.

Farinde, A. J., Owolarafe, O. K., & Ogungbemi, O. I. (2007). An overview of production, processing, marketing and utilisation of okra in egbedore local government area of Osun State, Nigeria. Agricultural Engineering International : the CIGR Ejournal. Manuscript No. MES 07 002. Vol. IX.

Farooq, U., Malviya, R., and Sharma,P. K. (2013). Extraction and Characterization of Okra Mucilage as Pharmaceutical Excipient. *Academic Journal of Plant Sciences* 6 (4): 168-172.

Fasasi, O. S., Eleyinmi, A. F., Fasasi, A. R., & Karim, O. R. (2004). Chemical properties of raw and processed breadfruit (*Treculia africana*) seed flour. In *African Crop Science Conference Proceedings*, 2 (1), 65-68.

Fedeniuk, R. W., & Biliaderis, C. G. (1994). Composition and physicochemical properties of linseed (Linum usitatissimum L.) mucilage. *Journal of Agricultural and Food Chemistry*, *42*, 240-240.

Fekadu, M., & Dandena, G. (2006). Review of the status of vegetable crops production and marketing in Ethiopia. *Uganda J. Agric. Sci*, *12*(2), 26-30.

Fennema, R. O. (1996). Food Chemistry,(3rd edn) pp. 365-369 Marcel Dekker, Inc. *New York. Basel. Hong Kong*.

Ferreira, I. C., Baptista, P., Vilas-Boas, M., & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food chemistry*, *100*(4), 1511-1516.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*, 239-247.

Florkowski J.W., Robert L. S., Bernhard B. and Stanley E. P. (2009). Postharvest Handling: A Systems Approach, Second Edition. Elsevier Inc. Academic Press.

Franklin W. M. (1982). Okra, Potential Multiple-Purpose Crop for the Temperate Zones and Tropics". Economic Botany, 36(3), 340–345.

Frontela, C., Ros, G., & Martínez, C. (2009). Effect of dephytinization on bioavailability of iron, calcium and from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterol*ogy, *15*(16), 1977-1984.

Fukumoto, L. R., & Mazza, G. (2000). Assessing antioxidant andprooxidant activities and phenolic compounds.Journal of Agriculturaland Food Chemistry, 48, 3597–3604.

Gallegos-Infante, J. A., Rocha-Guzman, N. E., Gonzalez-Laredo, R. F., & Pulido-Alonso, J. (2010). Effect of processing on the antioxidant properties of extracts from Mexican barley (*Hordeum vulgare*) cultivar. *Food Chemistry*, *119*(3), 903-906.

García-Arias, M., Alvarez-Pontes, E., García-Fernández, M. C., & Sánchez-Muniz, F. J. (2003). Freezing/defrosting/frying of sardine fillets. Influence of slow and quick defrosting on protein quality. *Journal of the Science of Food and Agriculture*, *83*(6), 602-608.

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules*, *15*(12), 8813-8826.

Gashu, D., Stoecker, B. J., Bougma, K., Adish, A., Haki, G. D., & Marquis, G. S. (2016). Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal*, *15*(1), 38.

Gebresamuel, N., & Gebre-Mariam, T. (2011). Comparative physico-chemical characterization of the mucilages of two cactus pears (Opuntia spp.) obtained from Mekelle, Northern Ethiopia. *Journal of Biomaterials and Nanobiotechnology*,3, 79-86.

Gebreselassie, S. (2006). Food Aid and Small-holder Agriculture in Ethiopia: Options and Scenarios. A Paper for the Future Agricultures Consortium Workshop, Institute of Development Studies 20-22 March Sussex, UK: Future Agricultures Consortium.

Gelmesa, D. (2010). Shifting to alternative food source: potential to overcome ethiopias'malnutrition and poverty problems. In emilie coudel, hubert devautour, christophe-toussaint soulard, bernard hubert. montpellier, france. Cirad-Inra-SupAgro.

Gemede, H. F., Haki, G. D., Beyene, F., Woldegiorgis, A. Z., & Rakshit, S. K. (2015a). Proximate, mineral, and antinutrient compositions of indigenous Okra (*Abelmoschus esculentus*) pod accessions: implications for mineral bioavailability. *Food science & nutrition*. 4(2): 223–233.

Gemede, H. F., Ratta, N., Haki, G. D., Woldegiorgis, A. Z., & Beyene, F. (2015b). Nutritional quality and health benefits of okra (Abelmoschus esculentus): A review. *Journal of Food Processing & Technology*, *6*(6), 1.

Giami, S. Y., & Alu, D. A. (1994). Changes in composition and certain functional properties of ripening plantain (Musa spp., AAB group) pulp. *Food chemistry*, *50*(2), 137-140.

Giami, S.Y. (2003). Effect of processing on the proximate composition and functional properties of cowpea (*Vigna Ungniculate*) flour. *Food Chemistry*, 47:153-158.

Gibson, R. S., Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *Proceedings of the Nutrition Society*, *65*(02), 160-168.

Gockowski, J., Mbazo'o, J., Mbah, G., & Moulende, T. F. (2003). African traditional leafy vegetables and the urban and peri-urban poor. *Food policy*, *28*(3), 221-235.

Gohari Ardabili, A., Farhoosh, R., & Haddad Khodaparast, M. H. (2011). Chemical composition and physicochemical properties of pumpkin seeds (Cucurbita pepo subsp. pepo var. Styriaka) grown in Iran. *Journal of Agricultural Science and Technology*, *13*, 1053-1063.

Golden, M. (2009). Proposed nutrient requirements of moderately malnourished populations of children. *Food Nutr Bull*, *30*(3), S267-S342.

Gómez-Alonso, S., Salvador, M. D., & Fregapane, G. (2002). Phenolic compounds profile of Cornicabra virgin olive oil. *Journal of agricultural and food chemistry*, *50*(23), 6812-6817.

González-Vallinas, M., González-Castejón, M., Rodríguez-Casado, A., & de Molina, A. R. (2013). Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. *Nutrition reviews*, *71*(9), 585-599.

Goplana, C., Sastri, SBV., & Balasubramanian, S. (2007). Nutritive value of Indian foods, National Institute of Nutrition (NIN), ICMR, India, *1*(2), 49-54.

Greiner, R., & Konietzny, U. (2006). Phytase for food application. *Food Technology and Biotechnology*, *44*(2), 123-140.

Grivetti, L. E., & Ogle, B. M. (2000). Value of traditional foods in meeting macro-and micronutrient needs: the wild plant connection. *Nutrition Research Reviews*, *13*(01), 31-46.

Gujska, E., Reinhard, W. & Khan, K. (1994). Physicochemical properties of field pea, pinto and navy bean starches. *Journal of Food Science*, *59*(3), 634-636.

Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, *217*(2), 213-220.

Gülçin, İ., Topal, F., Çakmakçı, R., Bilsel, M., Gören, A. C., & Erdogan, U. (2011). Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (*Rubus idaeus L.*). *Journal of food science*, *76*(4).

Gulsen, O., Karagul, S., & Abak, K. (2007). Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biologia*, *62*(1), 41-45.

Gupta, S., Lakshmi, A. J., Manjunath, M. N., & Prakash, J. (2005). Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT-Food Science and Technology, 38(4), 339-345.

Gupta, S., Parvez, N., & Sharma, P. K. (2015). Extraction and characterization of Hibiscus rosa sinensis mucilage as pharmaceutical adjuvant. *World Appl Sci J*, *33*(1), 136-141.

Gursoy, N., Sarikurkcu, C., Tepe, B., & Solak, M. H. (2010). Evaluation of antioxidant activities of 3 edible mushrooms: Ramaria flava (Schaef.: Fr.) Quél., Rhizopogon roseolus (Corda) TM Fries., and Russula delica Fr. *Food Science and Biotechnology*, *19*(3), 691-696.

Guyot, J.P., Rochette, I., and Treche, S., (2007). Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children. *Food Chemistry*, 100; 623–63.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry*, *4*(8), 142-151.

Hamzah, R. U., Jigam, A. A., Makun, H. A., & Egwim, E. C. (2013). Antioxidant properties of selected African vegetables, fruits and mushrooms: a review. *Licensee in Tech. Mycotoxin and Food Safety in Developing Countries*, 203-249.

Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S., & Rasheed, M. (2006). Use of vegetables as nutritional food: role in human health. *Journal of Agricultural and Biological Science*, *1*(1), 18-20.

Haruna, S., Aliyu, B. S., & Bala, A. (2016). Plant gum exudates (Karau) and mucilages, their biological sources, properties, uses and potential applications: A review. *Bayero Journal of Pure and Applied Sciences*, *9*(2), 159-165.

Hassan, M. A., & Ali, H. M. (2015). The Nutritional Composition of Three Cultivars of Okra (Abelmoschus esculentus L.) Seeds Flour. *World Journal of Dairy & Food Sciences*, *10*(2), 122-131.

Hayder, A., Bari, A., MY, R., & MA, A. (2009). Studying the effect addition of Okra-natural mucilage as drag reducing agent in different size of pipes in turbulent water flowing system, National Conference on Postgraduate Research (NCON-PGR), UMP Conference, Malaysia, Centre for Graduate Studies, Universiti Malaysia Pahang.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of nutritional biochemistry*, *13*(10), 572-584.

Hong, N. T., & Ibrahim, N. H. (2012). Extraction and characterization of mucilage from leaves of pereskia bleo (rose cactus). *Jurnal Teknologi dan Industri Pangan*, *23*(2), 210.

Hooda, S., & Jood, S. (2003). Effect of soaking and germination on nutrient and antinutrient contents of fenugreek (*Trigonella foenum graecum L.*). *Journal of food biochemistry*, *27*(2), 165-176.

Hoskin, P. W., & Ireland, T. R. (2000). Rare earth element chemistry of zircon and its use as a provenance indicator. *Geology*, *28*(7), 627-630.

Hotta, H., Nagano, S., Ueda, M., Tsujino, Y., Koyama, J., & Osakai, T. (2002). Higher radical scavenging activities of polyphenolic antioxidants can be ascribed to chemical reactions following their oxidation. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1572*(1), 123-132.

Hrastar, R., Petrišič, M. G., Ogrinc, N., & Košir, I. J. (2009). Fatty acid and stable carbon isotope characterization of Camelina sativa oil: implications for authentication. *Journal of agricultural and food chemistry*, *57*(2), 579-585.

Hu, L., Yu, W., Li, Y., Prasad, N., & Tang, Z. (2014). Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride. *BioMed research international*, 103, 1395-1402.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry*, *53*(6), 1841-1856.

Huang, Z., Wang, B., Eaves, D. H., Shikany, J. M., & Pace, R. D. (2007). Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast United States. *Food Chemistry*, *103*(4), 1395-1402.

Hue, S. M., Boyce, A. N., & Chandran, S. (2012). Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (Ipomoea batatas). *Australian Journal of Crop Science*, *6*(3), 375-380.

Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S. R., Dassenko, S. A., & Cook, J. D. (2003). Soy protein, phytate, and iron absorption in humans. *The American journal of clinical nutrition*, *56*(3), 573-578.

Hussain, J., Khan, A. L., Rehman, N., Hamayun, M., Shah, T., Nisar, M., *et al*., (2009). Proximate and nutrient analysis of selected vegetable species: A case study of Karak region, Pakistan. *African Journal of Biotechnology*, *8*(12).

Hussain, J., ur Rehman, N., Al-Harrasi, A., Ali, L., Ullah, R., Mabood, F., ... & Ismail, M. (2011). Nutritional prospects and mineral compositions of selected vegetables from Dhoda sharif Kohat. *Journal of Medicinal Plants Research*, *5*(29), 6509-6514.

Idouraine, A., Kohlhepp, E. A., Weber, C. W., Warid, W. A., & Martinez-Tellez, J. J. (1996). Nutrient constituents from eight lines of naked seed squash (Cucurbita pepo L.). *Journal of agricultural and food chemistry*, 44(3), 721-724.

Ihemeje, A., Ukauwa, O., & Ekwe, C. C. (2015). Effects of cooking and germination on physiochemical properties and sensory attributes of african walnut (*tetracarpidium conophorum*). *International Journal of Pharmacology, Phytochemistry and Ethnomedicine*, 1: 93-102.

Ijarotimi, O. S., Adeoti, O. A., & Ariyo, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringa oleifera seed flour. *Food science & nutrition*, *1*(6), 452-463.

Ijeh, I. I., Unaegbu, S. O., & Anaga, A. O. (2004). Studies on some nutritional and toxicological properties of Mucuna sloanei. *Bio-research*, *2*(1), 24-28.

Ikewuchi, C. C., & Ikewuchi, J. C. (2008). Chemical profile of Pleurotus tuberregium (Fr) Sing's sclerotia. *Pacific J Sci Technol*, *10*, 295-9.

Inekwe, U. V., Odey, M. O., Gauje, B., Dakare, A. M., Ugwumma, C. D., & Adegbe, E. S. (2012). Fatty acid composition and physicochemical properties of Jatropha curcas oils from Edo and Kaduna states of Nigeria and India. *Annals of Biological Research*, 3(10), 4860-4864.

Isaac, I. O., & Ekpa, O. D. (2009). Minerals and anti-nutrients in two varieties of African pear (Dacryodes edulis). *Journal of Food Technology*, *7*(4), 106-110.

Ismail, M. A., & Ibn Idriss, E. M. (2013). Mathematical modelling of thin layer solar drying of whole okra (Abelmoschosus esculentus (L.). Moench) pods. *International Food Research Journal*, *20*(4), 1983-1989.

Jacob, A. G., Etong, D. I., & Tijjani, A. (2015). Proximate, Mineral and Anti-nutritional Compositions of Melon (*Citrullus lanatus*) Seeds. *British Journal of Research*, *2*(5), 142-151.

Jannat, B., Oveisi, M. R., Sadeghi, N., Hajimahmoodi, M., Behzad, M., Choopankari, E., & Behfar, A. A. (2010). Effects of roasting temperature and time on healthy nutraceuticals of antioxidants and total phenolic content in Iranian sesame seeds (*Sesamum indicum L.*). *Iranian Journal of Environmental Health science and Engineering*, 7:97-102

Jarret, R. L., Wang, M. L., & Levy, I. J. (2011). Seed oil and fatty acid content in okra (Abelmoschus esculentus) and related species. *Journal of agricultural and food chemistry*, 59(8), 4019-4024.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food chemistry*, *83*(4), 547-550.

Jayewardenapura, N. (2000). Nutritional evaluation of protein and starch of mature Canavalia gladiata seeds. *International journal of food sciences and nutrition*, *51*, 289-294.

Jeff, S. (2002). Growing okra, Backyard gardener. Retrieved from http:// ag.arizona.edu/yavapai/anr/hort/byg/archive/growingokra.html.

Jenab M. and Thompson, L.U.(2002). Role of phytic acid in cancer and other diseases. In: Reddy, N.R., Sathe, S.K. (Eds.), Food Phytates. CRC Press, Boca Raton, FL, pp. 225–248. *Journal of Clinical Nutrition* 8, 64-74.

Jenkins, D. J., Kendall, C. W., Marchie, A., Faulkner, D. A., Wong, J. M., de Souza, R., ... & Lapsley, K. G. (2005). Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. *The American journal of clinical nutrition*, *81*(2), 380-387.

Jeong, S. M., Kim, S. Y., Kim, D. R., Nam, K. C., Ahn, D. U., & Lee, S. C. (2004). Effect of seed roasting conditions on the antioxidant activity of defatted sesame meal extracts. *Journal of Food Science Chicago*, 69:377-381.

Jideani, V. A., & Bello, B. M. (2009). Functional properties of okra protein products containing different levels of mucilage. *Journal of Food, Agriculture & Environment*, *7*(2), 252-255.

Jitngarmkusol, S., Hongsuwankul, J., & Tananuwong, K. (2008). Chemical compositions, functional properties, and microstructure of defatted macadamia flours. *Food chemistry*, *110*(1), 23-30.

Kaewmanee, T., Bagnasco, L., Benjakul, S., Lanteri, S., Morelli, C. F., Speranza, G., & Cosulich, M. E. (2014). Characterisation of mucilages extracted from seven Italian cultivars of flax. *Food chemistry*, *148*, 60-69.

Kajihausa, O. E., Fasasi, R. A., & Atolagbe, Y. M. (2014). Effect of different soaking time and boiling on the proximate composition and functional properties of sprouted sesame seed flour. *Nigerian Food Journal*, *32*(2), 8-15.

Kalava, V., & Menon, S. (2012). In-vitro free radical scavenging activity of aqueous extract from the mycella of volvariella volvacea (*bulliard ex fries*) singer. *Int J Current Pharmaceutical Res*, *4*, 7.

Kamga, R. T., Kouamé, C., Atangana, A. R., Chagomoka, T., & Ndango, R. (2013). Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research*, *21*(1), 99-106.

Kašparová, S., Brezová, V., Valko, M., Horecký, J., Mlynárik, V., Liptaj, T., *et al*. (2005). Study of the oxidative stress in a rat model of chronic brain hypoperfusion. *Neurochemistry international*, *46*(8), 601-611.

Kathirvel, P., & Kumudha, P. (2011). A Comparative Study on the Chemical Composition of Wild and Cultivated Germplasm of *Phaseolus Lnatus L*. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(4), 298.

Kaushal, P., Kumar, V., & Sharma, H. K. (2012). Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colocasia esculenta*), rice (Oryza sativa) flour, pigeonpea (*Cajanus cajan*) flour and their blends. *LWT-Food Science and Technology*, *48*(1), 59-68.

Kayode, R. M., Sani, O. A., Apata, D. F., Joseph, J. K., Olorunsanya, O. A., Amnongu, A. A., *et al*. (2011). Physico-chemical and anti-nutritional characterization of the kernels of some mango (*Mangifera indica*) cultivars grown in parts of Nigeria. *Bioresearch bulletin*, 61:1-8.

Keatinge D. (2012). Vegetables: Less visible, but vital for human health - Why nutrient-dense indigenous vegetables must be on the plate for economic development, food security, and health. AVRDC News Brief.

Keatinge, J. D., Waliyar, F., Jamnadas, R. H., Moustafa, A., Andrade, M., Drechsel, P., ... & Luther, K. (2010). Relearning old lessons for the future of food by bread alone no longer: diversifying diets with fruit and vegetables. *Crop Science*, *50*(1), 51-62.

Khan, M. N., Soherwardy, R., & Sarwar, A. (2015). Nutritional assessment of olive fruit (*Oleaeuropaea*) available at local market. *International Food Research Journal*, *22*(2), 526-531.

Khanzadeh, F., Haddad Khodaparast, M. H., Elhami Rad, A. H., & Rahmani, F. (2012). Physiochemical properties of Gundelia tournefortii L. seed oil. *Journal of Agricultural Science and Technology*, 14, 1535-1542.

Khattak, G. S. S., Ashraf, M., Zamir, R., & Saeed, I. (2007). High yielding DESI chickpea (*Cicer arietinum L.*) variety. *Pakistan Journal of Botany*, *39*(1), 93.

Kiin-Kabari, D. B., & Akusu, O. M. (2014). Effect of processing on the proximate composition, functional properties and storage stability of water melon (*Citrullus Lanatus*) seed flour. *International Journal of Biotechnology and Food Science*, *2*(7), 143-148.

Kim, S. Y., Jeong, S. M., Park, W. P., Nam, K. C., Ahn, D. U., & Lee, S. C. (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. *Food chemistry*, *97*(3), 472-479.

Knochel, J. P., 2006. Phosphorus. In: Shils, M. E., Shike, M., Ross, A. C., Caballero, B. and Cousins, R. J., eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams and Wilkins, pp. 211-222.

Knothe, G., & Steidley, K. R. (2005). Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. *Fuel*, 84(9), 1059-1065.

Kolakowska, A., & Sikorski, Z. E. (Eds.). (2003). *Chemical and functional properties of food lipids* (pp. 133-166). New York: CRC Press.

Kolawole, O. M., Ajiboye, A. E., Aturu, E. E., & Anibijuwon, I. I. J. (2011). Effect of solar drying on the proximate and microbial composition of *Abelmoschus esculentus*. *Microbiology and Biotechnology Research*, *1*(1), 71-81.

Kouakou, B., N'Da-Kouassi, A. M., Halbin, K. J., Tagro, G., N'Guessan, K. F., *et al*., (2013). Biochemical characterization and functional properties of weaning food made from cereals (millet, maize) and legumes (beans, soybeans). *Journal of Food Chemistry and Nutrition*, *1*(1), 22-32.

Kumar, D. S., Tony, D. E., Kumar, A. P., Kumar, K. A., Srinivasa, D. B., & Nadendla, R. (2013). A review on Abelmoschus esculentus (Okra). *International Research Journal of Pharmaceutical and Applied Science*, *3*, 129-32.

Kumar, R., Patil, M. B., Patil, S. R., & Paschapur, M. S. (2009). Evaluation of Abelmoschus esculentus mucilage as suspending agent in paracetamol suspension. *International Journal of PharmTech Research*, *1*(3), 658-665.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D., & Kouame, C. (2010). Okra (Abelmoschus spp.) in West and Central Africa: potential and progress on its improvement. *African Journal of Agricultural Research*, *5*(25), 3590-3598.

Kumar, S., Sandhir, R., & Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. *BMC research notes*, *7*(1), 560.

Kuwada, H. (1974). F1 Hybrids of Abelmoschus tuberculatus× A. Manihot, with Reference to the Genome Relationship: Studies on Interspecific and Intergeneric Hybridization in the Malvaceae XI. *Japanese Journal of Breeding*, *24*(5), 207-210.

Kwenin, W. K. J., Wolli, M., & Dzomeku, B. M. (2011). Assessing the nutritional value of some African indigenous green leafy vegetables in Ghana. *Journal of Animal and Plant Sciences*, *10*(2), 1300-1305.

Lamont, W. J. (1999). Okra - A versatile vegetable crop. *HortTechnology*, *9*(2), 179-184.

Lee, Y. R., Woo, K. S., Kim, K. J., Son, J. R., & Jeong, H. S. (2007). Antioxidant activities of ethanol extracts from germinated specialty rough rice. *Food Science and Biotechnology*, *16*(5), 765-770.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food chemistry*, *76*(1), 69-75.

Lewu, M. N., Adebola, P. O., & Afolayan, A. J. (2009). Effect of cooking on the proximate composition of the leaves of some accessions of *Colocasia esculenta (L.)* Schott in KwaZulu-Natal province of South Africa. *African Journal of Biotechnology*, *8*(8): 1619 - 1622.

Lge, M. N., Ogunsua, O., & Okon, O. L. (1984). Functional properties of the protein of some Nigeria oil seeds. Casophor seeds and three varieties of some Nigeria oil seeds. *Food Chemistry*, 32, 822-825.

Li, X., Wang, X., Chen, D., & Chen, S. (2011). Antioxidant activity and mechanism of protocatechuic acid in vitro. *Functional Foods in Health and Disease*, *1*(7), 232-244.

Liebman, M. (2002). The truth about oxalate: answers to frequently asked questions. *The Vulvar Pain Newsletter*, (22).

Lim, T. K. (2012). *Edible medicinal and non-medicinal plants* (Vol. 1, pp. 656-687). New York, NY, USA:: Springer.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, *134*(12), 3479S-3485S.

Liu, S. C., Lin, J. T., Wang, C. K., Chen, H. Y., & Yang, D. J. (2009). Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis Sonn.*) flowers. *Food Chemistry*, *114*(2), 577-581.

Loewus, F. A. (2002). Biosynthesis of phytate in food grains and seeds. *Food phytates*, 53-61.

Lola, A. (2009). The effect of boiling on the nutrients and anti-nutrients in two non conventional vegetables. *Pakistan Journal of Nutrition*, *8*(9), 1430-1433.

Lorenz, A. J., Scott, M. P., & Lamkey, K. R. (2007). Quantitative determination of phytate and inorganic phosphorus for maize breeding. *Crop science*, *47*(2), 600-604.

Lyimo, M., Temu, R. P. C., & Mugula, J. K. (2003). Identification and nutrient composition of indigenous vegetables of Tanzania. *Plant Foods for Human Nutrition*, *58*(1), 85-92.

Manal, A., Hassan, M. & Hend, M. (2015). The Nutritional Composition of Three Cultivars of Okra (*Abelmoschus esculentus*) Seeds Flour. *World Journal of Dairy & Food Sciences* 10 (2): 122-131.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2000). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in food science & technology*, *11*(9), 340-346.

Maramag, R. P. (2013). Diuretic potential of Capsicum frutescens L., Corchorus oliturius L., and Abelmoschus esculentus L. *Asian journal of natural and applied science*, 2 (1). 60-69.

Mariod, A. A., Ahmed, S. Y., Abdelwahab, S. I., Cheng, S. F., Eltom, A. M., Yagoub, S. O., & Gouk, S. W. (2012). Effects of roasting and boiling on the chemical composition, amino acids and oil stability of safflower seeds. *International Journal of Food Science & Technology*, *47*(8), 1737-1743.

Massey, L. K., Palmer, R. G., & Horner, H. T. (2001). Oxalate content of soybean seeds (Glycine max: Leguminosae), soyfoods, and other edible legumes. *Journal of agricultural and food chemistry*, *49*(9), 4262-4266.

Mathur, P., & Choudhry, M. (2009). Effect of domestic processing on proximate composition of fenugreek seeds. *Journal of Food Science and Technology (Mysore)*, *46*(3), 255-258.

Maxson, E. D., & Rooney, L. W. (1972). Evaluation of methods for tannin analysis in sorghum grain. *Cereal Chemistry*, *49*(6), 719.

Mbah, B. O., Eme, P. E., & Ogbusu, O. F. (2012). Effect of cooking methods (boiling and roasting) on nutrients and anti-nutrients content of Moringa oleifera seeds. *Pakistan Journal of Nutrition*, *11*(3), 211.

McWhorter, J. H. (2000). The missing Spanish creoles: recovering the birth of plantation contact languages. Univ of California Press.

MEF, (2013). Biology of Okra. Series of crop specific biology document. *Ministry of Environmental and Forest Government of India*. p1-8.

Mensah, J. K., Okoli, R. I., Ohaju-Obodo, J. O., & Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology*, *7*(14).

Mepba, H. D., Eboh, L., & Banigo, D. E. B. (2007). Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *African Journal of Food, Agriculture, Nutrition and Development*, *7*(1); 1-16.

Middha, S. K., Usha, T., & Pande, V. (2013). HPLC evaluation of phenolic profile, nutritive content, and antioxidant capacity of extracts obtained from Punica granatum fruit peel. *Advances in pharmacological sciences*, *2013*. http://dx.doi.org/10.1155/2013/296236.

Mirhosseini, H., & Amid, B. T. (2012). A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Research International*, *46*(1), 387-398.

Mlitan, A. M., Sasi, M. S., & Alkherraz, A. M. (2014). Proximate and Minor Mineral Content in Some Selected Basil Leaves of Ocimum gratissimum L, in Libya. *International Journal of Chemical Engineering and Applications*, *5*(6), 502.

Mohamed, E. M., & Girgis, A. Y. (2005). Utilization of mango seed kernels for improving stability of some oils and biscuit production. *J. Agricult. Sci., Mansoura University*, 30, 4625-4636.

Mohammed, A., Woldeyohannes, D., Feleke, A., & Megabiaw, B. (2014). Determinants of modern contraceptive utilization among married women of reproductive age group in North Shoa Zone, Amhara Region, Ethiopia. *Reproductive health*, 11(1), 13.

Mohammed, M. I., & Hamza, Z. U. (2008). Physicochemical properties of oil extracts from Sesamum Indicum L. seeds grown in Jigawa state–Nigeria. *Journal of Applied Sciences and Environmental Management*, 12(2).

Mohammed, R. F., & Jorf-Thomas, M. (2003). Determination of the lipid classes and fatty acid profile of Niger seed (*Guizotia abyssinica Cass*). *Phytochemical Anaysisl*, *14*, 366-370.

Mohini, S., Eram, S.R., (2005). Food Science – Experiments and Applications. CBS Publishers. p. 22, 161.

Morris, E. R., & Ellis, R. (1989). Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. *Biological Trace Element Research*, *19*(1), 107-117.

Motalleb, G., Hanachi, P., Kua, S. H., Fauziah, O. and Asmah,R. (2005). Evaluation of Phenolic contentand total antioxidant activity in Berberis vulgaris fruit extracts. *Journal of Biological Sciences*, 5(5) 648–653.

Mullie, P., & Clarys, P. (2011). Association between cardiovascular disease risk factor knowledge and lifestyle. *Food and Nutrition Sciences*, *2(10), 1048-1053*.

Murray R.K., Granner D.K., Mayes P.A. & Rodwell V.W. (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.

NARP (1993). National Agricultural Research Project, Horticultural crops. NARP, CSIR, Accra. Vol. 3.

Nasar-Abbas, S. M., Plummer, J. A., Siddique, K. H., White, P., Harris, D., & Dods, K. (2008). Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT-Food Science and Technology*, *41*(7), 1260-1267.

Nath, K. G., Vijayalakshmi, D., Yankanchi, G. M., & Patil, R. B. (2009). Proximate composition of underutilized green leafy vegetables in Southern Karnataka. *Asian Journal of Home Science*, *3*(2), 118-120.

National Research Council, (2006). "Okra". Lost Crops of Africa: Volume II: Vegetables. Lost Crops of Africa. 2. National Academies Press. ISBN: 0-309-66582-5, 378 pages.

Naveed, A., Khan, A. A., & Khan, I. A. (2009). Generation mean analysis of water stress tolerance in okra (Abelmoschus esculentus L.). *Pak. J. Bot*, *41*(1), 195-205.

Ndangui, C. B., Kimbonguila, A., Nzikou, J. M., Matos, L., Pambou-Tobi, N. P. G., Abena, A. A., *et al*., (2010). Nutritive composition and properties physico-chemical of gumbo (*Abelmoschus esculentus*) seed and oil. *Research Journal of Environmental and Earth Sciences*, 2(1), 49-54.

Ndhlala, A. R., Finnie, J. F., & Van Staden, J. (2010). In vitro antioxidant properties, HIV-1 reverse transcriptase and acetylcholinesterase inhibitory effects of traditional herbal preparations sold in South Africa. *Molecules*, *15*(10), 6888-6904.

Ndunguru, J., & Rajabu, A. C. (2004). Effect of okra mosaic virus disease on the above-ground morphological yield components of okra in Tanzania. *Scientia Horticulturae*, *99*(3), 225-235.

Nelson, DL., and Cox, MM., (2005). Lehninger's Principles of Biochemistry, 4th Edition. W. H. Freeman and Company, New York.

Nesamvuni, C., Steyn, N. P., & Potgieter, M. J. (2001). Nutritional value of wild, leafy plants consumed by the Vhavenda: research letter. *South African Journal of Science*, *97*(1-2), 51-54.

Nichols, D. S., & Sanderson, K. (2003). The nomenclature, structure, and properties of food lipids. *Chemical and functional properties of food lipids*, 29-59.

Nishaa, S., Vishnupriya, M., Sasikumar, J. M., Hephzibah, P. C., & Gopalakrishnan, V. K. (2012). Antioxidant activity of ethanolic extract of Maranta arundinacea L. tuberous rhizomes. *Asian Journal of pharmaceutical and clinical research*, *5*(4), 85-88.

Njoku, O. U., Muma, A. F., Ononogbu, I. C., & Eleanya, R. (2001). Preliminary Investigation on some nutritional and toxicological properties of Ricinodendron heudelotti seed oil, Nigerian *Journal of Biochemistry and Molecular Biology*, 16(3), 132-133.

Nnamani, C. V., Oselebe, H. O., & Agbatutu, A. (2009). Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. *African Journal of Biotechnology*, *8*(10).

Noorlaila, A., Siti Aziah, A., Asmeda, R., & Norizzah, A. R. (2015). Emulsifying properties of extracted Okra (Abelmoschus esculentus L.) mucilage of different maturity index and its application in coconut milk emulsion. *International Food Research Journal*, *22*(2).

Norhaizan, M. E., & Norfaizadatul, A. A. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malyasian Journal of Nutrition*, *15*(2), 213-222.

Nwachukwu, E. C., Nulit, R., & Go, R. (2014). Nutritional and biochemical properties of Malaysian okra variety. *Advancement in Medicinal Plant Research*, *2*(1), 16-19.

Nwofia, G. E., Victoria, N. N., & Blessing, K. N. (2012). Nutritional variation in fruits and seeds of pumpkins (*Cucurbita Spp*) accessions from Nigeria. *Pakistan Journal of Nutrition*, *11*(10), 848.

Nzikou, J. M., Mvoula-Tsieri, M., Matouba, E., Ouamba, J. M., Kapseu, C., Parmentier, M., & Desobry, S. (2006). A study on gumbo seed grown in Congo Brazzaville for its food and industrial applications. *African Journal of Biotechnology*, 5(24).

Nzikou, J. M., Mvoula-Tsiéri, M., Ndangui, C. B., Pambou-Tobi, N. P. G., Kimbonguila, A., Loumouamou, B., *et al*., (2010). Characterization of seeds and oil of sesame (*Sesamum indicum*) and the kinetics of degradation of the oil during heating. *Research Journal of Applied Sciences, Engineering and Technology*, 2(3), 227-232.

Obiegbuna, J. E., Ishiwu, C. N., Akubor, P. I., & Igwe, E. C. (2014). Effect of Processing and Storage Relative Humidity on Selected Functional Properties of Cocoyam (*Colocasia Esculenta*) Corm Flour. *Food Science and Quality Management*, 28: 123-136.

Oboh, G. (2006). Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes. *Journal of food biochemistry*, *30*(5), 579-588.

Oboh, G., Ademiluyi, A. O., & Akindahunsi, A. A. (2010). The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. *International journal of food science & technology*, *45*(6), 1236-1242.

Ochigbo, S. S., & Paiko, Y. B. (2011). Effects of Solvent Blending on the Characteristics of Oils Extracted from the Seeds of Chrysophyllum albidium. *International Journal of Science and Nature, IJSN*, 2(2), 352-358.

Oghbaei, M., & Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture*, *2*(1), 1136015.

Ogungbenle, H. N. (2003). Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. *International Journal of Food Sciences and Nutrition*, 54(2), 153-158.

Ogungbenle, H. N., & Omosola, S. M. (2015). The comparative assessment of nutritive values of dry nigerian okra (*Abelmoschus esculentus*) fruit and oil. *International Journal of Food Science and Nutrition Engineering*, 5(1), 8-14.

Oguntibeju, O. O., Esterhuyse, A. J., & Truter, E. J. (2013). The role of fruit and vegetable consumption in human health and disease prevention. *INTECH Open Access Publisher*.

Okaka, J. C., & Okaka, A. N. C. (2001). Food composition, spoilage and shelf life extension. *OCJANCO Acad. Publishers, Enugu-Nigeria*, *225226*.

Oladele, A. K., & Aina, J. O. (2007). Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). *African Journal of Biotechnology*, *6*(21), 2473-2476.

Olagbemide, P. T., Ojiezeh, T. I., & Adarabioyo, M. I. (2016). Essential vitamins and mineral salts in some extracts used in alternative medicine in Nigeria. *Der Pharma Chemica, 8 (14):10-18.*

Olana, G. (2001). Food source diversification: potential to ameliorate the chronic food insecurity in Ethiopia. In *The Potential of Indigenous Wild Foods Workshop Procedings*. USAID/OFDA, CRS.

Olanipekun, O. T., Omenna, E. C., Olapade, O. A., Suleiman, P., & Omodara, O. G. (2015). Effect of boiling and roasting on the nutrient composition of kidney beans seed flour. *Sky Journal of Food Science*, *4*(2), 024-029.

Olaofe, O., Ogungbenle, H. N., Akhadelor, B. E., Idris, A. O., Omojola, O. V., Omotehinse, O. T., & Ogunbodede, O. A. (2012). Physico chemical and fatty acids composition of oils from some legume seeds. *IJBPAS*, 1, 355-363.

Olaofe, O., Okiribiti, B. Y., & Aremu, M. O. (2008). Chemical evaluation of the nutritive value of smooth luffa (Luffa cylindrica) seed's kernel. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *7*(10), 3444-3452.

Olivera, D. F., Mugridge, A., Chaves, A. R., Mascheroni, R. H., & Viña, S. Z. (2012). Quality attributes of okra (Abelmoschus esculentus L. Moench) pods as affected by cultivar and fruit size. *Journal of Food Research*, *1*(4), 224.

Oluba, O. M., Ogunlowo, Y. R., Ojieh, G. C., Adebisi, K. E., Eidangbe, G. O., & Isiosio, I. O. (2011). Physicochemical properties and fatty acid composition of *Citrullus lanatus* (Egusi Melon) seed oil. *Journal of Biological Science*, 8(4), 814-817.

Oluwole, I. S., Adeoti, O. A., & Ariyo, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringa oleifera seed flour. *Food science & nutrition*, *1*(6), 452-463.

Omari, A., Mgani, Q. A., & Mubofu, E. B. (2015). Fatty Acid Profile and Physico-Chemical Parameters of Castor Oils in Tanzania. *Green and Sustainable Chemistry*, 5(04), 154.

Omotoso, O. T. (2006). Nutritional quality, functional properties and anti-nutrient compositions of the larva of Cirina forda (Westwood)(Lepidoptera: Saturniidae). *Journal of Zhejiang University Science B*, *7*(1), 51-55.

Ong, M. G., Mat Yusuf, S. N. A., & Lim, V. (2016). Pharmacognostic and Antioxidant Properties of Dracaena sanderiana Leaves. *Antioxidants*, *5*(3), 28.

Onyango, C., Noetzold, H., Bley, T., & Henle, T. (2004). Proximate composition and digestibility of fermented and extruded uji from maize–finger millet blend. *LWT-Food science and Technology*, *37*(8), 827-832.

Osada, K., Ogino, Y., Nakamura, S., Kanda, T., Yanagida, A., & Sugano, M. (2004). Effect of dietary apple polyphenol on metabolic disorder of lipid in rats given oxidized cholesterol. In *Proc. Jpn. Conf. Biochem. Lipids*, 39 (317), 20-31.

Oshodi, A. A., Ogungbenle, H. N., & Oladimeji, M. O. (1999). Chemical composition, functional properties and nutritionally valuable minerals of benniseed, pearl millet, quinoa seed flour. *International Journal of Food Science Nutrition*, 50, 325-331.

Osundahunsi, O. F., & Aworh, A. C. (2002). A preliminary study on the use of tempe-based formula as a weaning diet in Nigeria. *Plant foods for human nutrition*, *57*(3-4), 365-376.

Osungbaro, T. O., Jimoh, D., & Osundeyi, E. (2010). Functional and pasting properties of composite Cassava-Sorgum flour meals. *Agriculture and biology journal of north america*, *1*(4), 715-720.

Ouattara, C. A. T., Somda, M. K., Moyen, R., & Traore, A. S. (2015). Comparative physico-chemical and proximate analysis of oils of Shea nut, Sesamum indicum, Cucurbita pepo, Cucumis melo seeds commonly cultivated in West Africa. *African Journal of Biotechnology*, 14(31), 2449-2454.

Oulai, S. F., Ahi, A. P., Kouassi-Koffi, J. D., Gonnety, J. T., Faulet, B. M., Djè, M. K., *et al*., (2014). Treatments Effects on Functional Properties of Breadfruit (*Artocarpus altilis*) Pulp Flour harvested in Côte d'Ivoire. *International Journal of Rec. Biotech*, *2*(4), 1-12.

Oyelade, O. J., Ade-Omowaye, B. I. O., & Adeomi, V. F. (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. *Journal of Food Engineering*, 57(2), 111-114.

Ozsoy, N., Can, A., Yanardag, R., & Akev, N. (2008). Antioxidant activity of Smilax excelsa L. leaf extracts. *Food Chemistry*, *110*(3), 571-583.

Pandey, H., & Awasthi, P. (2015). Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed flour. *Journal of food science and technology*, *52*(2), 1054-1060.

Pandey, M., Abidi, A. B., Singh, S., & Singh, R. P. (2006). Nutritional evaluation of leafy vegetable paratha. *Journal of Humanity and Ecology*, *19*(2), 155-156.

Peace, O. E., & Aladesanmi, A. O. (2008). Effect of fermentation on some chemical and nutritive properties of Berlandier Nettle Spurge (*Jatropha cathartica*) and physic nut (Jatropha curcas) seeds. *Pakistan Journal of Nutrition, Faisalabad*, 7(2), 292-296.

Pérès, J. M., Bureau, F., Neuville, D., Arhan, P., & Bouglé, D. (2001). Inhibition of zinc absorption by iron depends on their ratio. *Journal of trace elements in medicine and biology*, *15*(4), 237-241.

Perez, V., & Chang, E. T. (2014). Sodium-to-potassium ratio and blood pressure, hypertension, and related factors. *Advances in Nutrition: An International Review Journal*, *5*(6), 712-741.

Pham, P. J., Peralta, M. M., & Pham, L. J. (2003). Okra (*Hibiscus esculentus*) seed oil: characterization and potential use for high value products. In *Advanced Research on Plant Lipids* (pp. 35-38). Springer Netherlands.

Ponka, R. O. G. E. R., Fokou, E., Fotso, M., Achu, M. B., & Tchouanguep, F. M. (2005). Methods of preparation and the energy, protein and mineral values of three Cameroonian dishes:″corn Chaff ″,″Nnam Owondo/Ebobolo ″and ″Nnam Ngon/Ebobolo ″. *African Journal of Food, Agriculture, Nutrition and Development*, *5*(1).

Prasad M.N.V. (2008). Consequences in Ecosystems and Human Health. *Wiley,New Jersey*, pp. 777.

Prasad, K., & Nath, N. (2002). Effect of pre-treatments and clarificants on sugarcane juice characteristics. *Asian Journal of Chemistry*, *14*(2), 723.

Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *HortScience*, *35*(4), 588-592.

Priya, S., Varun, C., Brahm, K., Shubhendra, SC., Sobita, S., Bilalc, S., & Abidia, AB. (2014). An Overview on Okra (Abelmoschus Esculentus) and It's Importance as a Nutritive Vegetable in the World. *International Journal of Pharmacy and Biological Sciences* 4(2):227-233.

Ramadan, M. F., Sharanabasappa, G., Seetharam, Y. N., Seshagiri, M., & Moersel, J. T. (2006). Characterisation of fatty acids and bioactive compounds of kachnar (*Bauhinia purpurea*) seed oil. *Food chemistry*, 98(2), 359-365.

Randhir, R., Kwon, Y. I., & Shetty, K. (2008). Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science & Emerging Technologies*, *9*(3), 355-364.

Rao, P. U. (1985). Chemical composition and biological evaluation of Okra (*Hibiscus esculentus*) seeds and their kernels. *Plant Foods for Human Nutrition*, *35*(4), 389-396.

Raven, P. H., Evert, R. F., & Eichhorn, S. E. (2005). *Biology of plants*. Macmillan.

Regassa, T., Ensermu, K., and Zemede, A. (2015). Ethnobotany of wild and semi-wild edible plants of Chelia District, West-Central Ethiopia. *Science, Technology and Arts Research Journal* 3 (4): 122-134.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.

Roger, A. B., Rebecca, R. A., Georges, A., & Mathias, I. O. (2010). Chemical characterization of oil form Germinated nuts of several coconut cultivars (*cocos nuciferh*). *European Journal of Scientific Research*, 391(4), 514-522.

Roy, A., Shrivastava, S. L., & Mandal, S. M. (2014). Functional properties of Okra Abelmoschus esculentus L.(Moench): traditional claims and scientific evidences. *Plant Science Today*, *1*(3), 121-130.

Rubatzky, V. E., & Yamaguchi, M. (2012). *World vegetables: principles, production, and nutritive values*. Springer Science & Business Media.

Sabitha, V., Ramachandran, S., Naveen, K. R., & Panneerselvam, K. (2011). Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus (L.) Moench*. in streptozotocin-induced diabetic rats. *Journal of Pharmacy and Bioallied Sciences*, *3*(3), 397-402.

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC complementary and alternative medicine*, *12*(1), 221.

Saha, J., Biswal, A. K., & Deka, S. C. (2015). Chemical composition of some underutilized green leafy vegetables of Sonitpur district of Assam, India. *International Food Research Journal*, *22*(4), 1466-1473.

Saifullah, M., & Rabbani, M. G. (2009). Evaluation and characterization of okra (Abelmoschus esculentus L. Moench.) genotypes. *SAARC Journal of Agriculture*, *7*(1), 92-99.

Sani, I., Owoade, C., Abdulhamid, A., Fakai, I. M., & Bello, F. (2014). Evaluation of Physicochemical Properties, Phytochemicals and Mineral Composition of Cocosnuciferal.(*Coconut*) Kernel Oil. *International Journal of Advanced Research in Chemical Science*, 1(8), 22-30.

Sankar, B., Jaleel, C. A., Manivannan, P., Kishorekumar, A., Somasundaram, R., & Panneerselvam, R. (2008). Relative efficacy of water use in five varieties of *Abelmoschus esculentus (L.) Moench* under water-limited conditions. *Colloids and Surfaces B: Biointerfaces*, *62*(1), 125-129.

Sayre, L. M., Smith, M. A., & Perry, G. (2001). Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Current medicinal chemistry*, *8*(7), 721-738.

Scherer, R., & Godoy, H. T. (2009). Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. *Food chemistry*, *112*(3), 654-658.

Schippers, R. R. (2000). African Indigenous Vegetables: An overview of the cultivated species. Chathan, UK: Natural Resources Institute/ACPEU Technical Centre for Agricultural And Rural Cooperation, 103–113 PP.

Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. (2009). Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus*) *Carbohydrate Research*, *344*(14), 1824-1832.

Şensoy, Í., Rosen, R. T., Ho, C. T., & Karwe, M. V. (2006). Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry*, *99*(2), 388-393.

Seyoum M. (2013).View of Okra: Un-tapping the potential of Local Food to Improve Food and Nutrition security in Ethiopia. *Food security in Ethiopia and Horn of Africa*.

Sharma, P., & Gujral, H. S. (2013). Extrusion of hulled barley affecting β-glucan and properties of extrudates. *Food and Bioprocess Technology*, *6*(6), 1374-1389.

Sharma, R. K., & Prasad, K. (2010a). Characterisation of promising okra genotypes on the basis of Principal Component Analysis. *The society for advancement of horticulture*, *12*(1), 71-74.

Sharma, R. K., & Prasad, K. (2010b). Classification of promising okra (Abelmoschus esculentus) genotypes based on principal component analysis. *Journal of Tropical Agriculture and Food Sc*ience, *38*(2), 161-169.

Sharma, V., Khan, A., & Shukla, R. K. (2016). Growth and Yield Attribute of Okra (Abelmoschus esculentus L.) Under the Application of Bio and Chemical Fertilizers either Alone or in Combination. *International Journal of Agricultural Science and Research (IJASR)*, *1*(6), 189-198.

Sheela, K., Nath, K. G., Vijayalakshmi, D., Yankanchi, G. M., & Patil, R. B. (2004). Proximate composition of underutilized green leafy vegetables in Southern Karnataka. *Journal of Human Ecology*, *15*(3), 227-229.

Shils, ME., (1999). Modern Nutrition in Health and Disease. 9th ed. Baltimore: Williams & Wilkins.

Shui, G., & Peng, L. L. (2004). An improved method for the analysis of major antioxidants of Hibiscus esculentus Linn. *Journal of Chromatography A*, *1048*(1), 17-24.

Siddhuraju, P., & Becker, K. (2001). Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian tribal pulse, Mucuna pruriens var. utilis. *Journal of Agricultural and food Chemistry*, *49*(6), 3058-3067.

Siddiq, M., Ravi, R., Harte, J. B., & Dolan, K. D. (2010). Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris L.*) flours. *LWT-Food Science and Technology*, *43*(2), 232-237.

Siegenberg, D., Baynes, R. D., Bothwell, T. H., Macfarlane, B. J., Lamparelli, R. D., Car, N. G., *et al.* (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *The American journal of clinical nutrition*, *53*(2), 537-541.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*, *101*(3), 1012-1018.

Simas-Tosin, F. F., Barraza, R. R., Petkowicz, C. L. O., Silveira, J. L. M., Sassaki, G. L., Santos, E. M. R., *et al*., (2010). Rheological and structural characteristics of peach tree gum exudate. *Food Hydrocolloids*, *24*(5), 486-493.

Šimić, D., Sudar, R., Ledenčan, T., Jambrović, A., Zdunić, Z., Brkić, I., *et al*. (2009). Genetic variation of bioavailable iron and zinc in grain of a maize population. *Journal of Cereal Science*, *50*(3), 392-397.

Simmone, E. H., Maynard, G. J., Hochmuth, D. N., Vavrina, C. S., Stall, W. M., Kucharek, *et al*., (2004). Okra Production in Florida. Horticultural Sciences Department document HS729. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Singh, N., Kaur, N., Rana, J. C., & Sharma, S. K. (2010). Diversity in seed and flour properties in field pea (*Pisum sativum*) germplasm. *Food chemistry*, *122*(3), 518-525.

Singha, P., Chauhana, V., Tiwaria, B. H., Chauhanb, S. S., Simonb, S. *et al*., (2014). An overview on okra (abelmoschus esculentus) and it's importance as a nutritive vegetable in the world. *International Journal of Pharmacy and Biological Sciences*, 4(2): 227-233.

Sinnadurai, S. (1971). Vegetable production in Ghana. Acta Horticulture (ISHS) 33:25-28.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science*, *4*(5), 200-222.

Sorkheh, K., Kiani, S., & Sofo, A. (2016). Wild almond (*Prunus scoparia*) as potential oilseed resource for the future: Studies on the variability of its oil content and composition. *Food Chemistry*, *212*, 58-64.

Sreeramulu, D., & Raghunath, M. (2010). Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. *Food Research International*, *43*(4), 1017-1020.

Stéphane (2004). Interaction of grape seed procyanidins with various proteins in relation to wine fining. *Journal of Science, Food and Agriculture*, 57:111-125.

Steve, I. O. (2011). Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (*Triticum aestivum*). *Journal of Cereals and Oilseeds*, *3*(3), 35-47.

Stevenson, D. G., Eller, F. J., Wang, L., Jane, J. L., Wang, T., & Inglett, G. E. (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *Journal of agricultural and food chemistry*, *55*(10), 4005-4013.

Sultana, B., Anwar, F., & Iqbal, S. (2008). Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. *International journal of food science & technology*, *43*(3), 560-567.

Suttirak, W., & Manurakchinakorn, S. (2014). In vitro antioxidant properties of mangosteen peel extract. *Journal of food science and technology*, *51*(12), 3546-3558.

Tachakittirungrod, S., Okonogi, S., & Chowwanapoonpohn, S. (2007). Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chemistry*, *103*(2), 381-388.

Taffesse, A. S., Dorosh, P., and Asrat, S. (2012). Crop Production in Ethiopia: Regional Patterns and Trends Summary of ESSP II Working Paper, Crop Production in Ethiopia: Regional Patterns and Trends, 74 (3): 53.

Temple, N. J. (2000). Antioxidants and disease: more questions than answers. *Nutrition research*, *20*(3), 449-459.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, *19*(6), 669-675.

Thampi, K. S., & Indira, V. (2000). Nutritive value and organoleptic evaluation of thamara venda genotypes (*Abelmoschus caillei*). *Journal of Tropical Agriculture*, *38*(1-2), 38-40.

Thanatcha, R., & Pranee, A. (2011). Extraction and characterization of mucilage in Ziziphus mauritiana Lam. *International food research journal*, *18*(1), 201-212.

Thidarat, S., Udomsak, M., Jindawan, W., Namphung, D., Suneerat, Y., Sawan, T., & Pisamai, T. (2016). Effect of roasting on phytochemical properties of Thai soybeans. *International Food Research Journal*, *23*(2).

Thompson, L. U. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Research International*, *26*(2), 131-149.

Tiamiyu, R. A., Ahmed, H. G., & Muhammad, A. S. (2012). Effect of sources of organic manure on growth and yields of okra (Abelmoschus esculentus L.) in Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, *20*(3), 213-216.

Tian, Z. H., Miao, F. T., Zhang, X., Wang, Q. H., Lei, N., & Guo, L. C. (2015). Therapeutic effect of okra extract on gestational diabetes mellitus rats induced by streptozotocin. *Asian Pacific journal of tropical medicine*, *8*(12), 1038-1042.

Tindall, H. D. (1983). Vegetables in the tropics. *Macmillan Press Ltd.*

Torreggiani, A., Tamba, M., Trinchero, A., & Bonora, S. (2005). Copper (II)–Quercetin complexes in aqueous solutions: spectroscopic and kinetic properties. *Journal of Molecular Structure*, *744*, 759-766.

Tripathi, K. K., Govila, O. P., Warrier, R. and Ahuja, V. (2011). Biology of Abelmoschus esculentus L. (Okra). Series of Crop Specific Biology Documents, Department of Biotechnology, Ministry of Science & Technology & Ministry of Environment and Forest, Govt. of India.

Tsado A. N., Lawa B., Santali E. S, Shaba A. M., Chirama D. N., Balarabe M. M., *et al*. (2013). Effect of Different Processing Methods on Nutritional Composition of Bitter Leaf (*vernonia amygdalina*). *IOSR Journal Of Pharmacy and Biological sciences, 5(6), 08-14.*

Tsado, E. K. (2015). Effect of Method of Drying Okra Fruits (Abelmoschus esculentus) on Proximate Composition of the Dried Product. *Donnish journal of Agricultural Research, 2*(8), 66-70.

Udensi, E. A. &Okoronkwo, K. A. (2006). Effects of fermentation and germination on the physicochemical properties of Mucuna cochinchinensis protein isolate. *African Journal of Biotechnology*, *5*(10), 896.

Ugwu, F. M., & Oranye, N. A. (2006). Effects of some processing methods on the toxic components of African breadfruit (Treculia africana). *African Journal of Biotechnology*, *5*(22).

Ukegbu, P. O., & Okereke, C. J. (2013). Effect of solar and sun drying methods on the nutrient composition and microbial load in selected vegetables, African spinach (*Amaranthus hybridus*), fluted pumpkin (*Telferia occidentalis*), and okra (*Hibiscus esculentus*). *Sky Journal of Food Science*, *2*(5), 35-40.

Ukpabi, U. J., & Ejidoh, J. I. (1989). Effect of deep oil frying on the oxalate content and the degree of itching of cocoyams (*Xanthosoma and Colocasia spp*). In *Technical paper presented at the 5th Annual Conference of the Agricultural Society of Nigeria, Federal University of Technology, Owerri, Nigeria* (pp. 3-6).

Umeta, M., West, C. E., & Fufa, H. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis*, *18*(8), 803-817.

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis*, *23*(6), 499-509.

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical biochemistry*, *175*(1), 227-230.

Valdés, L., Cuervo, A., Salazar, N., Ruas-Madiedo, P., Gueimonde, M., & González, S. (2015). The relationship between phenolic compounds from diet and microbiota: impact on human health. *Food & function*, *6*(8), 2424-2439.

Valvi, S.R. and Rathod, V.S. (2011). Mineral Composition of Some Wild Edible Fruits From Kolhapur District. *International Journal of Applied Biology and Pharmaceutical Technology,* 2(1).

Velasco, L., Rojas-Barros, P., & Fernández-Martínez, J. M. (2005). Fatty acid and tocopherol accumulation in the seeds of a high oleic acid castor mutant. *Industrial Crops and Products*, *22*(3), 201-206.

Verma, A. K., Singh, H., Satyanarayana, M., Srivastava, S. P., Tiwari, P., Singh, A. B., *et al*. (2012). Flavone-based novel antidiabetic and antidyslipidemic agents. *Journal of medicinal chemistry*, *55*(10), 4551-4567.

Walingo, M. K. (2009). Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population. *African Journal of Food Agriculture Nutrition and Development*, 9,523-535.

Walker, S. P., Wachs, T. D., Gardner, J. M., Lozoff, B., Wasserman, G. A., Pollitt, et al., (2007). International Child Development Steering Group. Child development: risk factors for adverse outcomes in developing countries. *The lancet*, *369*(9556), 145-157.

Wani, I. A., Gani, A., Tariq, A., Sharma, P., Masoodi, F. A., & Wani, H. M. (2016). Effect of roasting on physicochemical, functional and antioxidant properties of arrowhead (*Sagittaria sagittifolia L.*) flour. *Food chemistry*, *197*, 345-352.

Watts, D. L. (2010). HTMA Mineral Ratios. *A brief discussion of their clinical importance. Trace Elem Newsletter*, *21*, 1-3.

Win, M. M., Abdul-Hamid, A., Baharin, B. S., Anwar, F., & Saari, N. (2011). Effects of roasting on phenolics composition and antioxidant activity of peanut (*Arachis hypogaea L.*) kernel flour. *European Food Research and Technology*, *233*(4), 599.

Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food chemistry*, *105*(3), 940-949.

Woldegiorgis, A. Z., Abate, D., Haki, G. D., & Ziegler, G. R. (2014). Antioxidant property of edible mushrooms collected from Ethiopia. *Food chemistry*, *157*, 30-36.

Woldegiorgis, A. Z., Abate, D., Haki, G. D., & Ziegler, G. R. (2015). Major, minor and toxic minerals and anti-nutrients composition in edible mushrooms collected from Ethiopia. *Journal of Food Processing & Technology*, 6(3), 234-244.

Wood, J.A. and M.A. Grusak. (2007). Nutritional value of chickpea. p. 101-142. In Redden, W. Chen, B. Sharma and Y. Yadav (eds.) Chickpea breeding and management. Oxford University Press, New York, USA.

Wood, R. J. and Ronnenberg, A. G., 2006. Iron. In: Shils, M. E., Shike, M., Ross, A. C., Caballero, B., Cousins, R. J., eds. Modern nutrition in health and disease. 10th ed. Philadelphia: Lippincott Williams and Wilkins, pp. 248-270.

Woolfe, M. L., Chaplin, M. F., & Otchere, G. (1977). Studies on the mucilages extracted from okra fruits (Hibiscus esculentus L.) and baobab leaves (Adansonia digitata L.). *Journal of the Science of Food and Agriculture*, *28*(6), 519-529.

Workneh, A., Zewdie. W., & Habtemariam, K. (2007). Reducing Vitamin A Deficiency in Ethiopia, International Center on Research on Women. Research Report Series 4.

World Health Organization, (1998). Food and Agriculture Organization of the United Nations (FAO). *Vitamin and mineral requirements in human nutrition*, 2nd ed., WHO and FAO, Bangkok, Thailand, 1998, pp 1- 340.

Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of food science*, *72*(2), S159-S166.

Yadav, S. K., & Dhankhar, B. S. (1999). Performance of Varsha Uphar cultivar of okra (*Abelmoschus esculentus*) as affected by the sowing dates and plant geometry. Vegetable Science 27: 70 – 74.

Yang, R. Y., & Keding, G. B. (2009). Nutritional contributions of important African indigenous vegetables. *African indigenous vegetables in urban agriculture. Earthscan, London*, 105-143.

Yang, R. Y., Chang, L. C., Hsu, J. C., Weng, B. B., Palada, M. C., Chadha, M. L., & Levasseur, V. (2006). Nutritional and functional properties of Moringa leaves–From germplasm, to plant, to food, to health. *Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU. Paris*.

Yonas, M., Garedew, W., & Debela, A. (2014). Multivariate analysis among okra (*Abelmoschus esculentus*) collection in South Ethiopia. *Journal of Plant Sciences*, *9*(2), 43.

Yu, J., Ahmedna, M., & Goktepe, I. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food chemistry*, *90*(1), 199-206.

Zarena, A. S., & Sankar, K. U. (2009). A study of antioxidant properties from Garcinia mangostana L. pericarp extract. *Acta Scientiarum Polonorum Technologia Alimentaria*, *8*(1), 23-34.

Zhao, H., Lin, J., Grossman, H. B., Hernandez, L. M., Dinney, C. P., & Wu, X. (2007). Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. *International journal of cancer*, *120*(10), 2208-2213.

Zia-Ul-Haq, M., Ahmad, S., Bukhari, S. A., Amarowicz, R., Ercisli, S., & Jaafar, H. Z. (2014). Compositional studies and biological activities of some mash bean (*Vigna mungo (L.) Hepper*) cultivars commonly consumed in Pakistan. *Biological research*, *47*(1), 23.

Zia-Ul-Haq, M., Ahmad, S., Qayum, M., & Ercişli, S. (2013). Compositional studies and antioxidant potential of *Albizia lebbeck (L.) Benth*. pods and seeds. *Turkish Journal of Biology*, *37*(1), 25-32.

Ziyada, A. K., & Elhussien, S. A. (2008). Physical and chemical characteristics of Citrullus lanatus var. colocynthoide seed oil. *Journal of Physical Science*, *19*(2), 69-75.

Vegetables	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate	Calcium	Iron	Phosphorus
Okra pods♦	89.29	16.45	1.26	21.25	7.78	42.22	224.72	27.23	42.1
Cabbage*	93.30	1.10	0.10	1.30	0.90	23.70	43.00	0.07	37.00
Ethiopian kale*	87.60	2.80	0.80	1.50	1.90	46.00	260.00	4.10	64.00
Lettuce*	95.50	1.00	0.20	0.70	0.50	15.40	22.00	1.60	31.00
Swiss chard*	91.50	2.20	0.40	1.10	2.10	27.60	85.00	3.60	41.00
Carrot*	89.10	0.40	0.20	1.30	1.90	27.80	31.00	0.05	20.00
Tomato*	92.50	1.30	0.70	1.50	0.70	30.70	9.00	0.90	29.00
Celery*	86.70	3.30	0.05	1.90	2.00	47.70	317.00	5.20	52.00