ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Evaluation of Cassava Cultivars and Methods for Roots Processing in Development of Cassava Composite Flours for Production a Household Staple *Injera* in Ethiopia

By

Abebe Haile

A Thesis Submitted to

The Centre for Food Science and Nutrition

Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition

Addis Ababa, Ethiopia

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ABBREVIATIONS AND ACRONYMS

AA	Addis Ababa
AACS	American Association of Cereal Science
Ab	Absorbance of blank
ACIAR	Australian Centre for International Agricultural Research
ACTA	Anti-Counterfeiting Trade Agreement
ANFs	Anti-nutritional factors
As	Absorbance of sample
BD	Bulk Density
BTC	Bean-Tef-Cassava
BV	Biological Value
CABI	Centre for Advanced Biomedical Imaging
CCDNN	Cassava Cyanide Diseases and Neurolathyrism Network
CFSN	Centre for Food Science and Nutrition
CIAT	Centre international de agricultura tropica
CRC	Cyclic redundancy check
DB	Diet Bean
Dc	Diet casein
DF	Dilution Factor
Dm	Diet maize
DRI	Daily Requirement Intake
DMR	Duncan multiple range
Dst	Diet starch

EARO	Ethiopian Agriculture Research Organization
EHNRI	Ethiopian Health and Nutrition Research Institute
ENI	Ethiopian Nutrition Institute
F	Faecal nitrogen
FER	Feed Efficiency Ratio
F_k	Endogenous faecal nitrogen
HNL	Hydroxynitrilelyase
HSDB	Hazardous Substances Data Bank
I	In taken nitrogen
IAR	Institute of Agricultural Research
IITA	International Institute for Tropical Agriculture
ISBN	International Standard Book Number
ISHS	International Society for Horticultural Science
Kcal	Kilo calories
Kgfw	Kilo gram fresh weight
ME	Molar equivalent
mf	Mass of flour
mmol	Millimoles
MTC	Maize-Tef-Cassava
MW	Molecular weight
NPR	Net Protein Ratio
NPU	Net Protein Utilization
NR	Nitrogen Retention

NRI	Natural Resource Institute
NW	Nigerian White
Ox	Oxalate
PER	Protein Efficiency Ratio
PHCRR	Population and Housing Census Results Report
Ppm	Part per million
RDA	Recommended Dietary Allowance
RMRDC	Raw Materials Research and Development Council
SNNPRS	Southern Nations, Nationalities and Peoples' Regional State
Sp	Swelling power
SRMC	Standard Reference Materials Catalogue
T	Titre
TAN	Tropical Ataxic Neuropathy
TD	True Digestibility
TVA	Total volume of aliquot
U	Urinary Nitrogen
U_k	Endogenous urinary nitrogen
USDAH	United States Department of Agriculture Handbook
Vme	Volume-mass equivalent
W/v	Weight to volume
WBC	Water Binding Capacity
µL	Micro litre

ABSTRACT

Evaluation of Cassava Cultivars and Methods for Roots Processing in Development of Cassava Composite Flours for Production a Household Staple *Injera* in Ethiopia

Abebe Haile

Addis Ababa University, 2013

Locally grown cassava cultivars were subjected to study the effect of boiling, sun-drying and fermentation processing methods on nutritional quality, cyanide detoxification and production of quality cassava-based Injera. The proximate composition, minerals, vitamins and anti-nutritional factors, and physico-chemical properties of cassava root cultivars and composite flours were also analysed. Cassava-based food products (Injera) were prepared, from 10, 20, 30, 40, 45 and 50 % of cassava flour and 10 % quality protein maize/common bean flours for tef flour. The developed food products were subjected to sensory evaluation, chemical analyses and biological evaluation on mice using standard procedures. The data generated were statistically analyzed using statistical package for social scientists (SPSS Ver. 17) and Micro Soft Office excels 2007.

The percentage proximate composition contents of cassava root of seven cultivars were in the average of 10.43, 8.17 and 8.35 for moisture content; 2.0, 0.32 and 1.55 for ash; 3.1, 3.92 and 3.09 for fibre; 0.23, 0.22 and 0.35 for fat; 0.42, 0.24 and 0.32 for the protein; 68.88, 91.06 and 89.38 for carbohydrate for

the boiled, sun-dried and fermented flours, respectively. Out of the three processing techniques, fermentation of grated cassava roots for 72 hours sufficiently reduces HCN content to safe level value (<10 ppm, WHO) of human consumption. Reduction in phytate and tannin levels were highest for sun-dried followed by fermented and boiled flours. However, reduction in oxalate contents were highest for fermented followed by boiled and sun-dried flours.

Reduction in average vitamin C contents was significantly ($P<0.05$) highest for sun-dried followed by fermented and boiled flours. The β-carotene in unprocessed cassava root of seven cultivars was found to be in the average of 0.14 µg/100 g, appreciably low which may be due to the absence of yellow-orange colour in their flesh portion.

The bulk density and solubility values of boiled cassava root flours were significantly ($P<0.05$) higher than sun-dried and fermented flours, while, the water binding capacity and swelling power values of sun-dried cassava root flours were significantly ($P<0.05$) higher than boiled and fermented flours. Reduction in phosphorous and zinc contents were highest for fermented followed by boiled and sun-dried flours. While, reduction in calcium and iron contents were highest for boiled followed by sun-dried and fermented flours. The phytate to minerals molar ratio indices indicated that the bioavailability of Zn and Fe but not Ca was impaired by phytate in the cassava root flours.

The tannin content of BTC (45-68) mg/100g composite flour Injera is significantly ($P<0.05$) higher than MTC (6-10) mg/100g, while in the case of phytate it is slightly different from each other. The phytate to minerals molar ratio indicate the

bioavailability of Ca, Fe and Zn in the composite flours.

The body weight gain and length gain in all the group of mice were significantly increased after 21 days of feeding on test diets. The protein efficiency ratio (PER) value of mice fed on diets (Dm3 and Dm4) is significantly ($P<0.05$) higher than standard casein (Dc). The percentage net protein utilization (NPU) and biological value (BV) of mice fed on test diets DB3, DB4, Dm3 & Dm4 were significantly ($P<0.05$) higher than the mice fed on standard diets (Dc).

Key words: Cassava, Injera production, cassava composite flours, Nutritional performance

1. INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* C.), a dicotyledonous plant belonging to the family *Euphorbiaceae*, is one of the most important food crops in the world, especially in the tropics. Cassava represents the main source of energy for more than half a billion people all over the world. Brazil was the origin of cassava plant. Cassava is the fourth important source of carbohydrates in the tropics that occupy an exceptionally important position as a food security commodity for smallholder farmers (Onuweme, 1978; Bellotti, et al., 1999; Topouzis, 2003; Ademiliyi, et al., 2006). The world production of cassava root was estimated to be 281.7 million tonnes in 2012. The majority of production is in Africa where 153.8 million tonnes were grown (FAO, 2012).

However, much attention has continually been drawn to the cyanide in this crop, which could be lethal to man if consumed in large doses over a period. When raw cassava or inadequately processed cassava is consumed symptoms of discomfort happened to the consumer (Hahn, 1989; Banea-Mayambu, et al., 1990). Cassava-based diets have been associated with two neurological and iodine deficiency disorders including goitre, cretinism, mild mental disorders, and other related conditions (Howlett, et al., 1990; Cherinet, et al., 1998; Muquingue, et al., 2008). These occur among the rural poor people whose diets are largely restricted to high-cyanide containing cassava. Apart from cyanide, cassava contains anti-nutrients like tannin, oxalate and phytate that inhibit the absorption of minerals to the body (Omoruyi and Dilworth, 2007).

Cassava was introduced into Ethiopia over 60 years ago, although its cultivation was never generally accepted and appreciated until 1984 famine (Mulugeta, 1994). Cassava is also known as yucca (Spanish), mandioca (Portuguese) in different countries of the world. Similarly, in Ethiopia it is also known in different names such as "Yenchet Boye (Welayitigna)", "Muka Furno (Oromifa)" and "Tesike/Mogo (Koreegna)". However, in the past cassava in Ethiopia was not considered as useful crops, so that the crop was planted in areas of frequent moisture stress parts of the country, at the backyards and at farm boarders as fences. Presently, it is planted at field level and sometimes consumed as main dishes particularly in the Southern region of Ethiopia (Desalegn, 2007). Consumption and processing of cassava in the country is in a rather underutilized stage as compared to many African countries such as Uganda, Nigeria, Tanzania, etc., where people consume cassava leaf and tender shoots as vegetable. Therefore, consumption, productivity and processing issues are of major priorities in improving and promoting cassava production in the country. The crop has been found to have an excellent adaptation and growth performance in different agro ecologies of Ethiopia, with a total root yield ranging from 28 to 60 t/ha (Edossa, 1995). In Ethiopia, the consumption of cassava as human food is of immense importance, and regarded as the food security crop for millions of people. However, cassava as a food has its problems of protein deficiency and cyanide toxicity. Therefore, this calls for a technique that enhance the nutrient content at the same time reducing the anti-nutrients without adversely affecting the acceptability of cassava based traditional foods. The products could become even more important in feeding additional segments of the increasing

Ethiopian population in the future. Thus, investigation of the effect of common processing techniques on nutritional quality and developing food product (Injera) from cassava-supplemented flour is highly important.

1.2 Rationale

The Southern part of Ethiopia is accustomed to eating tuber crops as staple foods, among them cassava. The food availability of resource-poor households were influenced negatively by major environmental and demographic challenges including declining soil fertility, unreliable rainfall pattern, expensive farm inputs, rapid population growth, and the HIV pandemic. Therefore, cassava crops fill critical food shortage and particularly due to its characteristics of tolerance to drought conditions, and wide range of utilization for both food and animal feed (Ukwuru and Egbona, 2013). Thus, the cassava crops can contribute substantially to the food security and economic growth of the country.

Despite this obvious advantage, cassava remained a neglected crop in agricultural research and development activities to an extent not commensurate with its importance as food in Ethiopia. Little work has been done regarding the processing and utilization as supplementary crop, cyanide removal methods and food product development of cassava blend composite flour (Mulugeta and Eskinder, 1999; Mulugeta, 2000). In addition, there was report on the effect of inadequately processed cassava consumption on the prevalence of goitre at Southern part of Ethiopia (Cherinet, et al., 1998).

Moreover, there was no work done so far on the improvement of staple foods (Injera), by cassava flour blend with commonly consumed cereals and pulses.

Cassava nevertheless is widely consumed and studied, by mixing with grains to make foods according to the traditional recipes in the other parts of the world (Danster, et al., 2008; Arisa, et al., 2011). However, there is no comprehensive study on physico-chemical properties on different landrace cultivars of cassava and development of food recipes using cassava composite flours in Ethiopia. The nutritional content, functional properties, preservation and detoxification systems have also not well been studied and documented in this country. On the other hand, some people at the village level have eaten the root part boiled, sliced, ground, roasted and brewed as beverage for sometimes (researcher survey).

However, limited knowledge on the utilization and potential for cyanide free cassava as alternative staple foods, exist which can be a source of income generation for the small-scale entrepreneur and farmers. Meanwhile, it has been proved that tef is free of gluten protein, which is allergic to many people (Spaenij-Dekking, et al., 2005). Hence, there is a growing demand for tef in the developed countries. This has created an increase in the price of tef, so that consumption of tef in Ethiopia declining. The Ethiopians' are therefore, forced to seek an alternative for tef flour to prepare their staple food Injera.

1.3. Objectives of the study

There are four general objectives of this study.

A. Characterization of seven cassava roots cultivars.

B. Determine the effect of common cassava processing methods (boiling, sun drying and fermentation) on nutritional quality and physico-chemical properties in seven cassava roots cultivars in Ethiopia.

C. Development of composite flour with cassava, tef, common beans and quality protein maize for production of Injera.

D. Evaluation of nutritional performance and acceptance of developed composite flours.

E. Evaluation of nutritional performance of composite flour by biological assays using mice.

The specific objectives of this study were to:

- Assess the effect of the most common processing methods (boiling, sun drying and fermentation) on the nutritional quality and physico-chemical properties of the seven cassava cultivars,
- Determination of cyanide, phytates, tannins and oxalates levels and minerals bioavailability in cassava roots flour,
- Develop a staple traditionally fermented food product (Injera) based on nutrient enriched cassava composite flour,
- Determination of nutritional composition, functional and physical properties of the Injera made from cassava-legume-cereals composite flours,
- Analyze anti-nutritional factors (phytates & tannins) in the staple food product (Injera),
- Determine the contents of the minerals and their bioavailability in the Injera,
- Evaluation of the organoleptic properties of Injera prepared from cassava-based composite flours and
- Determination of nutritional performance of Injera by biological assay using mice.

2. LITERATURE REVIEW

2.1 OVERVIEW

2.1.1 Origin and history of cassava

Archaeological findings in the Amazon suggests that cassava is an ancient crop which was domesticated between 5000-7000 BC. Cassava is native to tropical South America and its evolutionary centre of origin is thought to be in Brazil (Olsen and Schall, 1999; Henry and Hershey, 2002). The Portuguese initially introduced cassava into Africa in the 16th century. Then it was first brought to the West coast of Africa via the Gulf of Benin and River Congo at the end of the sixteen century and to the East coast via the Reunion Island, Madagascar and Zanzibar at the end of the eighteen-century (Nweke, 1994; Allen, 2002). Cassava was first introduced in Ethiopia in 1948 and has since been cultivated, particularly, in South, South West, and Western parts of the country (Mulugeta, 1994; Amsalu, 2003; Desalegn, 2007). Spanish traders took the cassava crop to Asia during the 17^{th} century (Onwueme, 2002). At present, cassava is grown in over 90 countries as an essential part of the diet for more than half a billion people in Africa, Asia and Latin America (FAO, 2012).

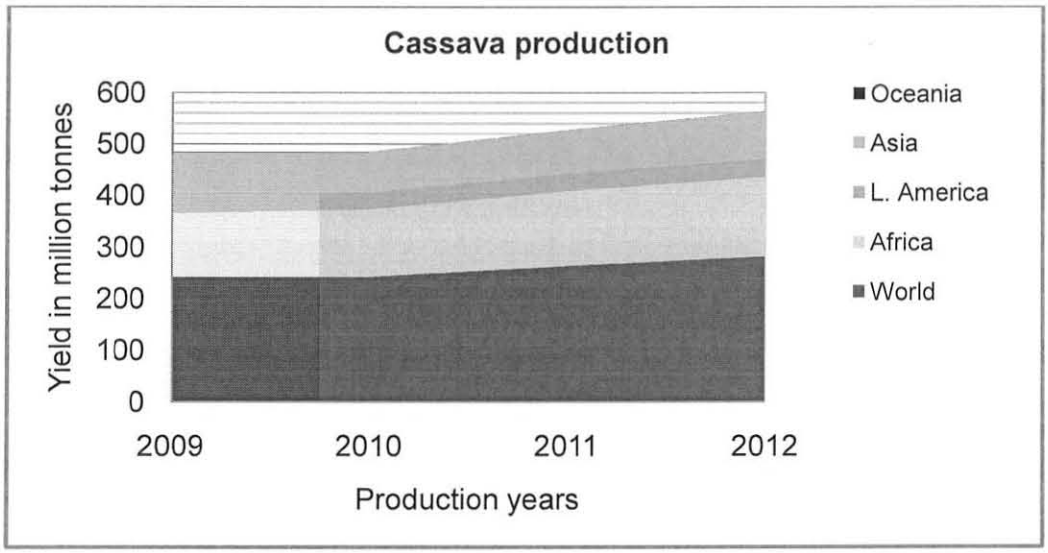
Cassava is adapted to the zone within latitudes 30°N and 30°S of the equator, at elevations of not more than 2,000 meters above sea level, in temperatures ranging from 18 to 25 °C, annual rainfall of 50 to 5,000 mm and in poor soils with a pH range from 4 to 9. Cassava grows to 1 to 4 meters in height, and has petiolated leaves with 5-7 lobes in a palmate orientation. The fibrous root system can develop 5-10 starchy roots with secondary thickening. Its

tuberous roots contain up to 85 % of their dry weight as starch. It is an important source of energy with a calorific value of 250 Kcal/ha/day as compared with the range of 110 - 200 Kcal/ha/day for cereals (Cock, 1982; RMRDC, 2004).

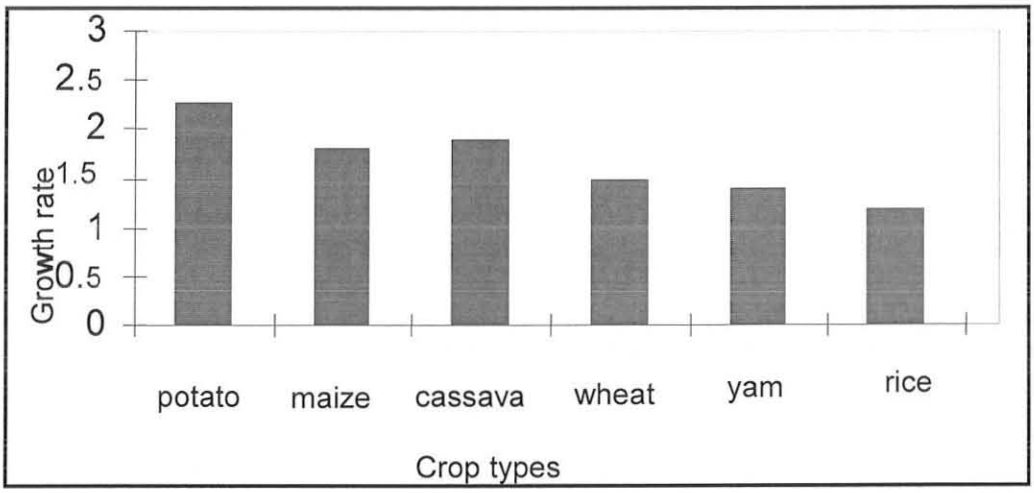
2.1.2 World production of cassava

The world production of cassava root was estimated to be 281.7 million tonnes in 2012. The main production is in Africa where 153.8 million tonnes were grown, while 34.7 million tonnes were grown in Asia, 93.1 million tonnes in Latin America and 0.2 million tonnes in Oceania (FAO, 2012). Accordingly, among the cassava-growing regions of the world, Africa accounts for more than 50 % of the global cassava production (Figure 2.1A). Nigeria is the world's largest producing country of cassava roots with a total of estimated yield more than 57.5 millions tones in the year 2012 (FAO, 2012). The world production of cassava has been projected to reach 290.8 million metric tons per year by 2020 (Scott, et al., 2000). According to (FAOSTAT, 2009), cassava production is projected to have a growth rate of 1.95 % per year in developing countries, being third behind potatoes and maize, but exceeding figures projected for other major cereals such as rice and wheat (Figure 2.1B).

Cassava is one of the most important crops in the developing countries as a source of calories and income for more than 500 million people (FAOSTAT) (Figure 2.2). The ability of cassava to withstand drought and to grow in extremely poor, exhausted soil make cassava suitable for growth in marginal areas unable to sustain many other crops (Bokanga, et al., 1994).



(A)



(B)

Figure 2.1. Cassava production (A) and projected % growth rates for major food crops in developing countries (B). Source: FAOSTAT, 2009; 2012.

Given above considerations cassava is considered as one of very important food security crop of choice for subsistence and small-scale farmers in developing countries (Bellotti, et al., 1999; FAO, 2000; Dejene, 2006).

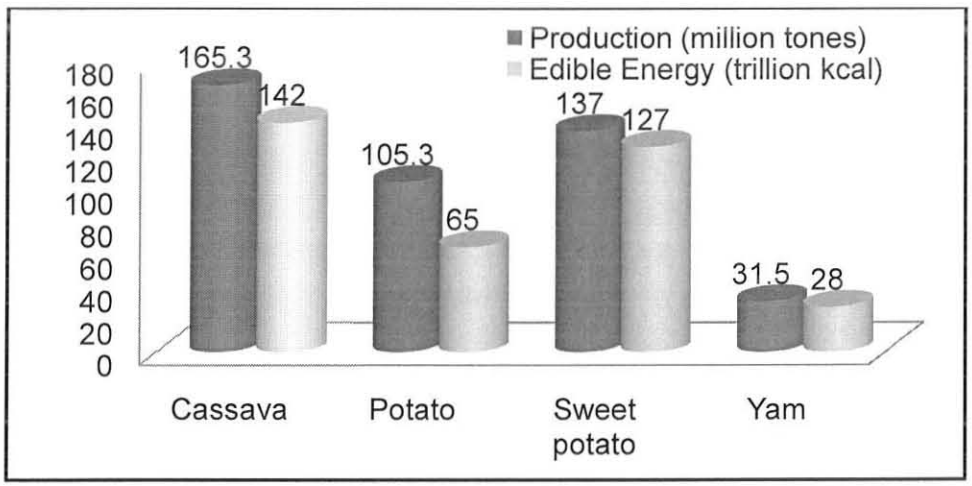


Figure 2.2. Production and edible energy of major root and tuber crops in developing world. Source: FAOSTAT (2009).

The flexible harvesting period of 8 to 24 months, makes cassava an excellent famine foodstuff. It is known as a 'food-bank' since it is amenable to partial harvest, where farmer can harvest only the required amount of roots, and leave the rest back in the soil for later harvest. Second only to sugarcane, cassava produces high amounts of calories per unit of land in form of starch (FAO, 2000; Scott, et al., 2000).

Moreover, in countries where HIV is prevalent, cassava has become a preferred crop. Where a series of natural famine/disasters exists, coupled with epidemic outbreak of HIV in the sub-Saharan countries which may have contributed to a diminished rural labour supply induces a move to labour saving, low-input crops like cassava (Topouzis, 2003; Chiwona-Karltun, 2005). However, due to its low protein content (1-2 %) it needs additional food sources and supplementation with protein rich crops to ensure balanced proteins.

2.2 POSTHARVEST HANDLING AND DETERIORATION FACTORS OF CASSAVA ROOTS

Post harvest deterioration during transport, storage and marketing of cassava is another problem (Wenham, 1995; Aerni, 2005). Even though roots can stay underground for extended periods, once harvested the roots deteriorate very rapidly and are unmarketable within 3-4 days after being detached from the plant. Careful handling and storage in high humidity can prolong the shelf life by one or two months, though this is not commonly practiced. The high perish ability of cassava roots has been attributed to storage organ, which has no function in propagation and possesses no bud primordia from which regrowth can occur (Onuweme, 1978; Cooke, et al., 1985; Cooke, et al., 1988; O'Hair, 1990). Several methods of storage have been proposed for cassava roots to reduce spoilage. For instance, in Amazon 300 years ago and in India 250 years ago fresh roots of cassava were successfully stored by burying them in the soil. In Mauritius they were stored in straw-lined trenches for periods of up to 12 months (June, et al., 1981; Westby, 2002). Storage methods, which results in a reduction of moisture loss from the roots, have good potential for pre-process storage on an industrial scale. Because of the large amounts of material required for industrial processing, two to three days of pre-process storage of cassava root is inevitable, during which time physiological changes reduce starch yield and the quality of processed cassava products. Thus making pre-process storage is the main problem of cassava utilization on an industrial scale (Cooke, et al., 1985; Mulugeta, 2000). Deterioration symptoms commonly occur in the parenchyma and xylogen bundles, which characteristically change colour. The two major identified deterioration factors of cassava roots are an endogenous physiological and microbiological

infection. Physiological or primary deterioration is the first to appear and is caused by rapid post harvest accumulation of phenols, (especially scopoletin), which in the presence of oxygen forms blue, black and brown pigments. Scopoletin can be detected by exposing roots to ultraviolet light. Symptoms include a white or coffee-colour ring because of desiccation at the periphery of the pulp and some blue-black streaks, especially near the xylogen (Rickard, 1985; Beeching, et al., 1994). Microbial or secondary deterioration appears, mainly in areas with physical damage 5 to 7 days after harvest, and are caused by fungi/bacteria. Deterioration is hastened in environments with high relative humidity and temperatures developing the symptoms of vascular streaking and later soft rot, with fermentation and maceration of tissues (FAO, 1995; Mulugeta, 2000).

2.3 MAJOR NUTRIENTS OF CASSAVA AND ITS COMPARISON WITH SELECTED CROPS

The chemical composition of cassava varies in different parts of the plant particularly in peel, leaves and roots, and according to cultivar, location, age, method of analysis and environmental conditions. The peel of cassava roots contains slightly more protein than is found in the flesh. Cassava root is reasonably rich in calcium and vitamin C, but the thiamine, riboflavin, and niacin contents are not as high as the leaves (Bradbury and Holloway, 1988). Large proportions of these nutrients have been reported to be lost during processing (Tilahun, 2009; Mulugeta and Eskindir, 1999). Some cultivars have yellow roots that contain appreciable amounts of β-carotene, (1mg/100 g, on a dwb) (McDowell & Oduro, 1983). Cassava leaves (5.1 % on dwb) are much richer in proteins than the roots (Westby, 2002). The amino acid profile

of the cassava root is very low in some essential amino acids, particularly lysine, methionine and tryptophan (Gil and Buitrago, 2002). Supplementation of cassava products such as roots-meal based with protein rich or any other nutrients to improve its biological value has been widely practiced in industries of food processing for human consumption and animal feeds. The root part of cassava has lower nutritional value than cereals, legumes, and even some other root and tuber crops such as yams (Mbofung, et al., 2006; Huang, et al., 2007; Bede & Okigbo, 2008) as shown in Table 2.1.

Table 2.1. Proximate composition (%) and selected nutrient content in cassava in comparison with some staple food crops in West Africa (minerals and vitamins in mgs).

Food Nutrients	Potatoes	Sweet Potatoes	Fresh Cassava	Yams	Taros	Maize	Sorghum	Cowpea
Energy (Cals)	82	117	146	105	104	363	335	340
Water	78	70	62.5	72.4	72.5	12	12	10.0
Carbohydrate	18.9	27.3	34.7	24.1	24.2	71	71	60.0
Protein	2.0	1.3	1.2	2.4	1.9	10.0	10.4	22.0
Fat	0.1	0.4	0.3	0.2	0.2	4.5	3.4	1.5
Calcium	8	34	33	22	23	12	32	90
Iron	0.7	1.0	0.7	0.8	1.1	2.5	4.5	5.0
Thiamine, B1	0.1	0.1	0.1	0.1	0.2	0.4	0.5	0.9
Riboflavin, B2	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.2
Niacin	1.4	0.6	0.1	0.5	0.9	2.0	3.5	17.0
Vitamin C	10	23	36	10	5	0	0	-

Source: USDA National Nutrient Database

2.4 Economic uses of cassava products

Cassava is one of the most important sources of industrial production of starch in tropical and subtropical countries (Ukwuru and Egbona, 2013; Moorthy, 2004). Several edible products like, porridges, garis, beverages,

chips, etc. are processed from cassava roots. In addition to these, cassava flour or starch can substitute wheat and rice flours at varying levels in baked products (Sakyi-Dawson, et al., 2006). The nutritional value of cassava was enhanced by legume fortification. It was reported that gari and fufu have wider range of consumption than other products of cassava (Emamnuel and Ramakrisha, 1985; Massaquoi, et al., 1990; CIAT, 2001). The root crop serves as a staple food for many families. It is also a source of many useful products as listed in Table 2.2 below. However, awareness and utilization of cassava in different products in sub-Saharan countries particularly in Ethiopia are insignificant.

Table 2.2. Cassava products for human food, animals' feed and industrial use.

	Major Product	
Industries	Animals' feed	Food for human
Alcohol	Cassava pellets	Raw cassava
Glucose	Cassava meal	Boiled cassava
Acetone	Cassava chips	Cooked cassava slices
Dextrins	Cassava slices	Fried cassava slices
Glues and pastes	Cassava peels	Cassava flakes
Binders	Cassava-leaf meal	Fermented cassava
Stabilizer	Broken roots	Cassava flour
Bodying agent (caramel)	Cassava silage	Macaroni
Fillers		Fufu, Gari
Dusting agent (chewing gum)		Ethanol
Single-cell protein		Composite flours, bread
Starch		Tapioca

Source: FAO, 2012; Ukwuru and Egbona, 2013.

2.5.1 Cyanogenic glycosides

Cyanogenic glycosides are compounds found in a number of pulses, roots and oil seeds (Conn, 1994; Francis, et al., 2001). The cyanogenic glycosides are not toxic by itself but hydrogen cyanide (HCN), which is the end product of their hydrolysis, is toxic. There are at least 25 cyanogenic glycosides known to be found in the edible parts of plants (Conn, 1979). The generation of cyanide from linamarin involves the initial deglycosylation of linamarin by linamarase and then the cleavage of acetone cyanohydrins. Linamarase is localized in the cassava cell wall and abundant in laticifers (Mkpong, et al., 1990; Poulton, 1990). Therefore, the release of cyanide occurs only after tissue damage when linamarin meets linamarase. The production of cyanide from acetone cyanohydrins is catalyzed by hydroxynitrile lyase (HNL), which also produces a ketone. This cleavage reaction also occurs spontaneously at temperatures greater than 35 ^{o}C (Jense, et al., 1974; White, et al., 1994) (Figure 2.3). Cyanohydrins produced as a result of linamarin activity is stable only under moderately acidic condition (pH = 4), while in neutral condition it undergoes spontaneous hydrolysis to yield HCN (Cooke, et al., 1985; McMahn, et al., 1995; Egan, et al., 1998). In spite of the relative instability of cyanohydrins, it coexists with intact glycoside and HCN in differently processed cassava products. Cyanogenesis has been shown to protect the plant against herbivore or fungal attack.

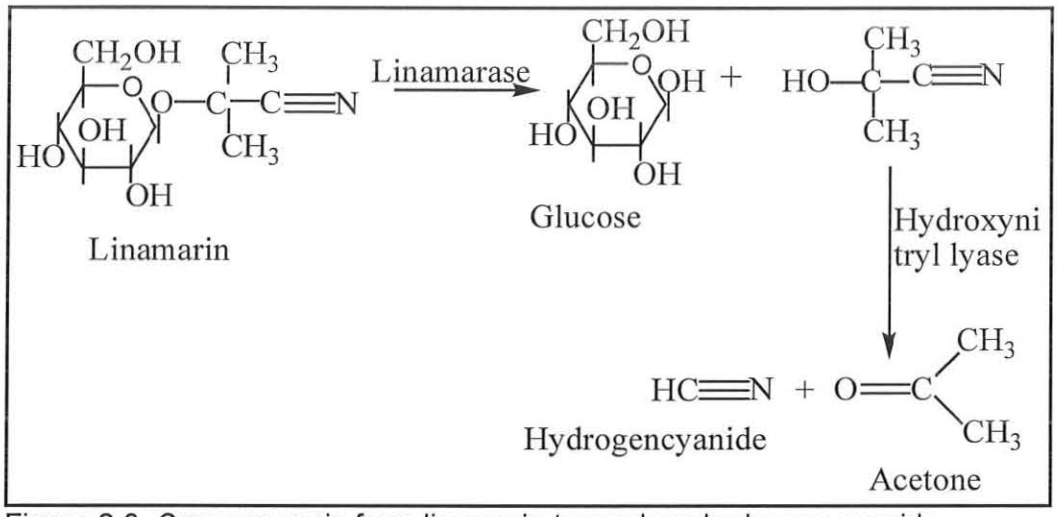


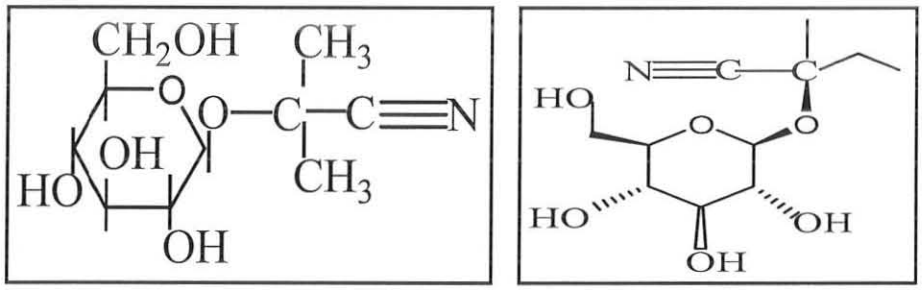
Figure 2.3. Cyanogenesis from linamarin to produce hydrogen cyanide.

Source: Kwok, 2008.

Performed feeding deterrent studies using insect pests of cyanogenic plants prove that cyanogenic glycosides function as natural defence mechanism from enemy (Nahrstedt, 1985; Bellotti and Arias, 1992; Hickel, et al., 1996).

2.5.2 Classification and cyanogenic levels in cassava plant parts

Cyanide, a by-product from cyanogenic glycosides is toxic to humans and most living organisms due to its ability of binding metals such as iron, zinc and copper functional groups of the ligands of most bio enzymes. Cassava contains cyanide in free and bound forms which are toxic substances But through the various processing operations, the level of this cyanic content can be reduced significantly. In the completely unbruised plant, the cyanogenic glucoside remains intact in the form of linamarin and lotaustralin (O'Hair, 1990; Massaquoi, et al., 1990; Bradbury, 2006) (Figure 2.4). Linamarin is chemically similar to glucose but with cyanide (CN ion) attached.



(A) (B)

Figure 2.4. Molecular structure of linamarin (A) and lotaustralin (B).

Unprocessed fresh cassava roots contain on average from 15 to 440 mg CN equivalents/kgfw, while high cyanide cultivars can contain up to 1500 mg CN equivalents/kgfw (O'Briens, et al., 1991; Egan, et al., 1998). Cultivars having root cyanide equivalents of less than 100 mg/kgfw cyanogens, are classified as low cyanogenic cultivars, whereas cultivars with cyanogenic levels greater than 500 mg/kgfw are referred to as high cyanogenic cultivars. A marked radial gradient in linamarin content exists from the outer peel to the inner parenchyma tissue. It has been shown that the outer peel can contain between 7- and 16- times of the linamarin in parenchyma tissue of the same variety (Bradbury and Egan, 1992). Cyanogenic glucosides are not uniformly distributed in the various tissues of cassava plants. The peel's cortex usually has the largest concentration followed by the leaves and the lowest in the seed (Figure 2.5). In the root, the section closest to the stem (proximal) contains more total cyanide than the middle and distal sections. While, there is a shallow longitudinal gradient from the proximal to the distal end (Kojima, et al., 1983).

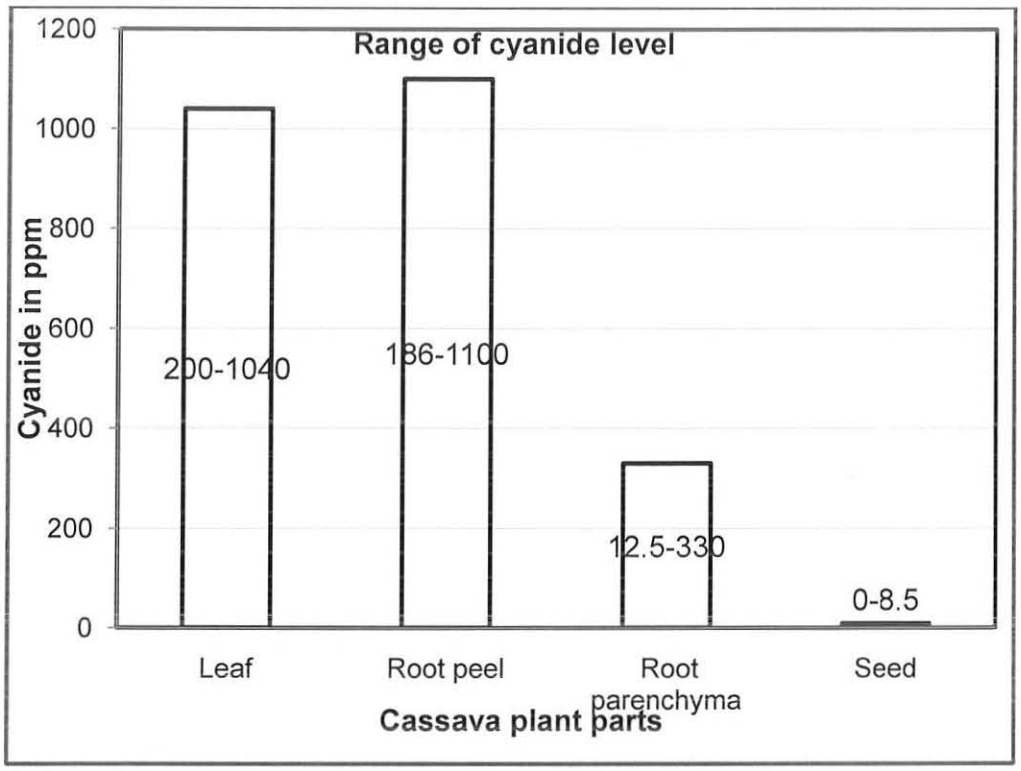


Figure 2.5. Cyanogenic levels in different parts of cassava.

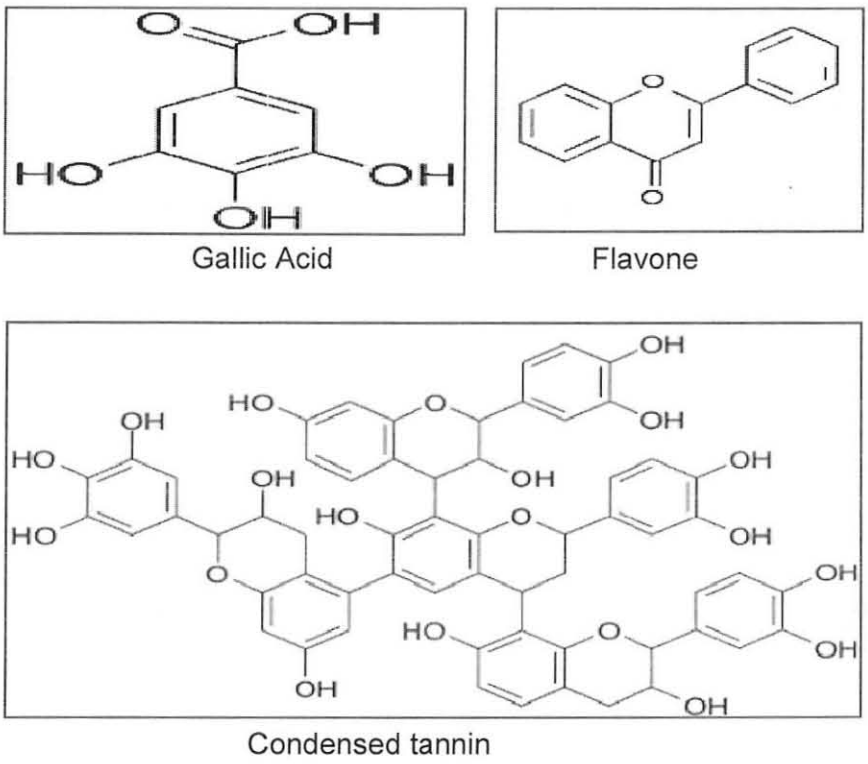
Source: from the reported cyanogenic content of cassava tissue by Makame, et al., 1987; Bradbury and Holloway, 1988; Mkpong, et al., 1990; Noomhorm, et al., 1992; Bradbury and Egan, 1992; 1994 and Eduehi, et al., 2005.

2. 6 Major Anti-nutritional factors of cassava

2.6.1 Tannin

Tannins are polyphenolic compounds of plant origin, having molecular weights between 500-3000 Kilo Daltons, and giving the usual phenolic reactions and special properties such as ability to precipitate alkaloids, gelatine and proteins. They can be classified into two groups, the proanthocyanidins or condensed or procyanidins which are derivatives of flavanols. The hydrolysable tannins are esters of a sugar, usually glucose and polyesters of Gallic acid and hexahydroxydiphenic acid (Mahmut and Ayhan,

2002) (Figure 2.6). The co-occurrence of both kinds of tannins in the same plant or plant tissue is often observed. Tannins were found in the leaves, fruits, barks, roots and wood of trees. Tannins are predominantly located in the pericarp and/or testa, particularly of pigmented cultivars of legumes and millets (Deshpande, et al., 1982; Egli, 2001; Kayode, 2006; Wobeto, et al., 2007).



Condensed tannin

Figure 2.6. Base structures of hydrolysable and non hydrolysable/condensed tannins. Source: Hagerman, 2002.

Tannins found in plants are used as natural protectors that protect kernels against attack by insects and microorganisms. Phenolic compounds cover a range of compounds including flavonoids, phenolic acid, polyphenols and condensed tannins. Phenolic compounds affect the biological availability or activity of metal ions by chelating the metal. Phenolic compounds can affect colour, flavour and nutritional quality of the grain and products prepared from

it (Gul, et al., 2010). They can have a large influence on the nutritive value of many foods eaten by humans such as vegetables, fruits, chocolate, tea, alcoholic and non-alcoholic beverages, etc. Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and iron absorption, which affect the utilization of vitamins and minerals from meals (Tinkilic and Uyanlk, 2001). The bioavailability of non-haem iron leading to poor iron and calcium absorption, and protein digestibility was adversely influenced by tannin (Asante, 1995; Adeparusi, 2001; Egli, 2001; Kayode, 2006).

2.6.2 Oxalate

Oxalate is simple di-carboxylic acid [$(COO)_2$-] found in some foods, which is common and widely spread in plants, and is found in almost all plant families usually at low levels. One major limiting factor in the utilization of leafy and roots plant including cassava is the presence of oxalates, which impart acrid taste or cause irritation when foods prepared from them are eaten (Bradbury and Nixon, 1998; Wobeto, et al., 2007). Ingestion of foods containing oxalates has also been reported to cause caustic effects, irritation to the intestinal tract and absorptive poisoning, and is also known to interfere with the bioavailability of calcium (Kelsay, 1985). Most crops cultivars having oxalate taste acrid and can cause swelling of lips and throat if eaten raw (FAO, 1992; Massey, 2007).

The highest levels of oxalates (400-900 mg/100 g) were found in the leaves and corms of plants such as amaranths, taros, beets, beetroots, spinaches, yams & rhubarbs and the daily consumption of these high oxalate

concentration foods affect health (Savage & Catherwood, 2007). The distribution of oxalic acid within plants is also uneven. In general, oxalic acid is highest in the leaves followed by seeds, but it is lowest in the stems. High oxalate levels in tropical plants are of concern. Taro and sweet potato were reported to contain 278-574 and 470 mg/100 g fresh weight, respectively (Noonan and Savage, 1999; Massey, 2007). The adverse effect of oxalate is greater if the oxalate: calcium ratio exceeds 9:4 (Hassan, et al., 2011). The oxalate can have deleterious effects on human nutrition and health, particularly by decreasing calcium and iron absorption, and aiding the formation of kidney stones (Wanasundera and Ravindran, 1992; Savage, et al., 2000; Albihn and Savage, 2001).

2.6.3 Phytate

Phytate is the primary storage form of phosphorus, formed during maturation of the plant seeds, and is associated with fibre in many foods such as soy- and cereal based products (Umeta, et al., 2005; Monica, et al., 2005). Phytate consists of a myo-inositol ring and six symmetrically distributed phosphate moieties (Figure 2.7). It is a major phosphorus compound in plant seeds and is also found in significant quantities in roots and tubers (FAO, 1990; Urbano, et al., 2000; Phillippy, et al., 2004). Depending on the amount of plant-derived foods in the diet and the level of food processing, daily intake of phytate can be estimated between 2000 & 2600 mg for vegetarian diets and diets of inhabitants of rural areas in developing countries, and about 150-1400 mg for mixed diets (Dicko, 2005). There is a large body of evidence that minerals are less available from foods of plant origin as compared to animal-based foods (Reddy, 2002).

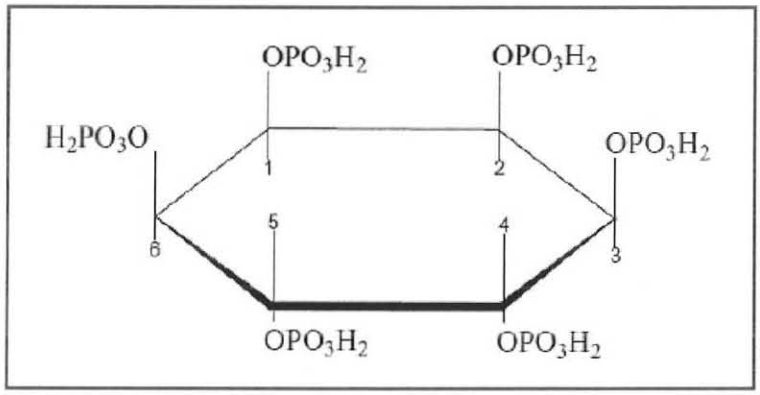
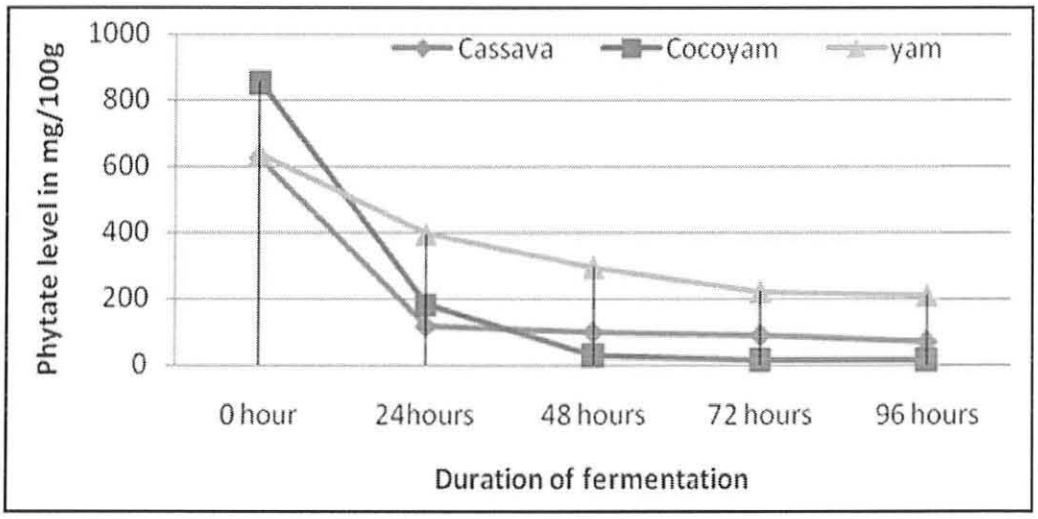
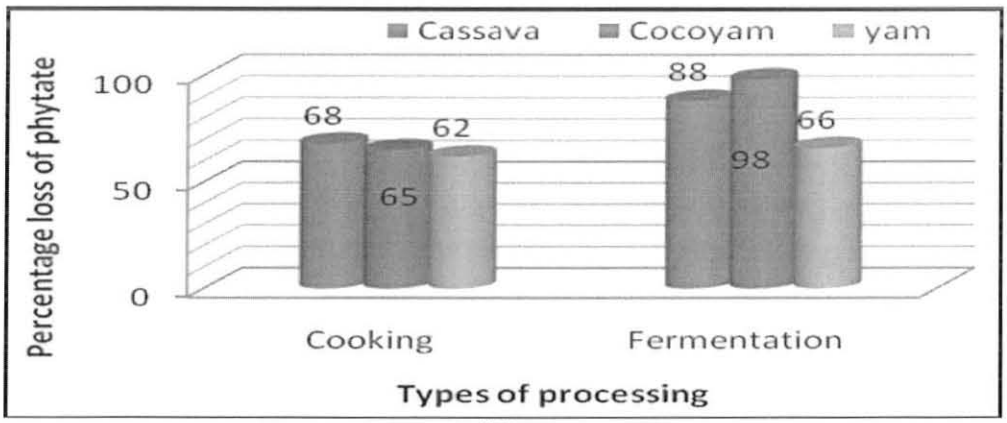


Figure 2.7. Molecular structure of phytate. Source: Graf and Eaton, 1984.

Phytate exhibits high affinity to all polyvalent cations. The inhibition of proteases may be partly responsible for the reduced protein digestibility. Processing of different root crops cultivars such as cassava, cocoyam and yam is reported to reduce phytate content to 624, 855 and 637 mg/100 g, respectively (Greiner and Konietzny, 2006; Adeyeye, et al., 2012). Thus, processing into fermented foods will reduce the phytate level of root crops sufficiently to nullify its adverse effect (Figure 2.8).



(A)



(B)

Figure 2.8. Comparing three root crops reduction of total phytate level due to: Fermentation (A) and Cooking & fermentation (B). Source: Marfo, et al., 1990.

However, beneficial properties of phytate have been observed like antioxidant activity, anticancer activity, prevention of renal stone formation and reduction of starch digestion along with slowing down of the glycemic index of foods (Hurrel, et al., 1999; Shamsuddin, 1999; Grases, et al., 2000). In addition to these, fibre-rich foods protect against diseases such as cardiovascular disease, colon and breast cancer (Howarth, et al., 2001; Slavin, 2004). The phytate content of many countries staple meals are different. For instance, the mg/100 g phytate content in Indian foods ranged from 480 to 520 (Pushpanjali and Santosh, 1995); Korean 191.7 - 973.3 for cereals and 508.5 - 1371.8 for legumes (Joung, et al., 2004) and Indonesia 8 - 319 for cereals, 24 - 1018 for legumes (Sanny, et al., 2007).

2.7 Cassava plantation in Ethiopia

2.7.1 Overview

Ethiopia is located within the cassava growing belt that is in areas between the latitude of 30 °N and 30 °S of the equator (Bokanga, 1994). Cassava plant was exotically introduced to Ethiopia at the middle of nineteenth century (Mulugeta, 1994; Desalegn, 2007). The introducer of cassava in Ethiopia is

However not well identified, some authors believing that it was first introduced by the British missionary (Mulugeta, 1994; Amsalu, 2003), while others say that a man called *Grazimach Damite Dawe*, an Aristocrat land lord of *Buriji*, introduced cassava from Kenya in 1948 (Desalegn, 2007). Although there are no reliable statistics and empirical evidence on the area and production of cassava in Ethiopia, the plant is grown and adapted in areas of an altitude range of 400-1800 m.a.s.l., annual temperature of 15-30 °C and annual rainfall of 600-1500 mm. The crop has been in cultivation, particularly, in South, South West, and Western parts since its introduction (Mulugeta, 1994; Asfaw, 2005) (Figure 2.9). The range of indigenous tuberous vegetables available in the country is extremely small, and estimated at about 0.7 million tonnes of root and tuber crops produced annually in this country (MoA, 1999).

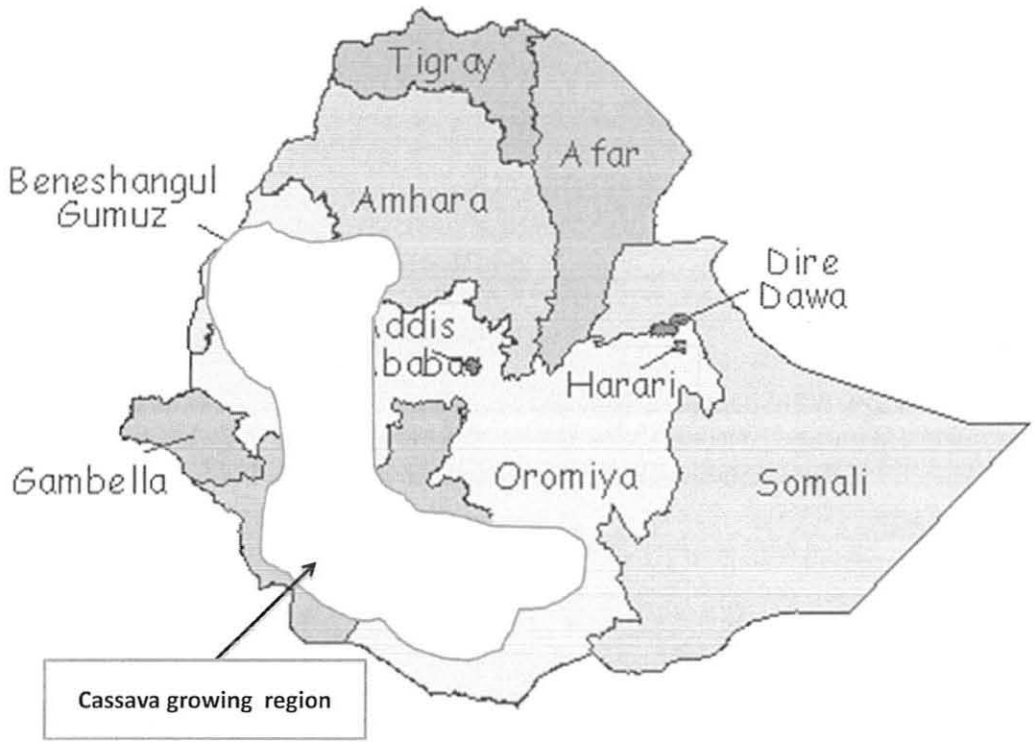


Figure 2.9. Geographical areas of cassava growing in Ethiopia.
Source: Mulugeta, 1994

Even though, the total cultivated area for cassava and total production in Ethiopia has not been recorded, the estimated yields for 2003/4 to 2010/11 for eight years in the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) region agricultural bureau are indicated in Figure 2.10. The highest production was found to be estimated at 2.5 million quintals in 2010/11 from 12,812 hectares of cultivated land. Generally, the data shows increasing in yield from year to year. There were over twenty local and identified cultivars that vary in their morphology, agronomic characters and cyanogenic glucosides content reported to be cultivated in some regions of Ethiopia (Mulugeta, 1995). Cassava was however not a priority crop for research in Ethiopia and very little research done on adaptability, yield potential, variety trial, spacing trial and harvesting trials.

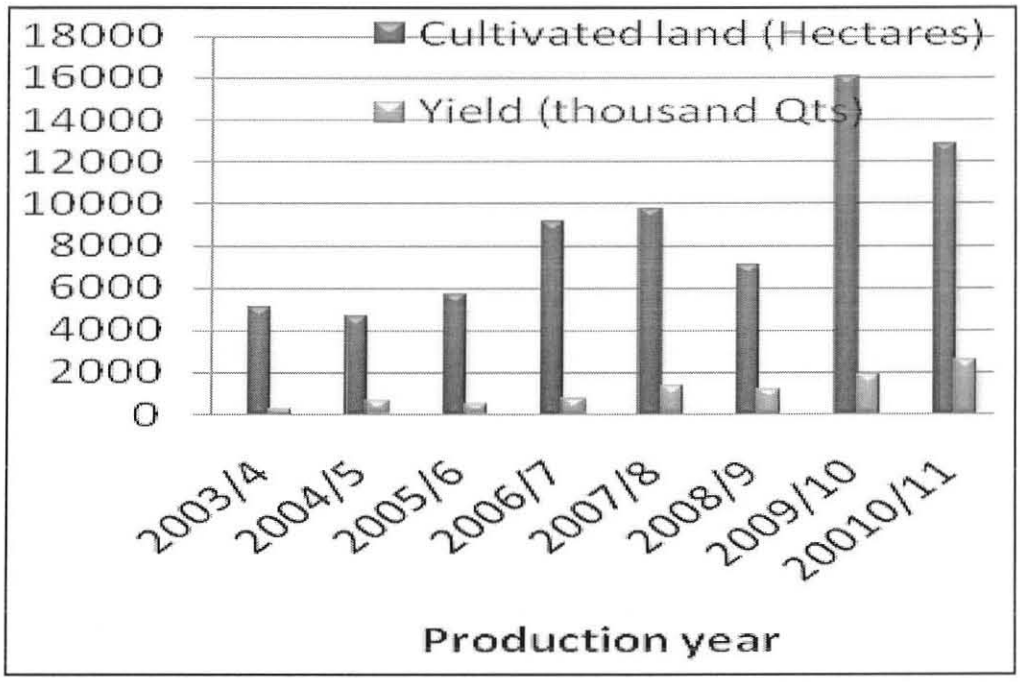


Figure 2.10. Yields in cassava over 8 years cultivation in quintals.

Source: from yearly (2003/4-2010/11) SNNPRS Agricultural Bureau reports.

2.7.2 Using cassava as food security crops in Ethiopia

Agriculture is the backbone of the Ethiopia's economy. It contributes about 50 % of the gross domestic product (GDP), 60 % of exports, provides a livelihood for 85 percent of the total population and generates nearly 90 % of foreign exchange earnings. Despite of this, the country suffers from serious food shortage and recurrent droughts. The majority of the Ethiopian population depend mainly on cereal crops as their main food source. The food potentials for most "horticultural" crops in particular root and tuber crops like cassava have not been fully exploited and utilized, despite their significant potential as food security crops for income generation, and as resource base conservation (EARO, 2000; Dejene, 2006; Desalegn, 2007).

In Ethiopia, cassava is cultivated poorly and extensively in densely populated and low rainfall areas of the Southern parts of the country, though an important food source among the communities that plays critical roles in rural diets. In some cases, it fills food shortage gaps during the months when maize and other foods run short and in year of drought (Simone, 1992; Edossa, et al., 1995; Asfaw, 2005). A preliminary survey, in Konso showed that the consumption of cassava is high among low-economic groups of families (Teshome, et al., 2003). Similarly, Amaro special woreda, revealed cassava to be the most frequently consumed root crop (field study of researcher). The main problems in the cassava consuming areas are the lengthy and tedious methods required to process it, and in particular cultivars that contain toxic substances. One of the methods of alleviation of toxic substances is blending the cassava flour with other protein foods crops

(Kebede, et al., 2012). Consumption of insufficiently processed cassava roots resulted in health complaints (Cherinet, et al., 1998).

2.8 Effect of anti-nutritional factors on humans health

Compounds, which act to reduce nutrient utilization and/or food intake, are often referred to as anti-nutritional factors. These anti-nutritional factors when consumed in foods may have adverse effects on health through inhibition of protein digestion and essential minerals absorption (Graf and Eaton, 1984; Omoruyi and Dilworth, 2007). Among cultivated varieties of most edible tubers and roots crops, some cassava cultivars contain cyanogenic glycosides at lethal levels and must be adequately processed before consumption (Mulugeta, 2000; Oboh and Akindahunsi, 2003; Oboh, 2005).

The residual cyanogens, linamarin and acetone cyanohydrins, are the apparent source of cyanide toxicity to animals when converted to cyanide inside the body. Cyanide is very poisonous because it binds to an enzyme called cytochrome oxidase and stops its action in respiration, which is a key energy conversion process in the body. The HCN toxicity is mostly due to its ability to combine reversibly with enzymes associated with cellular respiration thus suppressing natural respiration and causing cardiac arrest (Conn, 1979; Rosling, 1993; Francis, et al., 2001). Exposure to lower levels of cyanide can also cause a variety of symptoms, such as vomiting, nausea, palpitations, headaches and impaired vision (Hahn, 1983; Rosling, 1988).

The ingestion of large quantities of cassava or prolonged exposure to improperly processed cassava food has been associated with chronic cyanide

toxicity in several areas of Africa (Tylleskar, et al., 1992; Mlingi, et al., 1992). During drought cassava, associated cyanide poisoning is aggravated by the lack of firewood resulting in inadequate cooking and detoxification of cassava. Cyanide intake from cassava-dominated diet is a contributing factor in two forms of nutritional neuropathies and tropical ataxic neuropathy (TAN) described from Nigeria (Osuntokun, 1981; FAO, 1990); and epidemic spastic paraparesis described from Mozambique, Tanzania and Zaire (Ministry of Health, Mozambique, 1984).

2.9 Benefits of blending of flour

Enrichment of food products

Enrichment is defined as "the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups" (FAO/WHO, 2001). One of the problems affecting millions of people, and particularly children are lack of adequate protein intake, which is a consequence of mostly incidences of protein energy malnutrition. The nutritional quality of food can be improved by augmenting protein content and limiting amino acids especially lysine (Oyewole and Aibor, 1992; Anjum, et al., 2005). In food products manufacturing, it is important to balance the quality and quantity of protein, keeping the nutritional status of populations (Hung and Zayas, 1992; Pogna, et al., 1994; Vieira, et al, 2007).

Usually, cereal and root crops proteins are deficient in few essential amino acids like lysine and tryptophan, but these deficiencies are mainly related to

endosperm portion of the kernel. The bioavailability of proteins and energy from raw legumes and cereals is poor and require processing prior to consumption (Onigbinde and Onobum, 1993; Lijeberg, 1995; Melcion and Van der Pod, 1993; Taiwo, 2009). Cassava roots flour is deficient in some of the macronutrients which could be improved using nutrient rich crops blends (Kebede, et al., 2012). In general, cereals have low protein content, and enrichment of cereals with locally available legumes which have high protein, can increase protein content of the blended cereal and legume (Boonyaratpalin, et al., 1998; Ojinnaka, 2009; Bolaji, et al., 2010). The amino acid profile of quality protein maize germs is nutritionally valuable being balanced in all essential amino acids except for isoleucine absence. The maize germ protein contains 47 % albumin and amino acids particularly lysine and tryptophan are comparable with that of casein (Gupta and Eggum, 1998).

To solve the problem of nutrient deficiency, the enrichment of staple food is routine and has proven to be very efficient for certain macro and micronutrients (Darnton and Nalubola, 2002). Maize, sorghum, wheat, pulses and now tef are some of basic foods worthy of attention. These are some of the food materials commonly used throughout the developing countries (Uche, et al., 2008). To combat the macronutrient deficiency, the following processes were tried namely: production of composite breads and biscuits from mixed flours of wheat and plantain (Mepba, et al., 2007; Olaoye, et al., 2007). Most commonly, incorporation of cereals with tubers flour (potato) in cookie formulation has been reported by Singh, et al. (2008). Furthermore, a variety of wheat flour substitutes in bakery formulations such as soy flour

(Junqueira, et al., 2008), flaxseed (Koca and Anil, 2007) and sunflower seed (Skrbic and Filipcev, 2008) have been tried. In various research trials, layer cakes were successfully prepared from different cereals and legume composite flour blends (Salem, et al., 1999; Gomez, et al., 2008). The product from composite flour blends in food formulations could potentially supply most of the nutrients needed in human diets especially essential amino acids, minerals and dietary fibre (Elkhalifa and El-Tinay, 2002; Akubor and Ukwuru, 2003). Thus, formulation and fermentation of composite flour is vital for the development of value-added products with optimal functionality (Yigzaw, et al., 2001; Westby and Choo, 2002; Anton, et al., 2008).

2.10 Overview of experiment on animals (mice)

2.10.1 Biological evaluation of diets on mice

All living organisms need food for growth, work, repair and maintaining the life process. Using animal models to assess the safety of test components is usually focused on treatment-related values representing changes relevant to pharmacological effects. However, efficacy studies are carried out with multiple objectives like assessing the safety of products or specific ingredient, determining its specific quality such as protein and oil quality and in many instances health promoting potential of new food product (Cellini, et al., 2004; Malley, et al., 2007).

2.10.2 Body growth of mice

Growing mice require balanced dietary protein to meet their protein requirements. Dietary changes may lead to series of reactions which can cause disruption of normal physiological activity, bringing changes in

biochemical constituents of the body fluid of test animals. Body growth performance, biological evaluation and blood biochemical screening are useful indicators for nutritional research, and reliable diagnosis of various physiological disorders (White, et al., 2000; Schilter, et al., 2003). Furthermore, clinical pathological evaluation is being used as one of the safety assessment tools when some new food product sources are exploited for their appraisal as safe human food ingredient (Singh, et al., 2002; Adeyemi, et al., 2007).

2.10.3 Nutritional evaluation of protein *in vivo*

The nutritional quality of a protein was determined by the amino acid composition and the digestibility of that protein. This type of test is, in essence, an *in vivo* test conducted in a non-human animal model and, therefore, influenced by many of the same extrinsic factors as *in vivo* tests in human (Eggum and Pederson, 1983; Graf and Eaton, 1984; Myer, et al., 1996).

Biological assay measures the efficiency of biological utilization of dietary protein as source of the essential amino acid under a set of standard condition. Biological assay of particular protein quality can be measured as gain in weight of an animal per gram of the protein taken.

Moreover, the fundamental measurement of protein quality for human use depends on growth and/or other metabolic balance evaluation procedures performed in suitable subject of the target population. This procedure directly reflects the essential amino acid content, digestibility of the protein and bioavailability of the amino acids in foods or food products. Such studies are

only carried out using animal assay techniques that correlate closely with data from human experiments. Mice growth assays used in such work, have been widely used for predicting protein quality in foods, and numerous workers have discussed appropriateness of this method (Eggum and Pederson, 1983; Escudero, et al., 1999). Milk protein with special reference to casein is well known for its good nutritive functional properties in food formulations, and is usually used as reference to evaluate quality of other proteins (Friedman, 1996). Basal metabolism and body weight of test animals do not always exhibit linear relationship between different species, neither of test animals nor within the same species. For prediction of basal metabolism (BM), and in order to assess the safety aspects, weight of various organs and tissues together with their specific metabolic activity seems to be promising (Heusner, 1982; Even, et al., 2001; Olivera, et al., 2003; Petterino and Argentino-Storino, 2006).

3. MATERIALS AND METHODS

3.1 SOURCE OF MATERIALS AND STUDY AREAS

The study cassava samples were collected from Western (Jimma Agricultural Research Centre, 360 Km from A.A.), Northern (Hayik, 456 Km from A.A.) and Southern (Amaro, 470 Km from A.A.) parts of Ethiopia. These are located on the map (Figure 3.1). The seven cultivars of cassava roots were 12 to 14 months old, and with accession number or local name of 28, 192, 5538-19, 44/72-NW; Gamo (red skin) and Koree (white skin); and Hayik (red skin), taken from West, South and North, respectively. The cultivars were selected on the bases of most released varieties and highly grown areas of the country. The cassava roots were manually harvested, packed into a sack, and transported within one day to the laboratory of the Centre for Food Science and Nutrition (CFSN), Addis Ababa University.

The quality protein maize variety Melkasa 6Q *(Zea mays* L.) and dry common bean (*Phaseolus vulgaris*) were collected from Melkasa Agricultural Research Centre, while white Kuncho variety tef (*Eragrostis tef*) was collected from Debre Zeit Agricultural Research Centre for composite flour making (see Figure 3.2). These studies were carried out from 2010 -2012 as follows:

Cleaning, peeling, cutting into pieces and grating, fermenting, boiling, drying, grinding of dried chips and ingredients of composite flours, and Functional and physical properties were performed in the Centre for Food Science and Nutrition laboratory, Addis Ababa University (AAU), Addis Ababa, Ethiopia. Whereas, all chemical analyses, sensory evaluation and testing diets on mice were performed at the Ethiopian Health and Nutrition Research Institute

(EHNRI) laboratory, Addis Ababa, Ethiopia.

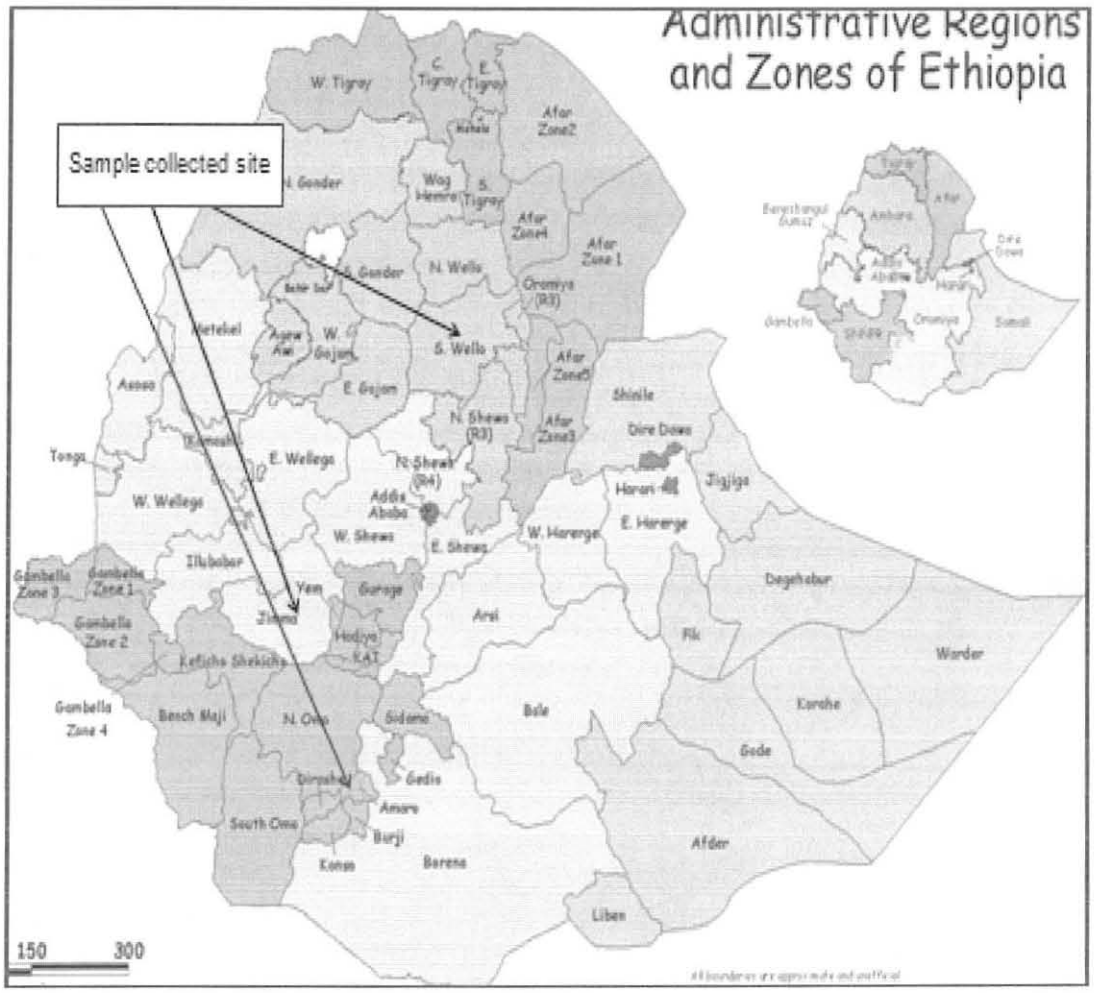
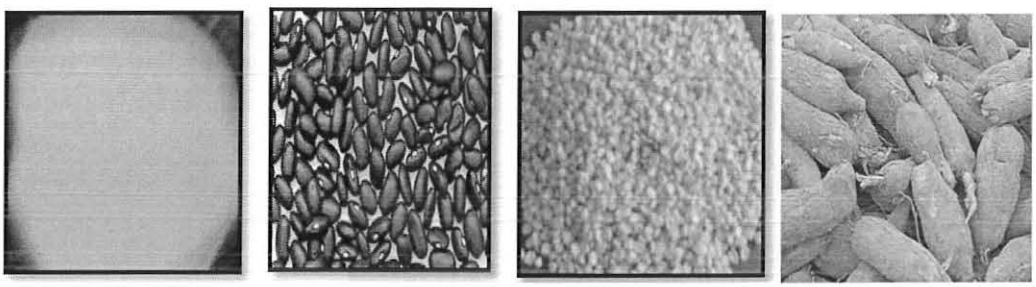


Figure 3.1. Ethiopian map showing areas where cassava root samples were collected (source: UN Emergencies Unit for Ethiopia, March 2000).



a. b. c. d.

Figure 3.2. Pictorial representation of raw ingredients for composite flour:

a = white tef, b = common bean, c = quality protein maize and d = cassava roots.

3.2 FRAME WORK OF THE STUDY DESIGN.

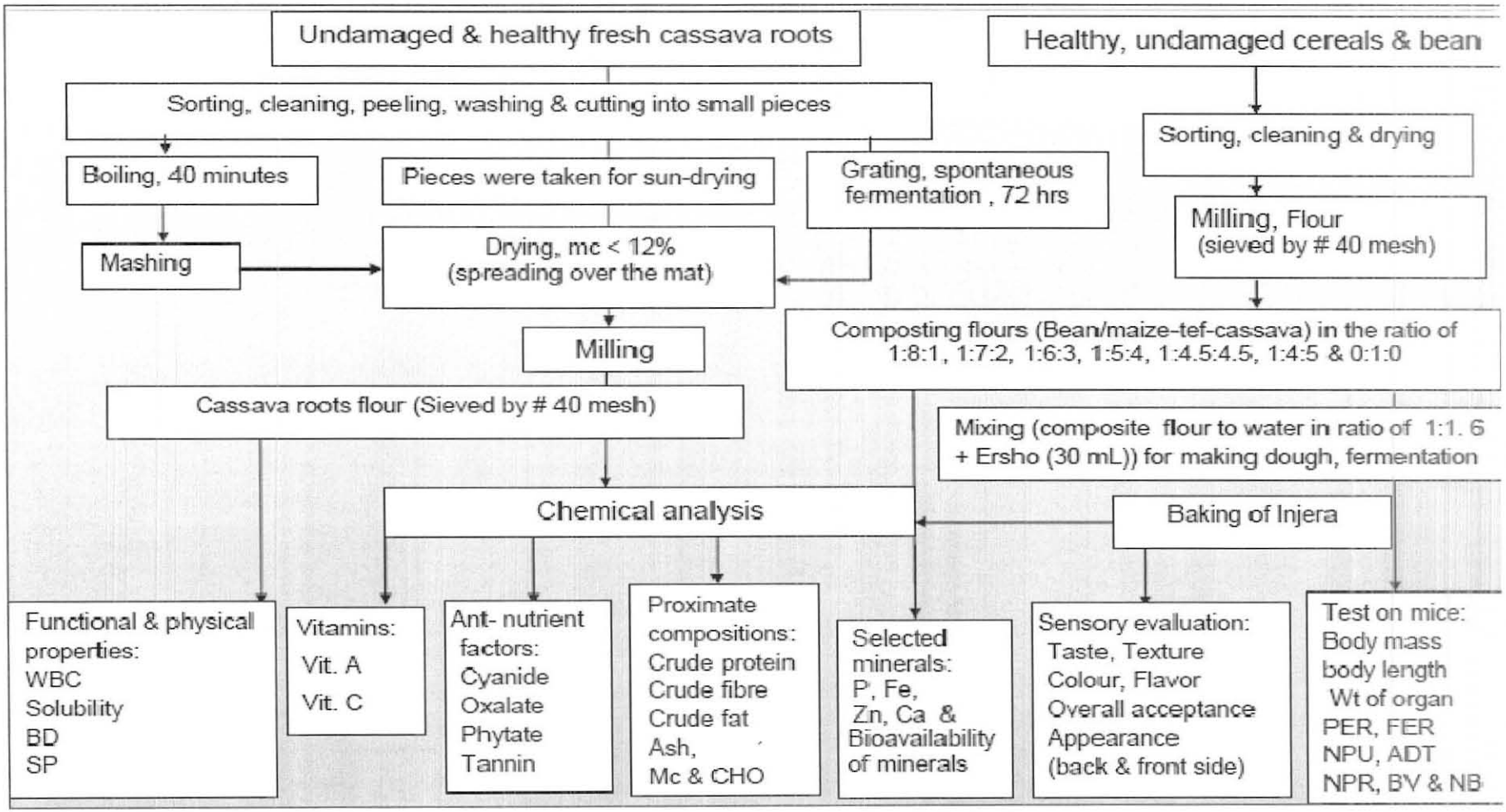


Figure 3.3. Frame work of the studied experiments.

3.3 SAMPLE PREPARATION BY THREE COMMON PROCESSING

3.3.1 Sun-drying

Undamaged and uniformly matured raw fresh cassava tubers were taken and then washed with potable water to remove dirt. The tubers were peeled and manually cut in to pieces on chopping board with a stainless steel knife. The pieces were sun-dried to less than 12 % moisture content (db) using the procedure of Gomez, et al. (1984). The dried chips were ground into flour by electrical grinder (Mouliex, A2424A, France) and then the flour was sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA) packed into polyethylene bags and stored in a cool and dry environment away from sunlight until analysis.

3.3.2 Boiling

Using methods described by Cooke and Maduagwu (1978), the raw fresh cassava roots were peeled, sized and placed into stainless steel pan and boiled for about 45 minutes. The cooked cassava roots were crushed and then sun-dried to less than 12 % moisture content. Furthermore, the dried cassava roots were ground into flour using electrical grinder (Mouliex, A2424A, France), sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), packed in polyethylene bags and stored in a similar manner like sun-dried flour until analysis.

3.3.3 Fermentation

Cassava fermentation was followed the Nigerian traditional cassava roots processing methods adopted by FAO (1998) with slight modification (see Appendix-I). Six Kg of each of the tuber roots from the seven cultivars were sorted, peeled, washed with tap water, cut into smaller pieces and grated

by electrical grinder. The grated pulps were put into a 1000 mL measuring cylinder and the cylinder was covered with aluminium foil and allowed to ferment naturally (spontaneously) at ambient temperature for 72 hours. After 72 hours of fermentation, the paste was spread over the tray and sun-dried to less than 12 % moisture content (db). The sun-dried paste was milled by electrical grinder (Mouliex, A2424A, France), sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), packed and stored in similar manner like other samples.

3.4 Determination of pH value

The pH of the dough was determined according to the method of AOAC (1984). The pH of the sample was measured by dipping the electrode in the batter using pH meter (MP 511 Lab. pH meter, China).

3.5 Chemicals and reagents

All chemicals and reagents used in laboratory analyses were of analytical grade or A.C. reagents.

3.6 Proximate composition analysis

The crude protein (N x 6.25), crude fat, crude fibre, ash and moisture content were determined using the method of AOAC (2000).

3.6.1 Determination of moisture content

The empty drying dishes (made of porcelain) were dried using drying oven (Memmert, Germany) for 1 hour at 100 °C, transferred to the desiccators (with granular silica gel) and cooled for 30 minutes, and weighed using digital analytical balance (Adventurer model, AR 2140, USA) to the nearest mg. The prepared fresh samples were mixed thoroughly and about 5.000 g of the fresh sample (in triplicate) were transferred to the dried and weighed

drying dishes. The dishes and their contents were placed in the drying oven and dried for 5 hours at 100 °C and transferred into the desiccators and cooled to room temperature and reweighed.

$$\%\text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

Where: $M_{INITIAL}$ and M_{DRIED} are the mass of the sample before and after drying, respectively.

3.6.2 Determination of total ash content

The ashing dishes (made of porcelain) were placed into a Muffle furnace (Carbolite model S302RR, USA), for 30 minutes at 550 °C. The dishes were removed and cooled in a desiccators (with granular silica gel) for about 30 minutes, and when cooled to room temperature, each dish was weighed to the nearest mg. About 2.500 g of fresh sample (in triplicate) were added into each dish. The dishes were placed on to a hot plate (Wagtech model ST15, Sweden), under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550 °C for 5 hours, and removed from the muffle and then placed in a desiccators for 1hour to cool. The ash was clean and white in appearance. When cooled to room temperature, each dish and ash was reweighed using digital analytical balance to the nearest mg.

$$\%\ \text{Ash (wet basis)} = \frac{M_{\text{ASH}}}{M_{\text{WET}}} \times 100$$

Where: M_{ASH} refers to the mass of the ash, and M_{WET} refer to the original masses of the fresh samples

3.6.3 Determination of crude fat

The extraction flasks were cleaned, dried in drying oven (Memmert model DIN 40050-IP 20, Germany) at 92 °C for 30 minutes, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2 cm layer of fat free cotton. About 5.00 g of fresh samples (in triplicate) were added into the extraction thimbles, and then covered with about 2 cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber (2055 Soxtec, Manual extraction unit Foss Tecator, Sweden). The cooling water was switched on, and a 70 mL of Petroleum ether was added to the extraction flask through the condenser. The extraction was conducted for about 3:30 hours (where it is immersed/soaked in the solvent for 50 minutes, for 2:30 hours dissolved/suspended and for 10 minutes recovery of the used petroleum ether) at 70 °C. The extraction flask with its content was removed from the extraction chamber and placed in the drying oven at 92 °C for about 30 minutes, cooled to room temperature in the desiccators for about 30 minutes and re-weighed.

$$\textbf{Fat g/ 100g fresh sample (W)} = (W_2 - W_1)*100/ W_D$$

Where: W = weight of fat; W_2 = weight of extraction flask after extraction (wt. of flask and fat); W_1 = weight of extraction flask before extraction (wt. of flask); W_D = weight of fresh sample

3.6.4 Determination of crude protein

The determination of crude protein was conducted by the method of Tecator method (Tecator manual E-DS 12.71)

Digestion: About 0.50 g of fresh samples (in triplicate) were taken in a Tecator tube and 6 mL of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulphuric acid) was added and mixed, and a 3.5 mL of 30 % hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shacked for a few times and placed back into the rack. A 3.00 g of the catalyst mixture (ground 0.50 g of selenium metal with 100 g of potassium sulphate) was added into each tube, and allowed to stand for about 10 minutes before digestion. When the temperature of the digester was at 370 ^{0}C, the tubes were lowered into the digester. The digestion was continued until a clear solution is obtained, for about 1hour. The tubes in the rack were transferred into the fume hood for cooling; a 15 mL of water was added, and shacked to avoid precipitation of sulphate in the solution.

Distillation and titration: The digested and diluted sample solution was distilled using boric acid and the distilled sample solution was titrated using 0.1N sulphuric acid to reddish colour using Kjeldehal apparatus (Kjeltec 2300 Analyzer unit, Foss Tecator, Sweden)

mg nitrogen in the sample = V x N x 14

g nitrogen/ 100 g = mg of nitrogen x 100/mg sample

Total nitrogen (%) = [(V-V_b) x N x 1.4)]/ W

Crude protein (%) = total nitrogen (%) x 6.25

Where: V = volume of sulphuric acid consumed to neutralized the sample; V_b= the volume of acid consumed to neutralize the blank; N = normality of the acid; 14 = Eq. wt of nitrogen; 6.25 = conversion factor from total nitrogen to crude protein

3.6.5 Determination of crude fibre content

Digestion: About 1.60 g of fresh sample (in triplicate) was placed into a 600 mL beaker; 200 mL of 1.25 % H_2SO_4 was added, and 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 minutes of heating, 20 mL of 28 % KOH was added and boiled gently for a further 30 minutes, with occasional stirring.

Filtration: The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible and then the vacuum pump (Robinar model 15601, Sweden), was turned on. The wall of the beaker was rinsed with hot distilled water several times, washings was transferred to crucible and filtered.

Washing: 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; and again washed with 1 % NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1 % H_2SO_4 and filtered. Finally the residue was washed with water- free acetone.

Drying & combustion: The crucible with its content was dried for 2 hours in the electric drying oven (Memmert model DIN 40050-IP 20, Germany), at 130 °C and cooled for 30 minutes in a desiccators (with granular silica gel), and then weighed (recorded as W_1). The crucible with dried content was transferred to Muffle furnace (Carbolite model S302RR, Sweden), and heated for 30 minutes at 550 °C. The crucible was cooled in a desiccators

and weighed (recorded as W_2).

Calculation:

$$\text{Crude fiber g/100g} = [(W_1-W_2)*100] / W_3$$

Where: W_1 = weight of (crucible + sample) after drying; W_2 = weight of (crucible + sample) after ashing; W_3 = fresh sample weight

3.6.6 Computation of carbohydrate

Total carbohydrate content was estimated by the difference, as % on db.

$$\text{Carbohydrate (Kcal/100g)} = 100 - \%\ (\text{fat} + \text{protein} + \text{mc} + \text{ash})$$

3.6.7 Computation of total energy

The energy values of different food formulations were determined by computation and expressed in calories. Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors: 1 g fat = 9 kcal, 1 g protein = 4 kcal, 1 g carbohydrate = 4 kcal and 1 g alcohol =7 kcal (Marero, et al., 1988).

$$1\text{Kcal/100g} = (4*\text{carbohydrate}) + (4*\text{protein}) + (9*\text{fat})$$

3.7 Minerals determination (Ca, Fe & Zn)

Ashes were obtained from dry ashing. Minerals were extracted from the sample by dry ashing according to the method of Hernandez, et al. (2004). The ash was wetted completely with 5 mL of 6N HCl, and dried on a low temperature hot plate (Wagtech model ST15, Sweden). A 7 mL of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature at open air in a

hood and filtered through a filter paper (Whatman 42, 125 mm) into a 50 mL graduated flask. A 5 mL of 3N HCl was added into each crucible dishes and heated until the solution just boil, cooled, and filtered into the flask. The crucible dishes were again washed three times with de-ionized water; the washings were filtered into the flask. A 2.5 mL of 10 % lanthanum chloride solution was added into each graduated flask. Then the solution was cooled & diluted the contents of the flask to the mark (50 mL) with de-ionized water. A blank which contain 12 mL 3N HCl and de-ionized water in 50 volumetric flask was prepared by taking the same procedure as the sample.

Standard solutions: Four series of working standard metal solutions (Table 3.1) were prepared by appropriate dilution of the metal stock solutions with de-ionized water containing 2.4 mL 3N HCl in 10 mL volumetric flask. After manipulating the instrument operation procedure, calibration graph (concentration versus absorbance) for each element using the prepared standard solutions was prepared.

The sample concentrations were analyzed using Flame Atomic Absorption Spectrophotometer (Buck scientific FAAS, model, 210, VGP, Canada) by aspirating de ionized water and the reagent and sample blank solution was run with the sample. A single mineral hollow cathode lamp was used for each element. Series of working standards solutions for essential minerals determination using flame atomic absorption spectrophotometer (FAAS) at their specific wavelength (Osborne and Voogt, 1978).

Table 3.1. Optimum working condition and calibration standards for minerals analysis.

Elements	Wave length (nm)	[t]Slit width (nm)	[t] (µg/mL)	Standard concentration (µg/mL)
Iron	248.3	0.2	0.06-15	0.0, 12.0, 24.0, 36.0, 48.0, 60.0
Zinc	213.9	1.0	0.01-2.0	0.0, 0.5, 1.0, 1.5, 2.0
Calcium	422.7	0.5	0.01-3.0	1.0, 1.5, 2.0, 2.5, 3.0

[t]FAAS optimum working range.

Metal content in mg/100g = [(a - b) x V]/(10 * W)

Where: W = Weight (g) of samples; V = Volume of extract;

a = Concentration (µg/mL) of sample solution; b = Concentration (µg/mL) of blank solution

3.8 TOTAL PHOSPHOROUS DETERMINATION

Ammonium vandate was used to determine phosphorous along with ammonium molybdate using the method of Chapman and Pratt (1982). One millilitre of the clear extract (sample solution prepared for mineral determination) was diluted into 100 mL with double distilled and deionised water. Five millilitre of the sample solution was added into test tubes. Exactly 0.5 mL of molybdate and 0.2 mL aminonaphtholesulphonic acid were added into the test tubes (sample solution) and mixed thoroughly. The solution was allowed to stand for 10 minutes. A series (0.0, 0.1, 0.2, 0.4, 0.6, 0.8 & 1.0 µg/mL) of working standard phosphorous solutions for calibration graph were prepared by appropriate dilution of the phosphorous stock solution (1000 µg P/mL of KH_2PO_4) with deionised water, using 10 mL volumetric flask. Absorbance (ab) of the sample solution was measured at

660 nm against distilled water using UV-Vis spectrophotometer (Model: CE1021, England). Calibration graph (concentration verses absorbance) for each element was prepared using the prepared standard solutions.

$$P \text{ in mg/100g} = \frac{(\text{sample ab.} - \text{blank ab.}) * \text{dilution factor} * \text{extracted volume}}{\text{Slope} * \text{weight of sample} * 10}$$

3.9 CYANIDE DETERMINATION

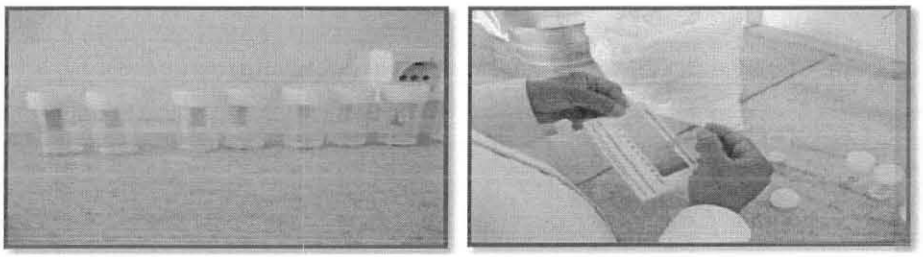
The total cyanide contents in the raw cassava root of the seven cultivars was analyzed by acid hydrolysis of cyanogenic glucoside as described by Bradbury, et al. (1991). Twenty gram of cassava roots sample was placed in extraction flask and followed by addition of 100 mL of distilled water and allowed to stand for two hours, in order to set free all the bound hydrocyanic acid, meanwhile keeping the flask connected with an apparatus for distillation. After two hours of maceration, 100 mL of distilled water was added to the slurry and steam distilled. The distillate was collected in 20 mL 0.01N $AgNO_3$ that has been acidified with 1 mL HNO_3. The distillation process was allowed to proceed for 40 minutes with vigorous boiling. After passing over of 150 mL of the distillate, the distillate was filtered through Gooch with little water and the excess $AgNO_3$ was titrated in combined filtrate and washings with 0.02N KSCN, using ferric alum indicator. The end point of titration was indicated by appearance of faint reddish colour up on addition of 0.02 N KSCN solution. The quantity of HCN present in the sample was calculated from the following relation.

Volume (mL) of $AgNO_3$ consumed to complex CN^- = 20 - 2V of the titter.

1mL 0.01 NAgNO3 = 0.27 mg HCN

In a parallel experiment the retained total cyanide level of the processed

cassava root flours samples were analyzed by Picrate kit protocol (Haque and Bradbury, 2002). A 100 mg flour sample was placed in a plastic vial, then a filter paper impregnated with linamerase plus pH 6 buffer added, followed by 0.5 mL of water and a yellow Picrate paper (Figure 3.4). The vial was immediately closed and left at room temperature for 24 hours. The next day the yellow brown Picrate paper was separated from the plastic backing strip and placed in 5.0 mL of water. The absorbance of the solution was measured at 510 nm by UV-Vis spectrophotometer (Model: CE1021, England) and the total cyanide content in ppm was calculated by multiplying with 396 according to (Bradbury, et al., 1999).



(A) (B)

Figure 3.4. Picrate kit detecting the level of total cyanide content of cassava roots flour: A) Picrate paper in the plastic bottle; B) Qualitative reading.

3.10 ANTI-NUTRITIONAL FACTORS DETERMINATION

3.10.1 Determination of phytate content

Phytate content of the raw and processed cassava flour samples were determined by using the method of Latta and Eskin (1980). About 0.05 g of fresh samples were extracted with 10 mL 2.4 % HCl in mechanical shaker for 1 hour at an ambient temperature and centrifuged at 3000 rpm for 30 minutes. The clear supernatant was used for phytate estimation. A 1 mL of

Wade reagent (containing 0.03 % solution of $FeCl_3.6H_2O$ and 0.3 % of sulfosalicilic acid in water) was added to 3 mL of the sample solution (supernatant) and the mixture were mixed on a vortex mixer for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA).

A series of standard solution were prepared containing 10, 20, 30, 40, 50, 60 µg/mL of phytic acid (analytical grade sodium phytate) in 2.4 % HCl. A 3 mL of standard were added into 15 mL of centrifuge tubes with 3 mL of water, which were used as a zero level (blank). A one 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 solutions were measured at 500 nm by using water to calibrate the spectrophotometer.

Phytic acid in µg/g = [(Ab. - Intercept)/(Slope * Density * Wt.)]*10

3.10.2 Determination of tannin content

Condensed tannin content of the cassava roots and composite flour samples were determined using the method of Maxson and Rooney (1972). About 2 g of sample was weighed in screw cap test tube. The sample solutions were extracted with 10 mL of 1 % HCl in methanol for 24 hours at room temperature with mechanical shaker. After 24 hours of shaking, the solution was centrifuged at 1000 rpm for 5 minutes. A 1 mL of supernatant was taken 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). A 40 mg of D-catechin was weighed and dissolved in 1000 mL of

1 % HCl in methanol, which was used as stock solution. Exactly 0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL of stock solution was taken into test tubes and the volume of each test tube was adjusted to 1 mL with 1 % HCl in methanol. A 5 mL of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of the solutions and the standard solution were measured at 500 nm using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA), and the calibration curve was constructed from the series of standard solution. Concentration of tannin was read as mg of D-catechin per g of sample from calibration curve as follows:

Tannin in µg/g = [(Ab.- Intercept)/(Slope * Density * Wt.)]*10

3.10.3 Determination of oxalate content

The oxalate content was determined using the method originally employed by Ukpabi and Ejidoh (1989). The procedure involves the following 3 steps.

Digestion: At this step, 2 g (db) of flour was suspended in 190 mL of distilled water contained in a 250 mL volumetric flask; 10 mL of 6M HCl was added and the suspension digested at 100 °C for 1 hour, followed by cooling, and then made up to 250 mL with distilled water before filtration.

Oxalate precipitation: A125 mL of the filtrate were measured into a beaker and four drops of methyl red indicator added [an acid-base indicator that turns Orange in an acidic solution and Yellow if basic.[(If the indicator indicates Yellow, insufficient HCl was added initially. Add one or two millilitres of additional 6M HCl)], followed by the addition of concentrated NH_4OH (used for persisting of Yellow colour) solution (drop wise) until the

test solution changed from its salmon pink colour to a faint yellow colour (pH 4-4.5), then heated to 90 °C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90 °C and 10 mL of 5 % $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5 °C. The solution was then centrifuged at a speed of 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 mL of 20 % (v/v) H_2SO_4, solution.

Permanganate titration: At this point, the total filtrate resulting from digestion of 2 g of flour was made up to 300 mL, Aliquots of 125 mL of the filtrate were heated until near-boiling, and then titrated against 0.5M standardized $KMnO_4$, solution to a faint pink colour which persisted for 30 seconds. The formula used to calculate calcium oxalate content is below:

$$\text{Oxalate (mg/100g)} = \frac{T \times (Vme)(DF) \times 105}{(ME) \times mf}$$

Where: **T** - the titre of $KMnO_4$ (mL), **Vme** - volume-mass equivalent (i.e. that 1 cm^3 of 0.05 M KMnO, solution is equivalent to 0.00225 g anhydrous oxalic acid), **DF** - dilution factor VTA [2.4, where: VT - total volume of filtrate (300 mL) & A - the aliquot used (125 mL)], **ME** - molar equivalent of $KMnO_4$ in oxalate [$KMnO_4$, redox reaction. (5)] & **mf** - mass of flour used.

3.10.4 Determination of bioavailability of minerals

Bioavailability of minerals was estimated on the basis of molar ratios. The molar ratios were calculated by dividing the mole of anti-nutritional factors to mole of minerals using the techniques of Morris and Ellis (1989). (atomic weights for: phytate: 660; oxalate: 88; Fe: 56; Zn: 65; Ca: 40 g/mol).

3.11 VITAMINS ANALYSIS

3.11.1 Vitamin C

Determination of vitamin C was measured spectrophotometrically at wavelength of 515 nm, according to vitamin assay procedure of Pearson (1981). Ascorbic acid solution was used as the standard values of vitamin C (Table 3.2).

Table 3.2. Calibration standards for vitamin C analysis.

Vitamin	Concentration (µg)
*V C	0, 10, 20, 30, 40, 50

* - Vitamin C

The VC content was calculated using the formula:

Ascorbic acid (AA) in mg/ 100g = $[(A_s - A_b)*10] / [A_{10\mu g\ Std} - A_b]$

Where: A_s - Absorbance of samples
A_b - Absorbance of blank
$A_{10\ \mu g\ Std.}$ - Absorbance of 10 µg A A standard

3.11.2 Pro-vitamin A (ß-carotene)

The ß-carotene content of cassava roots flour was analyzed following the procedure developed by Kimura & Rodriguez-Amaya (2003). About 20 g of a homogenous representative sample was weighed, blended and extracted with acetone in a mortar with a pestle. The extract was then filtered in to 100 mL conical flask. The procedure was repeated with the residue and the filtrates were combined. This process was repeated until the sample was devoid of any colour. Twenty-five mL of petroleum ether was placed in a separator funnel and to this acetone extract of the sample was added and the mixture was swirled, followed by the addition of small amount of distilled water, in order to separate the acetone and the petroleum ether phase. The

coloured material was in the petroleum ether phase. The acetone phase was collected in a conical flask. Then petroleum ether phase was washed with water to remove the residual acetone. The petroleum ether phase was collected in the volumetric flask. This process was repeated by returning the acetone phase in separator funnel until no colour was transferred in to the petroleum phase. The collected petroleum ether phase was evaporated to dryness on a rotary evaporator at 40°C. One mL of petroleum ether was added which was then introduced in to the open column chromatography, that was eluted with petroleum ether. The ß-carotene which went through the column as a yellow pigment was collected in a measuring cylinder and the volume was recorded until no colour is eluted through the chromatogram, then the absorbance was read at 440 nm using (Model: CE1021, England) spectrophotometer (Rodríguez-Amaya, et al., 1988). The β-carotene content was calculated using the formula:

$$\beta \text{ - Carotene content in } \mu g/g = \{ A * V (mL) * 10^{4} \} / \{ A^{1\%}_{1cm} \} * W$$

Where: A - absorbance, W - weight of sample in g.

V - the volume of β-carotene which goes through a column as a yellow pigment in mL.

$A^{1\%}_{1cm}$ - absorption coefficient of the carotenoids. In petroleum ether, its value is 2592.

3.12 Determination of physical and functional properties of flours

Physical and functional properties such as Bulk density (BD), water binding capacity (WBC), solubility (S) and swelling power (Sp) of cassava roots and composite flour were determined according to the respective procedures described below:

3.12.1 Bulk density

Bulk density was determined using the method of Oladele and Aina (2007). A sample of 7 g of cassava root flour was measured into a 50 mL graduated measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained to get tapped bulk density. The bulk density was calculated as weight of the cassava flour (g) divided by its volume in mL.

$$\text{Bulk density (g/mL)} = \frac{\text{Weight of cassava flour (g)}}{\text{Volume of cassava flour (mL)}}$$

3.12.2 Water binding capacity

Water binding capacity of flour sample was determined by using method of Buchat (1977). One gram of the cassava flour was mixed with 10 mL of distilled water (density 1 g/mL) in a centrifuge tube, mixed thoroughly and allowed to stand at room temperature for one hour. It was then centrifuged at 3500 rpm for 30 minutes and the supernatant was transferred in a 10 mL graduated cylinder. Water binding capacity was calculated as mL of water absorbed per gram of the flour.

Water Binding Capacity (WBC) was calculated from the equation:

$$\text{Water binding capacity in mL/g} = 10 - V$$

Where: V- volume of water left unabsorbed after centrifugation.

3.12.3 Swelling power and solubility

Swelling power and solubility determinations were carried out in the temperature of 80°C using the method described by Unnikrishnan and Bhattacharya (1981). One gram of cassava flour sample was taken in to a clear dried test tube and weighed (W_1). Fifty mL of distilled water was added and mixed gently at low speed for 5 minutes. The slurry was heated in a

thermostatic water bath (Wagtech, UK), at 80°C for 30 minutes with mixing the suspension intermittently. The test tube was cooled with its content to room temperature and the paste was centrifuged at 2200 rpm for 15 minutes. Five mL of aliquot of the supernatant was dried to constant weight at 120°C. The residue obtained after drying represented the amount of starch solubilised in water. Solubility was calculated as percentage of flour on dry basis. The dried residue obtained after centrifugation was transferred in pre-weighed clean dried test tube (W_1) and re-weighed (W_2) to give swollen mass.

$$\text{Swelling of flour (\%)} = \frac{(W2 - W1) \times 100}{\text{Weight of flour}}$$

3.13 Food product from traditionally fermented composite flour

3.13.1 Preparation of composite flour ingredients

The quality protein maize (*Zea mays* L.), tef (*Eragrostis tef*) and dry common bean (*Phaseolus vulgaris*) were cleaned manually to remove immature, damaged seed, stones and other foreign materials (Figure 3.2). The dried maize, tef and legume were milled using a laboratory Cyclotec sample mill (Tector-AB, Sweden) to fine powder and sieved through by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), following the procedure described by Oti and Akobundu (2007). Cassava roots flour was similarly sieved and prepared in the same way to get uniform size fine powder. The ground samples were transferred into polyethylene bags and kept in dark and dry places before analyses.

3.13.2 Formulation of composite flour

The flours were thoroughly mixed to obtain various proportions of homogeneous composite flour. The four types of flour (quality protein maize, common bean, cassava roots and white tef) were obtained to formulate composite flour blends. The composite flours samples were formulated as 10 % common bean/quality protein maize flour and 10, 20, 30, 40, 45 and 50 % of cassava flour substituted to tef flour while, 100 % whole tef flour considered as control (see Table 3.3). In this study 12 (for maize & bean) combinations were studied and the results of these combinations were reported with an emphasis on cassava to tef co-fermentation process.

Table 3.3. Formulation proportions of bean/maize- tef- cassava and whole tef, for composite flours.

Composite flour	Composite code	% blend proportion
Bean/Maize: Tef: Cassava1	B/MTC1	10:80:10
Bean/Maize: Tef: Cassava2	B/MTC2	10:70:20
Bean/Maize: Tef: Cassava3	B/MTC3	10:60:30
Bean/Maize: Tef: Cassava4	B/MTC4	10:50:40
Bean/Maize: Tef: Cassava5	B/MTC5	10:45:45
Bean/Maize: Tef: Cassava6	B/MTC6	10:40:50
Bean/Maize: Tef: Cassava7	B/MTC7	0:100:0

B/MTC - Bean/Maize: Tef: Cassava

3.13.3 Injera production

The baking of Injera was following the procedure described by ENI (1980) and adapted from Mogessie (2006). The mixing of composite flours to water 1:1.6 (w/v) was manually done to make the dough followed by the traditional fermentation process for all of the composite flours. The traditional starter (Ersho), leftover of the previous fermentation (30 mL), was added in to the

mixture of flours and water, and the dough allowed to ferment for 48 hours at room temperature. At the end of primary fermentation process, the liquid layer over the dough was poured-off and about 10 % of the fermented dough was taken for Absit mixed in the ratio of 1:6 (v/v) and boiled for 5 minutes, cooled to 45 ^{o}C and mixed with the rest of the fermented mass, followed by 3-4 hours of secondary fermentation. Then Injera was prepared on Metad (hot clay made oven) (see below Figure 3.5). The acidity (pH) was measured after 48 hours of fermentation.

3.14 Sensory evaluation of Injera made from composite flours

Injera samples prepared from cassava composite flours were evaluated for organoleptic properties according to procedures described by Larmond (1977) and Giami, et al. (2006). The sensory evaluations were done with 15 trained panellists from Ethiopian Health and Nutrition Research Institute (EHNRI) and graduate students from CFSN. The panellists were regular consumers of Injera and were not allergic to any food. The samples were evaluated on a desk placed in the air-conditioned laboratory of EHNRI, which provided a quiet and comfortable environment. The samples were arranged randomly in similar plates, each coded with 3-digit non-misleading or biasing numbers. Then the Injera were served on a white disposable plastic tray and tap water was provided for rinsing their mouths to clear the palate after each evaluation. Sensory evaluation was done on the same day that the Injeras were prepared. A 7-point Hedonic scale was used by panelists to score the Injera on the basis of like to dislike for the flavour, colour, texture, taste, back and front side appearance and overall acceptability (Appendix-II). On the scale score representation: 7= like

extremely, 6 = like moderately, 5 = like slightly, 4 = neither like nor dislike, 3 = dislike slightly, 2 = dislike moderately, 1 = dislike extremely. Mean of 15 evaluations were reported. Data generated from scores were analyzed for variance as described by Weaver & Daniel (2003).

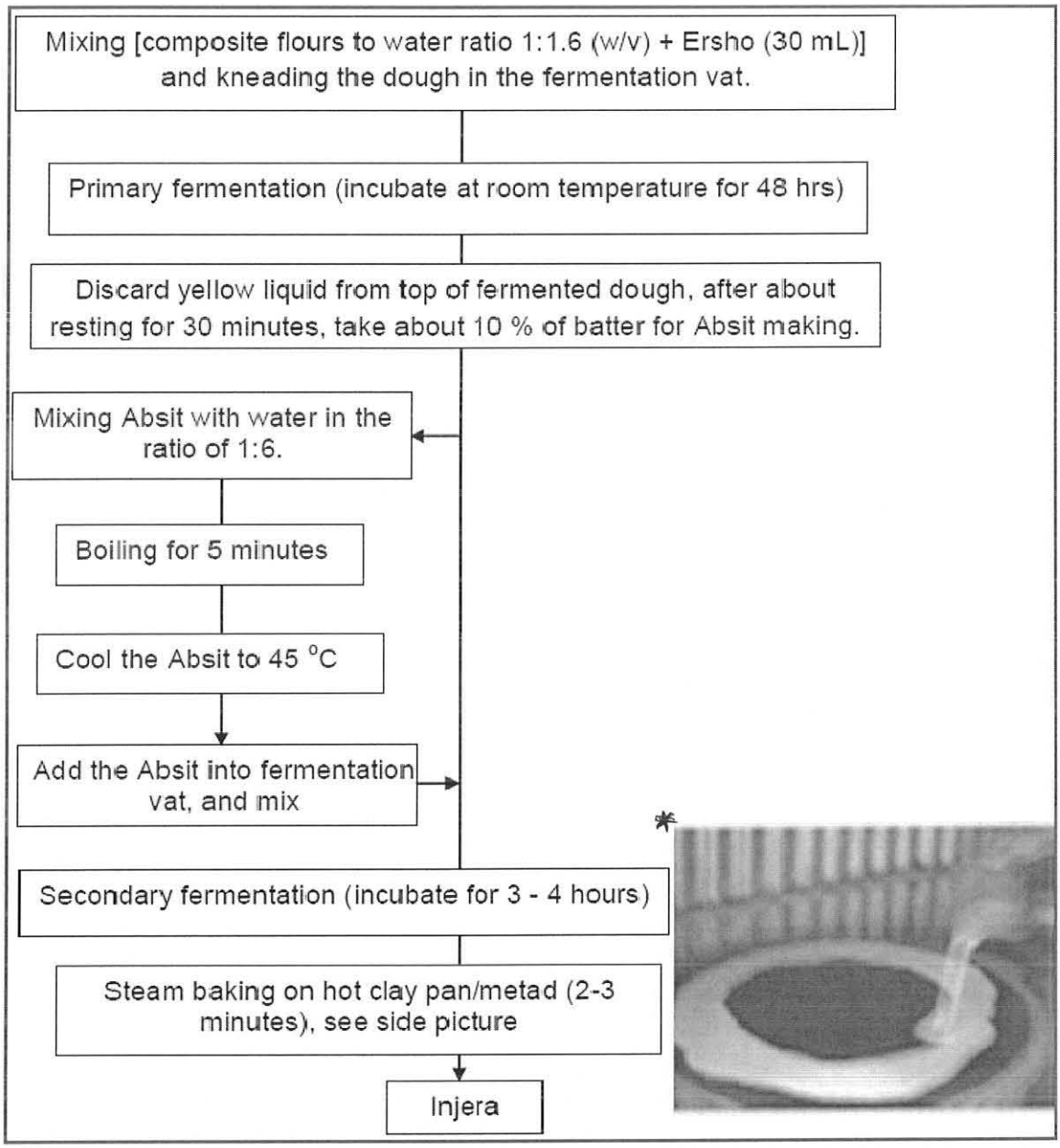


Figure 3.5. Flow diagram for traditional production of Injera by fermentation from composite flours. *represents a photograph for the traditional clay oven (Metad) used for baking.

3.15 Biological assays for composite flours Injera

The nutritional performance of Injera prepared from composite flours: 1:8:1, 1:7:2, 1:6:3 & 1:5:4 was analysed by biological assay techniques using mice. Injera prepared from these composite flours was found to be acceptable with scores more than average overall acceptability.

3.15.1 Formulation of diets to be tested on mice

The experimental diets were prepared according to table 3.4. The cassava-based composite flours diets were formulated to provide at least 10 % level of protein, while other ingredients such as vegetable oil, vitamins, minerals and cornstarch were added to balance the diets according to procedures described by Okpala and Okoli (2011). The diets were thoroughly mixed, labelled with designated names and stored in polyethylene bags. The polyethylene bags were kept in dry place until ready for use.

3.15.2 Ethical approval

All mice were treated in accordance with the Guide for the Care and Use of Laboratory Animals (1993). Ethical approval protocol was approved by the ethics committee of the Centre for FSN, AA University.

3.15.3 Biological tests for formulated diets

The eight composite flours were tested their effect on body growth of mice and protein utilization. This was denoted for composite flour blends from Quality Protein Maize: Tef: Cassava flour (MTC_1, MTC_2, MTC_3 and MTC_4 for 1:8:1, 1:7:2, 1:6:3 and 1:5:4 respectively), and Common Bean: Tef: Cassava composite flour (BTC_1, BTC_2, BTC_3 and BTC_4 for 1:8:1, 1:7:2, 1:6:3 and 1:5:4 respectively). Each formula was tested with replication, using weanling mice of Swiss albino mice strain, which were obtained from EHNRI.

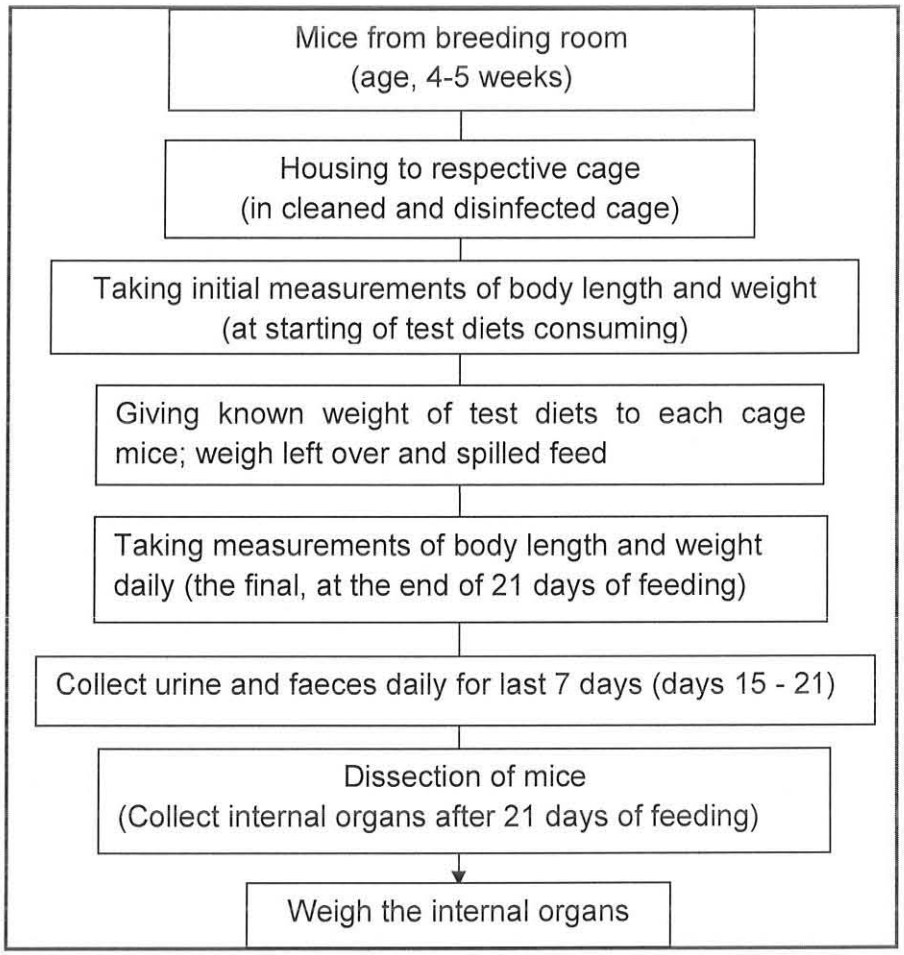


Figure 3.6. Flow chart for *in vivo* evaluation protocol for diets and composite flours using mice.

The mice used were 4-5 weeks old at the onset of the evaluation exercise. The experiment was a completely randomized design (CRD). The 30 mice of the same sex were allocated to 10 groups of 3 mice per group. The mouse was housed in individual stainless steel screen-bottom of metabolic cage (Model, 3700M022-MC, USA) with removable food cup in a room maintained at 18 to 22 °C with an alternate of 12 hours of light and dark, and relative humidity about 40 - 75 % (see below Figure 3.7). Food and water were provided *ad libiturn* to the mice for the 21-days of experimental period. Each mouse was acclimatized and given pellet (a commercial

laboratory mice chow diet) feed for 4 days before giving the test diets. On the basis of initial body weight such as the mean group weights were identical (22.38 - 22.77 g), and from the same stock of Swiss albino mice having equal age. Test diets, spilled and leftover feed were collected and weighed daily. Feeding trails lasted for 21 days, while at the last seven days (days 15 - 21) urine and faeces were collected daily from each cage separately according to the procedure of Al-Numair and Ahmed (2008). The faecal matter was dried at 65 °C in an oven (DHG-905A, Germany), milled and kept in a tight screw capped plastic bottles and frozen in deep freezer until required for analysis. The urine from each cubicle was collected into screw-capped plastic bottles and stored in deep freezer until analysis. About 1mL of concentrated sulphuric acid was added to each urine container as a preservative against fungal and other microbial growth (see below Figure 3.8). The concentration of nitrogen in urine and faeces were determined by the Kjeldlahl method (AOAC, 2000). The test diets were compared with standard proteins (casein) which was used as control diets due to its easiness for absorption and efficient utilization, while starch was used as protein-free (basal) diet reference for measuring endogenous faecal and urinary nitrogen (Friedman, 1996). Before the beginning of the test the mice that showed symptoms of ill health were excluded from the experiment. Each mouse per group was weighed using an electrical balance (SL3100S, Scientech, made in USA) and body length (transparent plastic ruler) were measured daily and the mean body weight and length per week for each group were calculated.

Table 3.4. Formulation of experimental diets on dry weight basis.

Ingredients	Casein	N-free	MTC1	MTC2	MTC3	MTC4	BTC1	BTC2	BTC3	BTC4
Corn starch	847.05	141.05	869.53	867.45	859.33	851.81	873.52	872.98	865.08	863.02
Casein	100	-	-	-	-	-	-	-	-	-
N_2-free	-	806	-	-	-	-	-	-	-	-
MTC1	-	-	77.52		-	-	-	-	-	-
MTC2	-	-	-	79.60	-	-	-	-	-	-
MTC3	-	-	-	-	87.72		-	-	-	-
MTC4	-	-	-	-		95.24	-	-	-	-
BTC1	-	-	-	-	-	-	73.53		-	-
BTC2	-	-	-	-	-	-	-	74.07	-	-
BTC3	-	-	-	-	-	-	-	-	81.97	-
BTC4	-	-	-	-	-	-	-	-	-	84.03
Vit. Mix [a]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Minls Mix [b]	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Oil (veg.)	50	50	50	50	50	50	50	50	50	50
Total diet wt (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Maize-Tef-Cassava composite flour (MTC_1, MTC_2, MTC_3 and MTC_4 for 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively),
Bean-Tef-Cassava composite flour (BTC_1, BTC_2, BTC_3, and BTC_4 for 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively),
[a] Vitamin mixture (ingredient/g): A (IU) 500; B1, B2, B6 and B12 (µg) 5 each; C (mg) 10; D3 (IU) 50; E (mg) 2.5; K3 (mg)1; Inositol (mg) 25; Pantothenic acid (mg) 10; Cholinchloride (mg) 100; Niacin (mg) 25; Folic acid (mg) 1; Biotin (µg) 250.
[b] Minls Mix- minerals mixture (mg/g): $CaCo_3$ 336; KH_2PO_4 502; $MgSO_4.7H_2O$ 162.

At the end of the experiments, the mice were anesthetized using diethyl ether and sacrificed, and then internal organs were collected by cervical decapitation. Hearts, spleen, liver, kidneys, lungs and pancreas of each mouse were removed and washed with normal saline and their weights determined gravimetrically using an electrical balance. The mean weight of each organ and its relative weight (in relation to mean live weight of each group) were calculated as percentages using the procedure of Adejumo (2004). These organs were visually inspected for possible abnormalities such as colour changes, lesions and fatty liver.

3.15.4 Nutritional evaluation of formulated diets

Based on nitrogen balanced studies, Net Protein Utilization (NPU), True Digestibility (TD), Biological Value (BV), Net Protein Ratio (NPR), Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) were computed using standard equations of Pirman, et al. (2007).

3.15.4.1 Determination of body growth, protein and feed efficiency ratios

The PER and FER values were calculated using the following formula.

$$\text{PER} = \frac{\text{Gain in body mass (g)}}{\text{Protein intake (g)}}$$

$$\text{FER} = \frac{\text{Gain in body mass (g)}}{\text{Feed intake (g)}}$$

3.15.4.2 Determination of metabolic and endogenous nitrogen

The protein quality measurements of individual mouse, was measured accordingly:

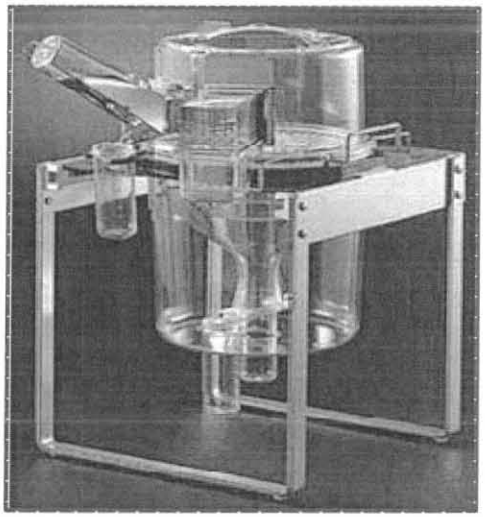


Figure 3.7. Metabolic cage used for *in vivo* experiment.

Following the determination of nitrogen in the feed, faeces and urine for the Nitrogen retention (NR): The nitrogen retained in the experimental mice trial calculated as the algebraic difference between the feed and the sum of both the faecal and urinary nitrogen for the collection period.

$$NR = I - (F + U)$$

Apparent nitrogen digestibility % (AND): The AND was determined by dividing the NR by I on a percentage basis.

$$AND\ \% = [I - (F + U)] / I \times 100$$

Net protein ratio (NPR): This was determined by finding the sum of weight gain of the test-protein group and the weight loss of the nitrogen-free diet group and then dividing the value by the protein intake.

$$NPR = \frac{[\text{wt gain of test - protein group + wt loss of the N - free diet group}]}{\text{Protein intake}}$$

$$NPU = \frac{\text{N - retained}}{\text{N-intake}} = \frac{I - (F - F_k) - (U - U_k)}{I}$$

$$TD = \frac{\text{N - retained}}{\text{N-intake}} = \frac{I - (F - F_k)}{I}$$

$$BV = \frac{N\text{ - retained}}{N\text{-intake}} = \frac{I - (F - F_k) - (U - U_k)}{I - (F - F_k)}$$

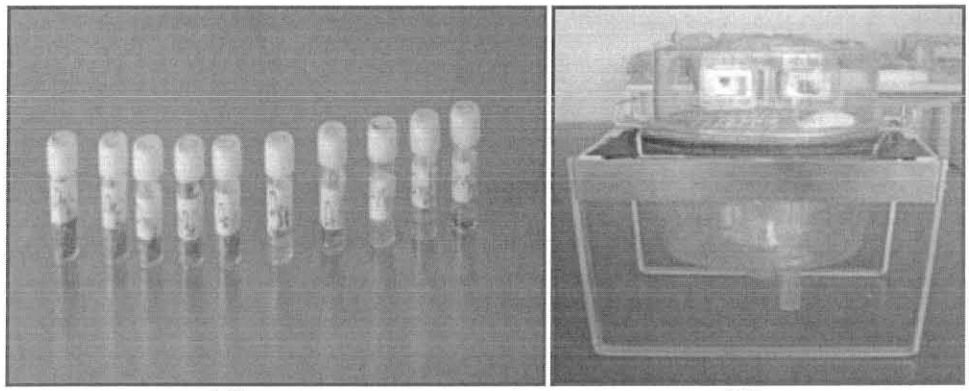
Where:

NR- nitrogen retention, I - the in taken nitrogen (nitrogen in the diet),

F- Faecal nitrogen, F_k - endogenous faecal nitrogen, U - urinary nitrogen,

U_k - endogenous urinary nitrogen, NPU - net protein utilization,

TD - true digestibility and BV- biological value



(A) (B)

Figure 3.8. Photo for the metabolic cage for biological diet evaluation using mice: (A) urine and faeces sample, (B) mouse in metabolic cage.

3.16 Statistical analysis

All the results obtained were statistically analyzed using statistical package for social scientists (SPSS) and Micro soft office excels 2007. The experimental design was arranged as a random block design with a 7*4 factorial arrangement of the following treatments:

Cultivars: cultivars of cassava roots (28, 192, 5538-19, 44/72-NW, Gamo, Koree and Hayik)

Processing methods: form of processing (fresh, boiled, sun-dried and fermented).

Data were analyzed by the General Linear Model option in the ANOVA program of the SPSS (vis.16). Sources of variation in the model were: cultivars, processing methods, interaction cultivars*processing methods and error. Duncan's multiple range (DMR) test was used to test for significant difference among the means ($P<0.05$). Similarly, all the animals experiments obtained data were subjected to the principles of variance. The significance of the difference between the treatments means were determined by ANOVA.

4. RESULTS AND DISCUSIONS

4.1 PROXIMATE COMPOSITION ANALYSIS OF CASSAVA CULTIVARS

Locally grown seven cultivars of cassava roots were subjected into three common processing techniques namely boiling, sun drying and fermentation methods. Moisture, total ash, crude fat, crude protein, crude fibre, total carbohydrate and total energy were analyzed and the results are presented in Table 4.1. The analysis reveals that the effect of cultivars, processing methods, and interaction of cultivars and processing methods on each of the dependent variable found to be significant ($P<0.05$) (see Appendix-V).

Moisture content

Moisture content of food is of great importance to every food processor as a number of biochemical reactions and physiological changes in food depend very much on the moisture content (Onwuka, 2005). The results for moisture content of the boiled, sun-dried and fermented cassava roots flours were found to be in the marginal means values of 10.43, 8.17 and 8.35 %, on dry weight basis, respectively. The, cell mean fermented cultivar 5538-19 and boiled cultivar 192 had the lowest (6.02 %) and highest (12.26 %) moisture content values, respectively.

The moisture content for the unprocessed cassava roots was in the average value of 60.89 % on fresh weight basis with Hayik cultivar having the lowest (59.80 ± 0.22 %) and cultivar 5538-19 having the highest (64.61 ± 0.69 %) cell mean value.

Among the processed seven cultivars, the fermented cassava flour for cultivar 5538-19 had the lowest (6.02 %) moisture content is an indication of a longer

stability. Off-course, in order to say the food is more stable water activity of the flour has to be measured. Thus, low moisture confers higher stability to the flour where microbial proliferation is minimum (Onitino, et al., 2007; Nwabanne, 2009). The result of this study is within the range of the recommended codex standard quality factor of edible cassava flour maximum value of (13 %) (CODEX STAN, 176-1989; Abass, et al., 1998). The seven cassava cultivars considered in this study have moisture content values (<13 %) similar to those for cereal flours (Sriroth, et al., 2000) desirable for stability of food product.

Ash

The ash content of a food denotes the presence of microelements in the food. Data in Table 4.1 shows the ash content of the seven processed cassava roots cultivars flour to be in the average values of 2.02, 0.32 and 1.35 % for the boiled, sun-dried and fermented flours, respectively. The marginal means of processing over levels of cultivars are significantly ($P<0.05$) different with the means for boiled (2.02) being the highest followed fermented and sun-dried flours. The interaction of cultivars and processing methods was significant ($P<0.05$). Similarly, the effect of cultivars across the processing methods were significantly ($P<0.05$) different with the marginal means for cultivar 44/72-NW (1.87) being the highest. The highest cell means ash content was found in boiled cultivar Gamo (red skin) (2.28 ± 0.15 %) and the lowest value in sun-dried cultivar 28 (0.26 ± 0.02 %). There was a significant ($P<0.05$) variation in the ash content among flours processed differently except for the sun-dried cultivars 44/72-NW and Koree, which had similar values. In this study, the average ash content (0.32 %) for sun-dried flour is

closer to that of the Nigerian cassava roots flour, reported to range from 0.33 to 0.77 % (Abass, et al., 1998; Eke, et al., 2007). Whereas, the percentage of ash for unprocessed cassava roots was found to be in the average value of 12.69. The highest and lowest cell means values belong to the cultivars of Hayik (3.72 ± 0.30) and Koree (1.48 ± 1.01), respectively. The obtained values are higher than those of peeled bitter cassava which had value (2.41 %) (Okigbo, 1980), and those of root and tubers value (0.84 %) reported by Bradbury and Holloway (1988). The marginal means of ash contents of sundried (0.32 %) and fermented (1.55 %) flours were found in line with the amount of regulatory standard values (<1.5 %) specified by standard organization of Nigeria (Sanni, et al., 2004) and Ethiopian food composition table (EHNRI, 1997). The average ash content was significantly ($P<0.05$) reduced by sun-dried flours, probably this is due to the loss of water-soluble minerals, which was an effect of slow drying process. The average ash content of the three common processing methods have significantly lower ($P<0.05$) than that of the unprocessed cassava roots flour, when compared. The decrease in the ash content after processing is due to leaching of some water soluble minerals and some elements consumed by microorganisms in fermentation. Similarly, the reduction of ash contents in processing of cassava roots were reported in several previous observations (Bradbury and Holloway, 1988; Oboh and Elusiyan, 2007 and Kebede, et al., 2012).

Table 4.1. Proximate compositions of raw and processed cassava roots from the seven cultivars (mg/100 g).

Pars[1]	Tres[2]	Cultivars*							Marginal			P of cultivar	P of processing methods	P of cultivar x Processing methods
		28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% cv			
Mc	Raw	61.20 ± 1.31	60.38 ± 1.24	64.61 ± 0.69	61.48 ± 1.98	59.84 ± 0.09	60.01 ± 0.20	59.80 ± 0.22	**60.89**	1.73	3.0	*	*	*
	Boiled	11.85 ± 0.18	12.26 ± 0.42	10.04 ± 0.09	8.81 ± 0.41	10.82 ± 0.18	10.07 ± 0.21	9.19 ± 0.43	**10.43**	1.25	1.2			
	Sun-dried	8.92 ± 1.00	9.28 ± 0.07	6.17 ± 0.25	9.29 ± 0.09	7.07 ± 0.12	8.07 ± 0.08	8.34 ± 0.39	**8.17**	1.13	1.4			
	Fermented	9.15 ± 0.08	10.02 ± 0.08	6.02 ± 0.12	9.29 ± 0.09	7.04 ± 0.07	9.93 ± 0.07	7.03 ± 0.08	**8.35**	1.54	1.8			
Marginal means		**22.90**	**22.86**	**21.58**	**22.09**	**21.22**	**21.97**	**21.10**						
Ash	Raw	2.48 ± 0.06	1.95 ± 0.05	3.36 ± 0.05	3.41 ± 0.19	2.5 ± 0.02	1.48 ± 0.20	3.72 ± 0.30	**2.68**	0.80	28	*	*	*
	Boiled	2.15 ± 0.10	1.84 ± 0.34	1.97 ± 0.17	1.92 ± 0.05	2.28 ± 0.15	1.96 ± 0.06	1.99 ± 0.01	**2.02**	0.18	9.0			
	Sun-dried	0.26 ± 0.02	0.34 ± 0.02	0.36 ± 0.02	0.34 ± 0.08	0.37 ± 0.03	0.29 ± 0.02	0.29 ± 0.01	**0.32**	0.05	16			
	Fermented	1.98 ± 0.09	1.45 ± 0.05	1.44 ± 0.07	1.79 ± 0.07	1.47 ± 0.01	1.36 ± 0.06	1.37 ± 0.09	**1.55**	0.23	15			
Marginal means		**1.71**	**1.38**	**1.78**	**1.87**	**1.66**	**1.27**	**1.84**						

[1]Results were mean values of triplicate determination (dwb) ± SD, * accession number of cultivars with treatments,

Mc-moisture content (%), cv- coefficient of variation, *- interactions significant at $P < 0.05$, Pars- parameters, tres - treatments.

Table 4.1. (Continued).

Pars[1]	Tres[2]	Cultivars*							Marginal			P of cultivar	P of processing methods	P of cultivar X processing methods
		28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% cv			
C. fibre	Raw	5.90 ± 0.07	5.13 ± 0.10	3.85 ± 0.02	3.60 ± 0.01	3.03 ± 0.06	2.82 ± 0.13	4.06 ± 0.07	**3.59**	0.72	20	*	*	*
	Boiled	2.83 ± 0.06	2.88 ± 0.03	2.53 ± 0.09	4.60 ± 0.06	3.16 ± 0.06	2.79 ± 0.02	2.93 ± 0.05	**3.10**	0.66	21			
	Sun-dried	5.09 ± 0.08	5.15 ± 0.01	3.76 ± 0.05	3.84 ± 0.13	4.10 ± 0.09	2.58 ± 0.03	2.44 ± 0.09	**3.92**	0.94	24			
	Fermented	3.43 ± 0.06	2.51 ± 0.01	2.93 ± 0.06	2.40 ± 0.04	4.02 ± 0.09	3.71 ± 0.10	2.69 ± 0.05	**3.09**	0.60	19			
Marginal means		**4.06**	**3.34**	**3.27**	**3.61**	**3.53**	**3.11**	**3.03**						
C. fat	Raw	0.23 ± 0.03	0.51 ± 0.01	0.24 ± 0.05	0.35 ± 0.04	0.40 ± 0.02	0.31 ± 0.02	0.22 ± 0.04	**0.32**	0.10	32	*	*	*
	Boiled	0.12 ± 0.02	0.48 ± 0.01	0.17 ± 0.00	0.25 ± 0.01	0.27 ± 0.00	0.25 ± 0.00	0.08 ± 0.01	**0.23**	0.12	52			
	Sun-dried	0.11 ± 0.01	0.47 ± 0.00	0.18 ± 0.01	0.15 ± 0.00	0.28 ± 0.00	0.25 ± 0.00	0.09 ± 0.01	**0.22**	0.12	57			
	Fermented	0.21 ± 0.00	0.15 ± 0.00	0.34 ± 0.00	0.32 ± 0.00	0.31 ± 0.01	1.01 ± 0.02	0.08 ± 0.02	**0.35**	0.29	85			
Marginal means		**0.17**	**0.40**	**0.23**	**0.27**	**0.32**	**0.45**	**0.12**						
C. protein	Raw	0.96 ± 0.05	0.95 ± 0.00	0.72 ± 0.03	0.80 ± 0.01	0.91 ± 0.02	0.79 ± 0.02	0.30 ± 0.04	**0.78**	0.22	28	*	*	*
	Boiled	0.28 ± 0.03	0.92 ± 0.00	0.13 ± 0.01	0.13 ± 0.01	0.79 ± 0.03	0.55 ± 0.01	0.11 ± 0.01	**0.42**	0.32	77			
	Sun-dried	0.10 ± 0.00	0.30 ± 0.00	0.41 ± 0.00	0.14 ± 0.01	0.38 ± 0.03	0.22 ± 0.02	0.11 ± 0.01	**0.24**	0.12	51			
	Fermented	0.69 ± 0.01	0.15 ± 0.02	0.45 ± 0.00	0.30 ± 0.00	0.41 ± 0.03	0.19 ± 0.02	0.11 ± 0.02	**0.32**	0.19	59			
Marginal means		**0.51**	**0.58**	**0.42**	**0.34**	**0.62**	**0.46**	**0.16**						

[1]Results were mean values of triplicate determination (dwb) ± SD, * accession number of cultivars with treatments, c.-crude, cv- coefficient of variation *- Interactions significant at P<0.05, Pars- parameters, tres - treatments.

Table 4.1. (Continued).

Pars[1]	Tres[2]	Cultivars*							Marginal			P of cultivar	P of processing methods	P of cultivar x processing methods
		28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% Cv			
CHO	Raw	85.49 ± 0.43	96 ± 0.00	95 ± 0.00	95 ± 0.00	96 ± 0.00	97 ± 0.00	95 ± 0.00	**95.71**	0.72	0.7	*	*	*
	Boiled	85.60 ± 0.06	84.50 ± 0.07	87.69 ± 0.08	88.89 ± 0.09	85.84 ± 0.07	87.17 ± 0.08	88.62 ± 0.05	**86.88**	1.61	1.8			
	Sun-dried	90.61 ± 0.10	89.61 ± 0.10	92.48 ± 0.06	90.08 ± 0.10	91.90 ± 0.01	91.17 ± 0.19	91.17 ± 0.93	**91.06**	1.09	1.2			
	Fermented	87.97 ± 0.09	88.23 ± 0.10	91.75 ± 0.10	88.30 ± 0.81	90.77 ± 0.81	87.51 ± 0.10	91.41 ± 0.10	**89.38**	1.73	1.9			
Marginal means		**90.02**	**89.58**	**91.83**	**90.65**	**90.99**	**90.69**	**91.55**						
Energy Kcal/100g)	Raw	391.00	395.00	388.00	388.00	392.00	396.00	386.00	**390.83**	3.47	1.0	*	*	*
	Boiled	344.60	346.00	352.81	358.33	348.95	353.13	355.72	**351.31**	5.09	1.0			
	Sun-dried	363.83	363.87	374.78	362.23	371.64	367.81	365.93	**367.16**	4.42	1.0			
	Fermented	356.53	354.87	371.86	357.28	367.51	359.89	366.80	**361.95**	6.21	2.0			
Marginal means		**364.00**	**364.90**	**372.00**	**366.80**	**369.50**	**369.00**	**368.30**						

[1]Results were mean values of triplicate determination (dwb) ± SD, * accession number of cultivars with treatments,

c-crude and CHO-carbohydrate, cv-coefficient of variation, *- Interactions significant at $P < 0.05$, Pars- parameters, tres - treatments.

Crude fibre

The results of crude fibre content determination are presented in Table 4.1. The marginal means for the crude fibre contents for the seven cultivars are 3.10, 3.92 and 3.09 % for the boiled, sun-dried and fermented, respectively. The marginal means of processing over levels of cultivars are significantly ($P<0.05$) different with the means for sun-dried (3.92) being the highest followed boiled and fermented flours. Fermentation reduces crude fibre content of food substances through the microbial degradation of non-dietary substances from the edible portion of food matrices by improving fibre digestibility. The interaction of cultivars and processing methods was significant ($P<0.05$). Similarly, the effect of cultivars across the processing methods were significantly ($P<0.05$) different with the marginal means for cultivar 28 (4.06) being the highest.

The lowest cell means crude fibre content obtained were for fermented 44/72-NW cultivar (2.40 ± 0.04 %), which falls within the range value (1.8 to 2.7 %) reported by Oboh and Elusiyan (2007). Out of the seven cassava cultivars the maximum cell mean fibre value is observed for sun-dried cultivar 192 (5.15 ± 0.1 %). The decrease in fibre content of fermented cassava flour of the seven processed cultivars is attributed to the possibility that the microorganisms could secrete hydrolytic enzymes. These enzymes are capable of hydrolyzing crude fibre into simple sugars. So that the organism use as its carbon source and change it to other macromolecules such as protein and fat (Oboh and Akindahunsi, 2003; Oboh, 2006). The nutritional role of fibre is said to reduce risk of cardiovascular disease, improve glycemic and glucose sensitivity, assist in weight management, improve bowel health and reduce the risk of

certain forms of cancer (Howarth, et al., 2001; Marlett, et al., 2002; Slavin, 2004; Anderson, et al., 2009).

The percentage of crude fibre content in unprocessed cassava roots was found to be in the average value of 3.59 %. The cell means of crude fibre values for the unprocessed seven cultivars were significantly different ($P<0.05$) from one another. Crude fibre cell means levels ranged from minimum value of 2.82 ± 0.13 mg/100 g for Koree cultivar to maximum value of 5.90 ± 0.07 for cultivar 28. These values are higher than the range values (1.10-1.4 %) reported by Bradbury and Holloway (1988); Buitrago (1990) for roots and tubers, but lower than for sweet cassava (10.31 %) and comparable to those of bitter cassava (3.09 %) reported by Okigbo (1980).

Crude fat

The fat content in the flours processed by the three methods (Table 4.1) were found to be in the average values of 0.23, 0.22 and 0.35 % for the boiled, sun-dried and fermented, respectively. The marginal means of processing methods over levels of cultivars are significantly ($P<0.05$) different with the means for fermented (0.35) being the highest followed boiled and sun-dried flours. The interaction effect of cultivars and processing methods was significant ($P<0.05$). Similarly, the effect of cultivars across the processing methods were significantly ($P<0.05$) different with the marginal means for cultivar Koree (0.45) being the highest. The variability of marginal means across processing might be attributed to genotypic, environmental and types of soils. The maximum value of fat cell means content was observed in fermented Koree cultivar (1.01 ± 0.02 %), while the minimum value was found in boiled Hayik cultivar flour (0.08 ± 0.01 %). However, these values were

higher than those of other cassava genotypes were namely nil to 0.01 % and 0.03-0.15 % as reported by Sriroth, et al. (1999) and Abera and Rakshit (2003), respectively. The cell mean of fat content obtained from the fermented Koree cultivar flour (1.01 ± 0.02 %) was significantly ($P<0.05$) higher than the fat content in boiled and sun-dried flours. The decrease in fat during boiling as compared to the fat content of the fresh cassava flour samples is most probably as a result of the hydrophobic nature of lipids which make them insoluble in aqueous solutions. The higher fat content of some varieties after fermentation could be attributed to the possibility that, microorganisms, which could secrete microbial oil, could possibly contribute for the increase in the fat content of fermented samples (Akindumila and Glatz, 1998; Oboh and Akindahunsi, 2003; Oboh and Elusiyan, 2007; Nevry, et al., 2007).

The fat content for unprocessed cassava roots was found to be in the average of 0.32 %, with the highest and lowest cell mean values observed for cultivar 192 (0.51 ± 0.01 %) and cultivar Hayik (0.22 ± 0.04 %), respectively. The seven cassava cultivars considered in this study have greater cell means fat content in their fresh form as compared to the fat content levels of 0.2 % reported by Bradbury and Holloway (1988). However, the results are comparable to previously reported values of 0.47 - 0.53 % in roots and tubers (Okigbo, 1980; Buitrago, 1990; Hoover, 2001).

Crude protein

The percentage of crude protein content for the seven cassava cultivars is given in Table 4.1. The effect of three different processing techniques on crude protein content was found to be in the marginal means of 0.42, 0.24

and 0.32 % for the boiled, sun-dried and fermented cassava root flours, respectively. The effect of processing methods across the levels of cultivars were significantly ($P<0.05$) varied to each other on the protein contents. The variation of protein content in processing might be due to some microorganisms that degrade cassava flour readily and could have secreted some extra cellular enzymes in the flour as it was reported by Reade and Gregory (1975); Oboh and Akindahunsi (2003). The highest and lowest cell means protein value was found for boiled cultivar 28 (0.92 ± 0.00 %) and sun-dried cultivar Hayik (0.10 ± 0.00 %), respectively. The observed reduction of crude protein content might be due to the maillard reaction of proteins and solubilisations of some nitrogenous compounds during cooking.

Whereas, the crude protein content of unprocessed cassava roots determined in the average of 0.78 %. The lowest and highest cell mean values found to be for cultivars of Hayik (0.30 ± 0.04 %) and 28 (0.96 ± 0.05 %), respectively. The crude protein content of the unprocessed cultivars is comparable to those of roots and tubers literature values (0.53 - 1.1 %) reported by Okigbo (1980); Buitrago (1990); Abera and Rakshit (2003). However, the processed crude protein content of all the seven cassava roots flour is reduced in the range values (0.10 - 0.92), when compared to the unprocessed one (0.30 - 0.96), which is contrary to the previous report of fermented protein content (Raimbault, 1998; Okafor, 1998; Oboh and Akindahunsi, 2003). This study result confirms the reduction of the nutrient contents of some roots and tubers products during processing (Bradbury and Holloway, 1988; Okaka, 2005).

Total carbohydrate content

Results in Table 4.1 shows that carbohydrate content of the seven cassava roots cultivar flours was found to be in the average of 95.71, 86.88, 91.06 and 89.38 for the unprocessed, boiled, sun-dried and fermented flours, respectively. The minimum and maximum cell means carbohydrates were found in the boiled cultivar 192 (84.50 %) and sun-dried cultivar 5538-19 (92.48 %), respectively. The total carbohydrate content of sun-dried cassava roots (89.61 - 92.48 %) is higher than oven dried value (80.1 - 86.3 %) reported by Charles, et al. (2005) for 5 genotypes.

The marginal means of processing over levels of cultivars are significantly ($P<0.05$) different with the means for boiled (86.88) being the lowest followed fermented and sun-dried flours. The possible reason for the decrease in the carbohydrate of the fermented cassava flour from other processing methods might be due to secretion of hydrolytic enzymes by microorganisms. These enzymes are capable of hydrolyzing carbohydrate into simple sugars, which was used by organism as its carbon source and changes it to other macromolecules (Oboh and Akindahunsi, 2003; Udensi and Okoronkwo, 2006; Oboh, 2006). The effects of interaction of cultivars and processing methods was significant ($P<0.05$). Similarly, the effect of cultivars across the processing methods were significantly ($P<0.05$) different with the marginal means for cultivar 5538-19 (91.83) being the highest.

Energy

The proximate composition result (see above Table 4.1) shows that the energy of cassava roots flour are (computed from Atwater's conversion factor), in the average value of 351, 367 and 362 in Kcal/100 g for boiled, sun-

dried and fermented flours, respectively. The maximum and minimum cell means energy content of processed cassava roots flour were obtained for sun-dried cultivar 5538-19 (374.78 Kcal/100 g) and boiled cultivar 28 (344.6 Kcal/100 g).

Whereas the energy content of unprocessed cassava roots was measured in the mean value of (391 kcal/100 g). The maximum and minimum cell means energy content belongs to cultivars of Koree (396 Kcal/100 g) and Hayik (386 Kcal/100 g), respectively. The energy content of processed cassava flours of all the seven cultivars are less than the value (580 Kcal/100 g) presented in food composition table of Bradbury and Holloway (1988). However, the highest amount of energy obtained (375 kcal/100 g) from processed cassava flour could be estimated to 15 % of the daily requirement of adult man (375 of 2500; age, 25-50). Thus, each individual's food energy intake must equal the energy expended, in order for the person to maintain his or her body weight. Like other root crops the content of energy demonstrated in the study confirms that cassava roots flour provide high amount of energy.

4.2 Effect of common processing methods on anti-nutritional factors

4.2.1 Total cyanide content

The total cyanide levels for the processed flours on the seven cassava cultivars roots are shown in Table 4.2. The Interaction of cultivars and processing methods, cultivars and processing effects are found to be significant ($P<0.05$) (see Appendix-V). The cyanide values for the marginal means were 136.62, 39.91, 17.44 and 4.93 ppm, for the raw, boiled, sun-dried and fermented flours, respectively. The highest and lowest level of the cell

means total cyanide obtained for boiled 5538-19 cultivar (90.33 ± 0.25 ppm) and fermented Gamo cultivar (1.09 ± 0.07 ppm), respectively. The total cyanide levels of commonly processed cassava roots flour was significantly varied ($P<0.05$) to each other. The pattern of total cyanide reduction was observed in the fermentation, followed by sun-dried and boiled. Of the three processing methods, fermentation significantly reduced the cyanide content ($P<0.05$) having the value below the recommended safe level value (10ppm) HCN, dwb (FAO/WHO 1991). This limit has been questioned because it was established with HCN as gas through inhalation instead of ingestion. Ramalho, et al. (2007) reported an experiment in which it is used linamarin extracted from cassava and given orally to mice. In such experiment the lethal dose (LD_{50}) was 324.86 ppm linamarin for body weight, a value three times higher than that recommend by WHO. The present study result of all fermented cassava roots flour is not associated with acute toxicity. Furthermore, it was observed to be below the lethal dose for humans' intake by mouth (0.5-3.5 mg/kg body weight for a 60 kg adult) which amount to 30-210 mg HCN (Montgomery, 1980; Solomonson, 1981). While in other processing methods (see Table 4.2) the total cyanide cell means levels are above 10 ppm except for the two cultivars 192 (sun drying=8.27 ppm) and Gamo (sun-drying=4.60 ppm; boiling=8.23 ppm) which are less than recommended value (10ppm) of WHO. This might be due to the soil condition and genotypic difference. The effect of common processing techniques such as boiling is reported to reduce the cyanide levels (Mahungu, et al., 1987; Rodríguez-Sandoval, et al., 2008; Kebede, et al., 2012). Boiling in addition to drying, steaming and frying is a common method of processing cassava roots

for consumption which significantly reduce ($P<0.05$) the cyanide content. The total cyanide content reduction of boiled and sun-dried cassava flour are not in agreement with the values (boiled=50-70 and sundried=30-70 %) reported by Jense, et al. (1974) a loss of HCN. However, the cyanide level of the seven cultivars of sun-dried results (4.60 to 26.84 ppm) are in between for the 5 genotypes oven dried results observed value (8.33 - 28.8 ppm) reported by Obilie, et al. (2004); Charles, et al. (2005). The percentage reduction of total cyanide level in all the seven cultivars after three days of fermentation and sun-dried are in agreement with the previous values (70 - 99 %) reported by Hahn (1983); Kobawila, et al. (2005); Nwokoro, et al. (2005).

The result in Figure 4.1 shows that, the total cyanide content of unprocessed cassava roots of the seven cultivars was ranged from 48.00 to 247.20 ppm. The concentration of raw cassava roots total cyanide level in the present study is greater than the values (27.6 - 28.5 ppm) reported by Enidiok, et al. (2008); Irtwange and Achimba (2009). The unprocessed cassava roots of Gamo cultivar (48.00 ± 1.10 ppm) which belong to category of sweet has its cyanide content value (<50 ppm) is non-toxic. According to the cassava toxicity category (Bourdoux, 1982; White, et al., 1994) the cassava roots having cyanide content of 50 to 200 ppm belongs to the bitter (moderately toxic) categories such as cultivars of Hayik (78.07 ± 0.16 ppm), 28 (83.70 ± 0.40 ppm), 44/72-NW (129.20 ± 0.18 ppm) and Koree (159.00 ± 1.07 ppm). Cultivars of 192 (211.17 ± 1.04 ppm) and 5538-19 (247.20 ± 0.40 ppm) belong to highly bitter (highly toxic) cultivar too. The seven unprocessed cultivars result represented in Figure 4.1 shows the means of total cyanide

content which are significantly ($P<0.05$) different from one another. Furthermore, it show high variability of HCN content in cassava roots, this might be due to genotypic difference and others. However, the study result is in line with previous observation of roots and tubers in animal feed with raw fresh weight values (88.3 - 416.3) and fresh pulp values (34.3 - 301.3) ppm (FAO, 1990; Westby, 2002).

Out of the three common processing techniques, total cyanide reduction was highest for fermented cassava roots flour The maximum and minimum percentage reduction was observed to be for fermented cultivars of Gamo and 5553-19 (98 %), and boiled cultivar Hayik (51 %), respectively. For the seven cultivars the pattern of cyanide reduction were highest for fermented followed by sun-dried and boiled flours except for cultivars 192. This is might be due to presence of several large sized granules over the mat that could not be fully penetrated by radiation (see below Figure 4.2).

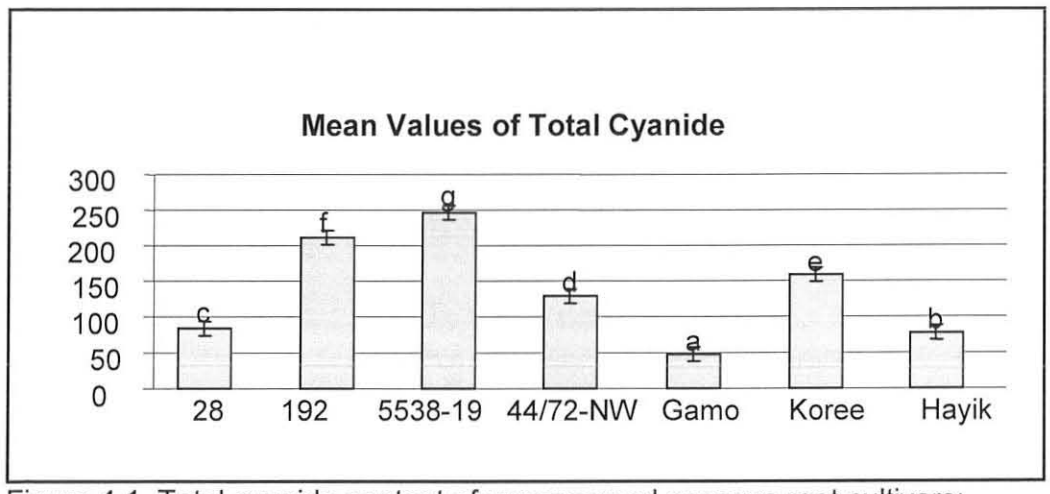


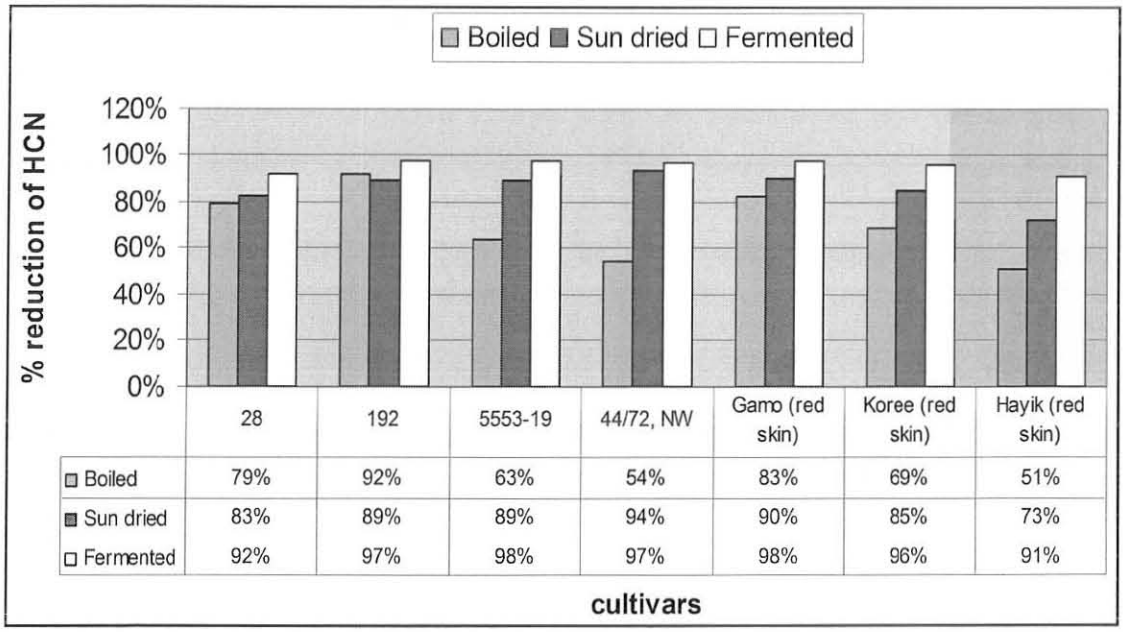
Figure 4.1. Total cyanide content of unprocessed cassava root cultivars: vertical = total cyanide in ppm and horizontal = types of cultivars.

Note: Statistical analysis was done using the one way ANOVA and DMR tests. Figures in column that share different letter superscripts are significantly different with a<b<c<d<e<f<g ($P<0.05$).

Table 4.2. Total cyanide levels in seven cassava cultivars roots of raw and processed in to three different methods (in ppm).

Tres[1, 2]	Cultivars*							Marginal			P of cultivar	P of processing method	P of cultivar x processing method
	28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% Cv			
Raw	83.7 ± 0.40d	129.2 ± 0.18d	247.2 ± 0.4d	211 ± 0.104d	48 ± 1.10d	159 ± 1.07d	78.07 ± 0.16d	**136.61**	69.74	51.05	*	*	*
Boiled	17.58 ± 0.56c	16.26 ± 0.06c	90.33 ± 0.25c	59.17 ± 0.20c	8.23 ± 0.18c	49.77 ± 0.75c	38 ± 0.12c	**39.90**	27.59	69.15			
Sun-dried	14.20 ± 0.74b	8.27 ± 0.41b	26.78 ± 0.82b	23.34 ± 0.33b	4.60 ± 0.41b	23.45 ± 0.65b	21.45 ± 0.58b	**17.44**	8.08	46.32			
Fermented	6.57 ± 0.30a	3.39 ± 0.18a	5.19 ± 0.15a	5.37 ± 0.49a	1.09 ± 0.07a	6.20 ± 0.11a	6.70 ± 0.43a	**4.93**	1.94	39.45			
Marginal means	**30.51**	**50.01**	**92.37**	**64.03**	**15.48**	**59.60**	**36.05**						

Results are mean values of three replicates ± SD, *- Interactions significant at $P < 0.05$, [1] means with the same letters within a column are not significantly different with a<b<c<d ($P>0.05$), [2] tres - treatments/processing methods, * accession number/name of cultivars,*- Interactions significant at $P<0.05$, cv- coefficient of variation.



	28	192	5553-19	44/72, NW	Gamo (red skin)	Koree (red skin)	Hayik (red skin)
Boiled	79%	92%	63%	54%	83%	69%	51%
Sun dried	83%	89%	89%	94%	90%	85%	73%
Fermented	92%	97%	98%	97%	98%	96%	91%

Figure 4.2. Effect of processing techniques on percentage reduction of total cyanide in cassava root flours.

4.2.2 Phytate, tannin and oxalate levels

Phytate

The Phytic acid is considered as an anti-nutritional factor, it is a common storage form of phosphorus in plant seeds, tubers and fruits. The phytate, tannin and oxalate level obtained from seven cassava roots cultivars and common processing are indicated in Table 4.3. The interaction effect due to processing methods and cultivars were found to be significant ($P<0.05$). Similarly, the cultivars and processing effects were significant ($P<0.05$) (see Appendix-V). The marginal means of phytate levels of processed cassava roots flour was found to be in the values of 655.15, 403.80 and 425.80 mg/100 g, for the boiled, sun-dried and fermented flours, respectively. The marginal means of processing methods over levels of cultivars are significantly ($P<0.05$) different with the mean for processed sun-dried (403.80

mg/100 g) being the lowest followed fermented and boiled. The cell means phytate level was highest for boiled cultivar of Gamo (910.66 ± 0.40 mg/100 g) followed by Koree (818.70 ± 1.56 mg/100 g) and the lowest belongs to fermented cultivar 44/72-NW (108.57 ± 2.1 mg/100 g). The marginal means of cultivars over levels of processing methods are significantly ($P<0.05$) different with the cultivar Hayik (269 mg/100 g) being the lowest. The cell means phytate content of processed cassava flour was significantly different ($P<0.05$) from one another except for the phytate content of boiled (44/72-NW) and sun-dried (Hayik). The complexing of phytate with nutritionally essential elements and the possibility of interference with protolytic digestion have been suggested as responsible for anti-nutritional activity. One of the factors is the presence of phytate, which is negatively charged phosphate compound that binds minerals and inhibits absorption (Howarth, et al., 2001; Anderson, et al., 2009). The processed cassava roots cultivars in the present study (Table 4.3) shows different value from the two local varieties of Qulle and Kello values (168.24 - 543.97 mg/100 g) and (31.3 - 60.4 mg/100 g) reported by Fasuyi (2005) and Tilahun (2009), respectively.

The variability of phytate content in cassava roots is not only due to cultivars factors but also it might be due to the total phosphorous content, found in soil and fertilizers, which can influence the phytic acid concentration (Maga, 1980). The phytate content in the present study is in line with the phytate content of Korean foods with the range values 191.7 to 973.3 mg/100 g for cereals and 508.5 to 1371.8 mg/100 g for legumes (Joung, et al., 2004). However, it shows little difference from the phytate content of Indian foods which ranged from 480 to 520 mg/100 g (Pushpanjali & Santosh, 1995) and

Indonesian's foods which ranged between 8 to 319 mg/l00 g for cereals and 24 to 1018 mg/100 g for legumes (Greiner, et al., 2006; Sanny, et al., 2007). Moreover, this study result is lower than the rice varieties of phytic acid content value (1976 - 2170 mg/100 g) reported by Saikia (1999).

However, the phytate intake of Ethiopian's food is still unknown consequently; the effect of phytate in local food of the country is not predictable. The phytate content of unprocessed cassava roots was significantly ($P<0.05$) higher than processed one, which had means value of 947.41 mg/100 g. The phytate contents of seven unprocessed cassava roots varieties presented in this investigation are found to be higher than the phytate content values (253 - 624 mg/100 g) of cassava roots reported by Oke (1990); Edeogu and Ekuma (2007).

Among the processing methods, sun-drying and fermentation are appeared to be effective to reduce the phytate levels, when compared to the boiling. The decrease in the phytate content of the fermented cassava flour for all the seven cultivars could possibly be attributed to the secretion of the enzyme phytase. This enzyme is capable of hydrolyzing phytate, thereby decreasing the phytate content of the cassava flour (Oboh and Akindahunsi, 2003; Nwokoro, et al., 2005). The decrease in phytate content during fermentation and boiling may be partly due to either the formation of insoluble complexes, such as phytate-protein and phytate-mineral (Badifu, 2001). Moreover, the Inositol hexaphosphate might also be hydrolyzed to penta- and tetra-phosphates and then leached out when boiled and fermented. The high content of phytate of nutritional significance is lowering the availability of many other essential dietary minerals. Thus, reduction of phytate is expected to

enhance the bioavailability of dietary minerals of the cassava (Siddhuraju and Becker, 2001). On the other hand, there is evidence that dietary phytate at low level may have beneficial role as an antioxidant, anti carcinogens and likely play an important role in controlling hypercholesterolemia and atherosclerosis (Slavin, 2004).

Tannin

The result shown in Table 4.3 signifies that the tannins level of processed cassava roots cultivars of boiled, fermented and all of the sun-dried flours were obtained below detectable limit. However, the marginal means of tannin level of boiled three cultivars (28, 192 and 5538-19) and fermented two cultivars (192 and 5538-19) of cassava roots flour were found to be in the value of 4.37 and 4.16 mg/100 g, respectively. The study result is in line with the tannin content value (3.6-6.9 mg/100 g) of cassava tubers and products reported by Oboh, et al. (2002); Fasuyi (2005). However, in some cultivars, tannins were not completely removed during fermentation and boiling, which might be due to the genotypic difference, environmental or combination effects.

The tannin levels of unprocessed cassava root flours were found to be in the means values of 66.89 mg/100 g. The interaction effect of cultivars and processing methods on dependent variable (tannin) was significant ($P<0.05$). The effect of cultivars and processing methods on tannin content were significant ($P<0.05$) (see Appendix-V). The observed tannin content of unprocessed cassava root is different with the report (Sarkiyayi and Agar, 2010), whose values are 0.40 and 0.6 mg/100 g for sweet and bitter cassava, respectively. The tannin content of the study is lower than that of rice value

(513 - 572 mg/100 g) reported by Saikia (1999). The presence of tannins can cause browning problems in both fresh food and processed products, and they act as anti-nutritional factor by provoking an astringent reaction in the mouth there by making the food unpalatable, they also form complex with proteins, precipitate proteins in the gut, inhibits digestive enzymes and microorganisms (Oboh and Elusiyan, 2007). This has nutritional implication for both human and livestock in that there is damage to the intestinal tract through absorption of tannic acid toxicity in the gut, also interference with the absorption of iron and a possible carcinogenic effect (Rao and Desothale, 1982; Onimawo and Akubor, 2005).

Oxalate

The ingestion of oxalic acid cause series health problem due to formation of calcium oxalate which is insoluble at physiological pH and can be deposited in the brain and kidney tubules. The lethal dose for oxalates in adults is estimated (143 - 428 mg/kg) (Libert and Franceschi, 1987). The result Table 4.3 shows the marginal means of processed cassava roots flour oxalate levels were found to be in the values of 27.16, 17.75, 18.13 and 10.28 mg/100 g for the raw, boiled, sun-dried and fermented flours, respectively. The effects of cultivars and processing methods were found to be significant ($P<0.05$). Similarly, the interaction of cultivars and processing methods was significant ($P<0.05$) (see Appendix-V). The three common processing techniques are found to be effective in reducing the oxalate levels of seven cassava root cultivars. The highest marginal means oxalate levels reduction are found to be for fermented followed by boiled and sun-dried flours. The possible reason to the observed high reduction in oxalate level is due to both fermentation and

boiling cause considerable cell rupture and facilitate the leakage of soluble oxalate into fermenting and cooking water (Savage, et al., 2000; Albihn and Savage, 2001; Bhandari and Kawabata, 2004). These average oxalate level variation observed is much higher than the range reported in previous study value (1.35 - 2.88 mg/100 g) for leaves (Correa, 2000; Wobeto, et al., 2007). The cell means oxalate level was recorded lowest for cultivar 44/72-NW (3.18 ± 0.00 mg/100 g) on dry weight basis for fermented followed by sun-dried cultivar 44/72-NW (3.35 ± 0.22 mg/100 g) and highest level observed for boiled cultivar Koree (29.00 ± 0.00 mg/100 g) cassava roots flour. The processed cultivars flour has shown significant ($P<0.05$) variability in cell means oxalate levels except for the cultivar Hayik (13.04 ± 0.06 mg/100 g). Whereas, the highest and lowest cell means oxalate levels found were 30.63 ± 0.21 and 19.81 ± 0.26 mg/100 g flour in Koree and 192 unprocessed cassava cultivars, respectively.

Table 4.3. Raw and processed cassava root cultivars flour phytate, tannin and oxalate levels in mg/100 g.

Pars[1]	Tres[2]	Cultivars*							Marginal			PoC	Po Pm	PoC x Pms
		28	192	5538 -19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% cv			
Phytate	Raw	724.98 ± 23.80d	741.84 ± 44.10d	871.53 ± 23.15d	622.24 ± 11.10c	1087.38 ± 21.68d	868.33 ± 6.25d	711.34 ± 0.69c	**803.95**	12.75	43.6	*	*	*
	Boiled	547.74 ± 26.47c	506.80 ± 28.47a	154.55 ± 21.35a	526.13 ± 28.75b	910.66 ± 0.40c	818.7 ± 1.56c	121.47 ± 0.54a	**655.15**	318.85	48.7			
	Sun-dried	137.76 ± 0.02a	569.82 ± 7.00b	225.88 ± 35.14b	535.21 ± 9.60c	713.32 ± 0.57b	521.70 ± 0.51b	122.91 ± 0.21b	**403.80**	224.46	55.6			
	Fermented	518.38 ±103.56b	720.41 ± 0.00c	745.57 ± 14.01c	108.57 ± 2.10a	611.79 ± 0.21a	153.78 ± 0.60a	122.13 ± 0.97b	**425.80**	275.91	64.8			
Marginal means		**482.22**	**634.72**	**749.38**	**448.04**	**830.79**	**590.63**	**269.46**						
Tannin	Raw	73.54 ± 0.00b	74.18 ± 4.40c	87.15 ± 2.31c	62.22 ± 1.10	47.74 ± 1.09	57.83 ± 0.11	65.58 ± 0.41	**66.89**	12.11	18.1	*	*	*
	Boiled	14.59 ± 0.47a	3.88 ± 0.97a	12.12 ± 0.37a	Nd^C	Nd^C	Nd^C	Nd^C	**4.37**	6.02	37.7			
	Sun-dried	Nd^C	Nd^C	Nd^C	Nd^C	Nd^C	Nd^C	Nd^C	**-**	-	-			
	Fermented	Nd^C	4.82 ± 1.60b	24.32 ± 4.03b	Nd^C	Nd^C	Nd^C	Nd^C	**4.16**	8.62	207.2			
Marginal means		**22.03**	**20.83**	**30.81**	**15.56**	**11.93**	**14.46**	**16.39**						
Oxalate	Raw	27.84 ± 0.50d	19.81 ± 0.26d	26.70 ± 0.29d	29.99 ± 0.24d	25.11 ± 0.28d	30.63 ± 0.21d	30.05 ± 0.34b	**27.16**	3.63	13.4	*	*	*
	Boiled	13.61 ± 0.22b	10.63 ± 0.21b	21.20 ± 0.21c	24.34 ± 0.00b	12.49 ± 0.40a	29.00 ± 0.01c	13.04 ± 0.06a	**17.75**	6.70	37.8			
	Sun-dried	16.31 ± 0.24c	17.99 ± 0.24c	16.03 ± 0.25b	27.29 ± 0.26c	18.15 ± 0.16c	18.01 ± 0.05a	13.07 ± 0.08a	**18.13**	4.22	23.3			
	Fermented	7.14 ± 0.51a	4.14 ± 0.53a	3.35 ± 0.02a	3.18 ± 0.00a	15.04 ± 0.08b	26.00 ± 0.12b	13.10 ± 0.11a	**10.28**	7.98	77.6			
Marginal means		**16.22**	**13.09**	**16.62**	**21.20**	**17.70**	**27.91**	**17.31**						

Results were mean values of triplicate determination (dwb) ± SD, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms - P of cultivar x processing methods, [1]means with the same letters within a column of respective group of parameters are not significantly different with a<b<c<d (P>0.05), [2] trets-treatments, pars-parameters, *accession number of cultivars, Nd^C -below detectable limit (Nd taken as=0 value).
*- Interactions significant at P < 0.05, cv- coefficient of variation.

4.2.3 Bioavailability of Ca, Zn & Fe in the 7 cultivars of cassava root flours

Mineral deficiencies, especially of iron, calcium, and zinc, have a negative effect on human health. However, to maintain mineral balance, it is not only the intake of a mineral is important, but more importantly the amount that is available to be absorbed. Thus, the bioavailability of minerals could be predicted by molar ratio of anti-nutritional factors to mineral value (Barbara, 1989). Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones.

4.2.3.1 Molar ratio of oxalate: calcium (ox to ca)

In Table 4.4 the value for molar ratio of oxalate: calcium cassava roots cultivars marginal means were calculated in the values of 0.12, 0.19, 0.16 and 0.10 for the raw, boiled, sun-dried and fermented flours, respectively. The interaction between cultivars and processing effects were significant ($P<0.05$). Similarly, the effect of cultivars and processing methods were significant ($P<0.05$). The average values of molar ratio of ox: ca were found to be increment for boiled and sun-dried flours when compared to raw. This is might be due ca ions attracted by other anti-nutrient factors in the flours. The highest and lowest cell means molar ratio values belongs to boiled cultivar 44/72-NW (0.45 ± 0.00) and fermented cultivars 5538-19 and 44/72-NW which had similar value (0.03 ± 0.00), respectively. In the present study result of the processed and unprocessed seven cassava roots cultivars show lower molar ratio of oxalate to calcium which is less than critical value (<2.5) reported by Hassan, et al. (2011). Therefore, the oxalate levels found in these

cultivars of cassava roots do not endanger the uptake of the calcium contained in it. Furthermore, the result is in agreement with molar ratio of oxalate to calcium on six Nepalese wild yams with value (1.1-2.2) reported by Megh, et al. (2004). In general, the reduced oxalate content by processing could have positive impact on the health of consumers, such as enhancing the bioavailability of essential dietary mineral and reduce the risk of kidney stones formation.

4.2.3.2 Molar ratio of phytate to Ca, Zn & Fe cassava roots flour

The results of molar ratio of phytate to minerals of the seven cultivars of cassava root values are presented in Table 4.5. The interaction between cultivars and processing effects were significant ($P<0.05$). Similarly, the effect of cultivars and processing methods on dependent variables were significant ($P<0.05$) (see Appendix-V). The molar ratio of phytate to zinc was calculated to be in the marginal means values of 48.89, 41.23, 38.44 and 24.52 for the raw, boiled, sun-dried and fermented flours, respectively. The maximum and minimum cell means molar ratio values belongs to unprocessed cultivar Koree (92.46 ± 0.06) and fermented cultivar 28 (0.51 ± 0.03) flours, respectively. The study result shows that the phytate: zinc molar ratio is above critical value (>1.5) which is in line with the previous observation value (>15) (Sandberg, et al., 1987; Morris & Ellis, 1989; Walter, et al., 2002). However, to say zinc is bio available in the food it is important to calculate the molar ratio of phytate x calcium to zinc. The molar ratio of phytate x calcium to zinc of seven cassava roots cultivars were in the marginal means values of 3.95, 1.88, 8.77 and 1.01 which belongs to raw, boiled, sun-dried and fermented flours, respectively. The average value for sun-dried molar ratio was significantly ($P<0.05$) higher

than the raw. This might be due to the reaction of Zn ion to other anti-nutrients factors in the flour. The highest and lowest cell means molar ratio of phytate * calcium to zinc value belongs to sun-dried cultivar 5538-19 (30.80 ± 0.03) and boiled cultivar 28 (0.02 ± 0.01), respectively. In addition to this, except for cultivars of 28 (raw, boiled & fermented=0.02); Hayik (boiled=0.28, sundried=0.31 & fermented=0.30); 44/72-NW (fermented=0.30) and Koree (fermented=0.37) the phytate x calcium: zinc cell means molar ratios are above the critical value (>0.5) which is in agreement with the reported value (>200) of Davies, et al. (1985) and Bindra, et al. (1986). The present study result shows limited Zn bioavailability in most of the seven-cassava root cultivars flour. Therefore, that zinc is none available in the flour for absorption.

The molar ratio of phytate to iron cassava roots cultivars were calculated in the marginal mean values of 35.24, 30.52, 23.08 and 17.82 for the raw, boiled, sun-dried and fermented flours, respectively. Among the average values of processed flours the molar ratio of phytate to Fe fermented flours were significantly ($P<0.05$) lower than others. The maximum and minimum cell means molar ratio values belongs to boiled cultivar Gamo (83.08 ± 0.01) and fermented cultivar Koree (0.12 ± 0.03), respectively. The phytate to iron molar ratio is found to be above critical value (>0.4) which is in agreement with the reported value (>1) of Morris & Ellis (1985) and Hallberg, et al. (1989). The availability of iron content to absorption is less; in order to correct, this it is necessary to fortify the food with iron to overcome the shortage.

The molar ratio of phytate to calcium of seven cassava roots cultivars were in marginal means values of 0.22, 0.42, 0.22 and 0.23 for the raw, boiled, sun-dried and fermented cassava roots flours, respectively.

Table 4.4. Raw and processed cassava root cultivars oxalate to calcium molar ratio* (ox: ca) [b]

Treatments [a]	Cultivars							Marginal			PoC	PoPm	PoC x Pms
	28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Mean	SD	% cv			
Raw	0.14 ± 0.00d	0.06 ± 0.00b	0.20 ± 0.01b	0.13 ± 0.00b	0.07 ± 0.00a	0.09 ± 0.00a	0.13 ± 0.00a	**0.12**	0.05	41.6	*	*	*
Boiled	0.08 ± 0.01b	0.04 ± 0.00a	0.41 ± 0.02d	0.45 ± 0.00d	0.07 ± 0.00a	0.15 ± 0.01c	0.13 ± 0.00a	**0.19**	0.17	89.4			
Sun-died	0.12 ± 0.01c	0.11 ± 0.00c	0.31 ± 0.01c	0.18 ± 0.01c	0.18 ± 0.00c	0.12 ± 0.00b	0.13 ± 0.00a	**0.16**	0.07	43.7			
Fermented	0.04 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.00a	0.03 ± 0.00a	0.17 ± 0.00b	0.26 ± 0.00d	0.13 ± 0.00a	**0.10**	0.09	90.0			
Marginal means	**0.09**	**0.06**	**0.24**	**0.20**	**0.12**	**0.15**	**0.13**						
Critical value [p]	=2.5	=2.5	=2.5	=2.5	=2.5	=2.5	=2.5						

Values of molar ratio of the same column with different superscript letters are significantly, different from each other with a<b<c<d (P<0.05),

[a] = methods of processing, [b] = results were mean values of triplicate determination, *- Interactions significant at P<0.05,

* = mg of oxalate/MW of oxalate: mg of calcium/MW of calcium, cv- coefficient of variation, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms - P of cultivar X processing methods. [p] = Critical values were sourced from Hassan, et al. (2011).

The effect of processing on molar ratio of phytate to calcium were found to be significantly ($P<0.05$) higher than the raw. The highest and the lowest cell means molar ratio value of phytate to calcium belongs to boiled cultivar 5538-19 (1.36 ± 0.02) and fermented cultivar 44/72-NW (0.07 ± 0.01) flours, respectively. On the other hand, except for two boiled cultivars of 5538-19 (1.36) and 44/72-NW (0.59) all other values of phytate: calcium molar ratio is below the critical value (<0.5) which is an indication of the availability of calcium in most of the seven cultivars of cassava root flours.

4.3 Effect of processing methods on minerals content in cassava flours

Our body in small amounts needs minerals, which classified as micronutrients. Deficiency in minerals however can have a major impact on health such as anaemia and osteoporosis that commonly occur in both developed and developing countries. This study focused on iron (Fe), zinc (Zn), calcium (Ca) and phosphorous (P) contents of the seven cassava roots cultivars. The interaction effect of cultivars and processing methods were found to be significant ($P<0.05$) for dependent variable response. Similarly, the effect of cultivars and processing methods were found to be significant ($P<0.05$) (see Appendix-V).

Zinc

The results in Table 4.6 show the minerals composition of cassava roots. The zinc content for the seven processed cassava roots cultivars marginal means values of 1.00, 1.01 and 0.99 mg/100 g for the boiled, sun-dried and fermented flours, respectively. The average sun-dried flours zn contents were

significantly ($P<0.05$) higher than other processed flours. The highest and lowest cell means values were referred to sun-dried cultivar 44/72-NW (1.26 ± 0.00 mg/100 g) and fermented cultivar 5538-19 (0.77 ± 0.02 mg/100 g) flours, respectively. The cell means of processed cassava roots flour zinc levels were significantly different ($P<0.05$) from one another except for Gamo, Koree and Hayik cultivars which have similar value. The zinc content of the seven processed cassava roots flour is found to be having more value than the value stated in the Food Composition Table of Ethiopia for cassava (0.20 mg/100 g) (EHNRI, 1997), while the result is within the value (1.41 - 4.25 mg/100 g) reported by Charles, et al. (2005). The low level and the variations in the composition of some minerals determined in this study from others obtained in previous literatures report could be attributed to many factors. As with all crops, the nutritional composition of root and tuber crops varies from place to place depending on the climate, the soil, the crop varieties (cultivars) and others (FAO, 1990; Njoku and Ohia, 2007). Although, the effect of age and soil management condition was assumed negligible and the dominant factor for mineral content variability is mainly attributed to the environmental and genetic variations of the cultivars. Whereas, the zinc content of unprocessed cassava roots was measured in the marginal mean value of 1.47 mg/100 g fresh weight basis. The zinc content of unprocessed cassava roots found to be significantly higher than processed one ($P<0.05$). The unprocessed cassava roots lowest cell mean value is obtained for cultivar Hayik (red skin)(1.16 ± 0.06 mg/100 g), this result is different with the locally grown cultivar Boloso Taro zinc content value (43.08 mg/100 g) reported by Adane (2009).

Calcium

The calcium content of the processed cassava roots flour are in the marginal means value of 143.44, 109.39 and 114.93 mg/100 g for the boiled, sun-dried and fermented flours, respectively. Among the three processing methods of the average ca content of boiled flours were found to be having the largest value of all other methods. The highest and lowest cell mean values found for boiled cultivar 192 (254.43 ± 4.8 mg/100 g) and sun-dried cultivar 5538-19 (50.81 ± 3.8 mg/100 g), respectively. The calcium content of all processed cassava roots flour show significant ($P<0.05$) difference between processing methods except for boiling and sun-dried cultivar (5538-19), and in all the three processing methods of Hayik cultivar. However, the calcium content of the seven cultivars was lower than the processed cassava leaves flour mean value (210 mg/100 g) reported by Fasuyi (2005).

The calcium content of unprocessed cassava roots was observed to be in the marginal mean value of 259.08 mg/100 g. The lowest cell mean value was belongs to cultivar 5538-19 (136.08 ± 1.67 mg/100 g). The cell mean calcium content of cultivar Koree (white skin) (342.14 ± 0.92) was significantly ($P<0.05$) higher than other cultivars. The calcium content of unprocessed cassava roots flour found to be significantly higher than processed one ($P<00.5$). The calcium content in unprocessed cassava flours were different from the value (10.9 - 50.0 mg/100 g) reported by Perera, et al. (1989) and Charles, et al. (2005).

Phosphorus

The phosphorus content of processed cassava roots flour was measured to

be in the marginal means value of 15.88, 16.63 and 15.68 mg/100 g, which was referred to, boiled, sun-dried and fermented flours, respectively. The average P content of sun-dried (16.63 mg/100 g) flours were significantly ($P<0.05$) higher than fermented and boiled flours. The highest and lowest cell means values were for sun-dried cultivar 28 and boiled cultivar 192 flours, respectively. The seven cassava root cultivars cell means phosphorous levels were significantly different ($P<0.05$) from one another except for the cultivars of Koree, 192, 5538-19 and Hayik having the similar value (Table 4.6). The phosphorus content in the seven unprocessed cassava roots cultivars was measured in the marginal mean value of 18.58 mg/100 g. The maximum and minimum cell means values were belongs to cultivars of Koree (19.34 ± 0.06 mg/100 g) and Hayik (17.31 ± 0.39 mg/100 g), respectively. The unprocessed cassava roots cultivars of phosphorous content was significantly higher than processed cultivars, except for the raw cultivar Hayik, which was significantly lower than processed sun-dried and fermented phosphorous value ($P<0.05$). The possibility of variation might be due to soil type, genetic, management and others factors. The phosphorus content of all the seven unprocessed cassava root cultivars was less than the phosphorus content value (42.6 mg/100 g) stated in the Food Composition Table of Ethiopia (EHNRI, 1997) and value (35.7 - 40 mg/100 g) reported by Perera, et al. (1989) and Nevry, et al. (2007).

Iron

The Iron content of processed cassava root flours were obtained in the marginal means value of 1.20, 1.22 and 1.36 mg/100 g that corresponds to boiled, sun-dried and fermented flours, respectively. The average Fe content

of fermented flours were significantly ($P<0.05$) higher than boiled and sun-dried flours. The cell means of iron content of seven cassava root cultivars flours were significantly different ($P<0.05$) from one another except for the 192, 5538-19, 44/72-NW and Hayik cultivars. The highest and lowest cell means Fe content were measured for fermented cultivar 28 (2.46 ± 0.22 mg/100 g) and boiled cultivar Gamo (0.93 ± 0.04 mg/100 g), respectively.

On the other hand, the iron content of processed cassava roots flour value (0.93-2.46 mg/100 g) is in agreement with the report of Charles, et al. (2005), which was in the range of 1.2 - 4.44 mg/100 g, for the oven dried 5 genotype cassava roots. However, it is not in line with the iron content value (0.16-0.24 mg/100 g) for tuber and roots reported by Bradbury and Holloway (1988).

The Iron content of unprocessed cassava root flours were found to be in the marginal means value of 1.44 mg/100 g. The highest and lowest cell means values belongs to cultivars of 5538-19 (1.83 ± 0.00 mg/100 g) and 192 (1.26 ± 0.03 mg/100 g), respectively. The unprocessed cassava roots cultivars of iron content was significantly ($P<0.05$) higher than processed one except for the fermented cultivars 28 and 192 having the same value (see below Table 4.6).

Table 4.5. Molar ratio of Phy.: Fe, Phy.: Zn, Phy.: Ca, [Ca]x[Phy.]: Zn contents of seven cultivars of cassava roots flour [b]

Molar ratio	Treatment	Cultivars							Marginal			PoC	Po Pm	PoC x Pm
		28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	means	SD	% cv			
[Ψ] Phytate: Zn	Raw	0.52 ± 0.40b	58.47 ± 0.04c	0.46 ± 0.02a	44.73 ± 0.02c	85.00 ± 0.04c	92.46 ± 0.06d	60.58 ± 0.35c	**48.89**	38.82	79	*	*	*
	Boiled	0.51 ± 0.31a	51.99 ± 0.04a	1.10 ± 0.03c	63.84 ± 0.24d	89.70 ± 0.09d	69.52 ± 0.02c	11.94 ± 0.01a	**41.23**	36.29	88			
	Sun-dried	15.77 ± 0.44c	57.26 ± 0.07b	26.43 ± 0.16d	41.87 ± 0.90b	68.85 ± 0.18b	46.73 ± 0.03b	12.19 ± 0.18ab	**38.44**	21.24	55			
	Fermented	0.51 ± 0.03a	77.91 ± 0.13d	0.95 ± 0.02b	12.29 ± 0.01a	52.40 ± 0.01a	15.15 ± 0.06a	12.40 ± 0.03b	**24.52**	29.28	119			
Marginal means		**4.33**	**61.41**	**7.24**	**40.68**	**73.99**	**55.97**	**24.28**						
[e] Phytate: Fe	Raw	44.59 ± 0.06c	49.96 ± 0.01d	0.40 ± 0.01a	36.16 ± 0.02d	70.97 ± 0.01c	1.17 ± 0.02d	43.42 ± 0.02d	**35.24**	25.89	73	*	*	*
	Boiled	47.90 ± 0.01d	38.05 ± 0.02b	0.57 ± 0.02c	33.09 ± 0.03c	83.08 ± 0.01d	0.52 ± 0.06c	10.41 ± 0.02a	**30.52**	29.80	97			
	Sun-dried	8.47 ± 0.09a	43.56 ± 0.03 c	14.18 ± 0.03d	32.44 ± 0.04b	51.78 ± 0.05a	0.38 ± 0.05b	10.74 ± 0.02b	**23.08**	19.54	84			
	Fermented	17.8 ± 0.13b	34.33 ± 0.03a	0.47 ± 0.03b	8.69 ± 0.03a	52.43 ± 0.01b	0.12 ± 0.03a	10.91 ± 0.03c	**17.82**	19.22	107			
Marginal means		**29.69**	**41.48**	**3.91**	**27.60**	**64.57**	**0.55**	**18.87**						
[Δ] Phytate: Ca	Raw	0.22 ± 0.01d	0.14 ± 0.02b	0.40 ± 0.01b	0.17 ± 0.01b	0.19 ± 0.01a	0.33 ± 0.01d	0.18 ± 0.01b	**0.22**	0.10	45	*	*	*
	Boiled	0.19 ± 0.01c	0.12 ± 0.01a	1.36 ± 0.02d	0.59 ± 0.010d	0.31 ± 0.01b	0.27 ± 0.01c	0.08 ± 0.01a	**0.42**	0.45	107			
	Sun-dried	0.14 ± 0.01a	0.21 ± 0.01c	0.26 ± 0.01a	0.22 ± 0.01c	0.43 ± 0.01d	0.22 ± 0.01b	0.08 ± 0.01a	**0.22**	0.11	50			
	Fermented	0.17 ± 0.01b	0.38 ± 0.01d	0.41 ± 0.01c	0.07 ± 0.01a	0.42 ± 0.01c	0.09 ± 0.01a	0.08 ± 0.02a	**0.23**	0.16	69			
Marginal means		**0.18**	**0.61**	**0.20**	**0.26**	**0.34**	**0.23**	**0.11**						
[Phy.]x[Ca]:Zn (Mol / Kg)	Raw	0.02 ± 0.01a	4.75 ± 0.06d	1.55 ± 0.30b	2.65 ± 0.03d	7.22 ± 0.05d	7.91 ± 0.03d	3.53 ± 0.02d	**3.95**	2.89	73	*	*	*
	Boiled	0.02 ± 0.00a	3.31 ± 0.09c	1.42 ± 0.02a	0.86 ± 0.06b	4.04 ± 0.07c	3.25 ± 0.06c	0.29 ± 0.02a	**1.88**	1.62	86			
	Sun-dried	22.90 ± 0.18b	2.34 ± 0.07b	30.80 ± 0.03d	1.56 ± 0.02c	1.74 ± 0.06b	1.72 ± 0.02b	0.31 ± 0.04c	**8.77**	12.57	143			
	Fermented	0.02 ± 0.01a	2.25 ± 0.02a	2.69 ± 0.05c	0.30 ± 0.01a	1.15 ± 0.03a	0.37 ± 0.02a	0.30 ± 0.02b	**1.01**	1.06	105			
Marginal means		**5.74**	**9.12**	**3.16**	**1.34**	**3.54**	**3.31**	**1.11**						

[b]results were mean values of triplicate determination (dwb) ± SD, values of respective molar ratio of the same column with different superscript, letters are significantly different from each other with a<b<c<d (P<0.05), [e] = mg of Phytate/MW of Phytate: mg of iron/MW of Iron, [Ψ] = mg of Phytate/MW of Phytate: mg of Zinc/MW of Zn, [Δ] = mg of Phytate/MW of Phytate: mg of Calcium/MW of Calcium, [ω] = (mol/kg Calcium) x (mol/kg Phytate): (mol/kg Zn), PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms-P of cultivar X processing methods, *- Interactions significant at P<0.05, cv-coefficient of variation.

Table 4.6. Effect of processing on Zn, Ca, P and Fe levels in seven cultivars of cassava root flours in mg/100 g.

		Cultivars*							Marginal			PoC	PoP	PoCxPms
Pars	Tres[2]	28	192	5538-19	44/72- NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% cv			
Zn	Raw	1.38 ± 0.02c	1.25 ± 0.01d	1.88 ± 0.01d	1.37 ± 0.05d	1.26 ± 0.03c	1.99 ± 0.02b	1.16 ± 0.06c	**1.47**	0.31	21	*	*	*
	Boiled	1.06 ± 0.05b	0.96 ± 0.00b	1.03 ± 0.00c	0.81 ± 0.00a	1.0 ± 0.01a	1.16 ± 0.19a	1.0 ± 0.02b	**1.00**	0.12	12			
	Sun-dried	0.86 ± 0.01a	0.98 ± 0.00c	0.87 ± 0.03b	1.26 ± 0.00c	1.02 ± 0.08a	1.1 ± 0.02a	0.98 ± 0.00a	**1.01**	0.13	13			
	Fermented	1.00 ± 0.22b	0.91 ± 0.00a	0.77 ± 0.02a	0.87 ± 0.00b	1.15 ± 0.06b	1.00 ± 0.03a	0.97 ± 0.06a	**0.99**	0.23	23			
Marginal means		**1.14**	**1.02**	**1.14**	**1.08**	**1.11**	**1.32**	**1.03**						
Ca	Raw	200.91 ± 0.09d	325.35 ± 0.75d	136.08 ± 1.67c	236.60 ± 0.60d	338.94 ± 0.11d	342.14 ± 0.92d	233.55 ± 0.52b	**259.08**	74.84	29	*	*	*
	Boiled	179.87 ± 2.84b	254.43 ± 4.80c	51.39 ± 1.14a	53.79 ± 0.18a	180.09 ± 0.90c	187.07 ± 1.00c	97.64 ± 0.47a	**143.44**	73.02	50			
	Sun-dried	56.33 ± 1.78a	163.62 ± 0.10b	50.81 ± 0.38a	148.93 ± 1.18c	101.07 ± 1.02b	146.9 ± 0.16b	98.04 ± 0.12a	**109.36**	43.06	39			
	Fermented	191.83 ± 1.30c	115.66 ± 0.12a	112.79 ± 0.30b	99.78 ± 0.93b	88.02 ± 1.69a	99.00 ± 0.10a	97.44 ± 0.59a	**114.93**	33.41	29			
Marginal means		**157.20**	**214.70**	**87.77**	**134.70**	**177.00**	**193.80**	**131.70**						

Results were mean values of triplicate determination (dwb) ± SD, [1]means with the same letters within a column of respective group parameters are not significantly different with a<b<c<d (P>0.05), *accession number of cultivars, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms - P of cultivar X processing methods, *- Interactions significant at P<0.05, Pars-parameters, Tres-treatments, cv-coefficient of variation.

Table 4.6.Continued

Pars	Trets	Cultivars*							Marginal			PoC	PoP	PoCxPms
		28	192	5538-19	44/72- NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	% cv	SD			
	Raw	19.1 ± 0.06c	17.99 ± 0.38b	19.05 ± 0.61b	18.31 ± 0.09d	18.97 ± 0.17d	19.34 ± 0.06b	17.31 ± 0.39a	**18.58**	0.74	4	*	*	*
	Boiled	15.44 ± 0.58b	10.31 ± 2.27a	18.69 ± 0.56b	15.45 ± 0.48b	15.68 ± 0.30b	18.06 ± 0.05a	17.53 ± 0.48a	**15.88**	2.77	17			
P	Sun-dried	18.96 ± 1.43c	17.58 ± 4.39b	15.35 ± 1.20a	12.12 ± 0.52a	16.13 ± 0.04c	17.90 ± 0.09a	18.37 ± 0.11b	**16.63**	2.33	16			
	Fermented	12.43 ± 1.71a	17.22 ± 2.16b	14.54 ± 1.37a	16.55 ± 0.29c	12.95 ± 0.09a	17.92 ± 0.12a	18.18 ± 0.43b	**15.68**	2.45	16			
Marginal means		**16.47**	**15.78**	**16.91**	**15.61**	**15.93**	**18.30**	**17.84**						
Fe	Raw	1.38 ± 0.02b	1.26 ± 0.03ab	1.83 ± 0.00c	1.46 ± 0.00b	1.30 ± 0.01d	1.35 ± 0.06c	1.39 ± 0.05b	**1.44**	0.18	13	*	*	*
	Boiled	0.97 ± 0.11a	1.13 ± 0.00a	1.71 ± 0.05b	1.35 ± 0.23ab	0.93 ± 0.04a	1.32 ± 0.09c	0.99 ± 0.02a	**1.20**	0.30	25			
	Sun-dried	1.39 ± 0.00b	1.11 ± 0.00a	1.33 ± 0.00a	1.40 ± 0.01ab	1.17 ± 0.01c	1.14 ± 0.00b	0.97 ± 0.02a	**1.22**	0.15	13			
	Fermented	2.46 ± 0.22c	1.78 ± 0.63b	1.32 ± 0.03a	1.06 ± 0.13a	0.99 ± 0.03b	1.01 ± 0.02a	0.95 ± 0.06a	**1.36**	0.56	41			
Marginal means		**1.56**	**1.32**	**1.55**	**1.32**	**1.11**	**1.20**	**1.08**						

Results were mean values of triplicate determination (dwb) ± SD, [1]means with the same letters within a column of respective group parameters are not significantly different with a<b<c<d (P>0.05), *accession number of cultivars, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms - P of cultivar X processing methods, *- Interactions significant at P<0.05, Pars- parameters, Tres – treatments, cv-coefficient of variation.

4.4.1 Vitamin C

The influence of processing on vitamin c content of cassava roots is shown in Table 4.7. The amount of vitamin c for processed cassava roots was observed to be in the marginal means of 2.82, 0.19 and 0.70 mg/100 g for the boiled, sun-dried and fermented flours, respectively. The interaction effect due to processing and cultivars on vitamin c contents were found to be significant ($P<0.05$). Similarly, the cultivars and processing methods effects were found to be significant ($P<0.05$) (see Appendix-V). The highest and the lowest cell means value belongs to boiled cultivar 192 (3.36 ± 0.24 mg/100 g) and sun-dried cultivar 28 (0.06 ± 0.01 mg/100 g), respectively. The processed cassava roots vitamin c level cell mean values were significantly different ($P<0.05$) from one another except for cultivar Hayik (0.10 ± 0.01 mg/100 g) which has similar value. The percentage reduction of vitamin c in processing of the seven cassava roots cultivars were found to be in the range of 5-99 % (Figure 4.3). In general, the vitamin c levels were significantly ($P<0.05$) reduced in all the processed flours. The reduction of vitamin c in processing is due to the sensitivity of this vitamin to different conditions such as light, heat, water and combination effect (Richardson and Finley, 1985). In this experiment, the retention of vitamin c decreases in the following order boiling, fermentation and sun-drying.

The unprocessed cassava roots vitamin c content obtained in marginal means of 4.74 mg/100 g. The highest and lowest cell mean values measured for cultivars of 5538-19 (6.87 ± 0.12 mg/100 g) and Gamo (3.36 ± 0.15 mg/100 g), respectively.

Table 4.7. Effect of processing on vitamin c content of seven cultivars of cassava root (mg/100 g).

	Treatments[2]				
Cultivars*	Raw	Boiled	Sun-dried	Fermented	Marginal means
28	4.03 ± 0.05d	2.98 ± 0.15c	0.06 ± 0.01a	2.71 ± 0.18b	**2.44**
192	4.59 ± 0.03d	3.36 ± 0.24c	0.08 ± 0.02a	0.88 ± 0.05b	**2.69**
5538-19	6.87 ± 0.12d	3.07 ± 0.05c	0.60 ± 0.08b	0.15 ± 0.07a	**2.65**
44/72-NW	5.41 ± 0.53c	3.06 ± 1.18b	0.16 ± 0.02a	0.17 ± 0.02a	**2.20**
Gamo (red skin)	3.36 ± 0.15d	2.11 ± 0.09c	0.22 ± 0.01a	0.69 ± 0.08b	**1.74**
Koree (white skin)	3.89 ± 0.10c	3.00 ± 0.02b	0.10 ± 0.01a	0.20 ± 0.01a	**1.79**
Hayik (red skin)	5.03 ± 0.09b	0.11 ± 0.01a	0.10 ± 0.01a	0.10 ± 0.01a	**1.34**
Marginal means	**4.74**	**2.82**	**0.19**	**0.70**	
SD	1.13	1.23	0.18	0.89	
% cv	23.80	43.60	96.70	127.20	
P of cultivar	*				
P of processing methods	*				
P of cultivar x Processing methods	*				

Results were mean values of triplicate determination (dwb) ± SD, [1] means with the same letters within a row are not significantly different with a<b<c<d (P>0.05), [2] processing methods,
* - accession number of cultivars, *- interactions significant at P<0.05, cv-coefficient of variation.

The result in Table 4.7 shows the vitamin c content of fresh unprocessed cassava roots cultivars found to be lower than the value (15 - 45 mg/100 g) reported by Perera, et al. (1989) and Charles, et al. (2001). The highest cell mean vitamin c content is found to be for cultivar 5538-19 (7.0 mg/100 g) which is about 10 % of recommended dietary allowance (RDA) value of daily requirement of adult man (7 of 60, 15-50 age). The lowest vitamin c content value covers about 5 % of recommended dietary allowance (RDA) value of daily requirement of adult man (3 of 60, 15-50 age).

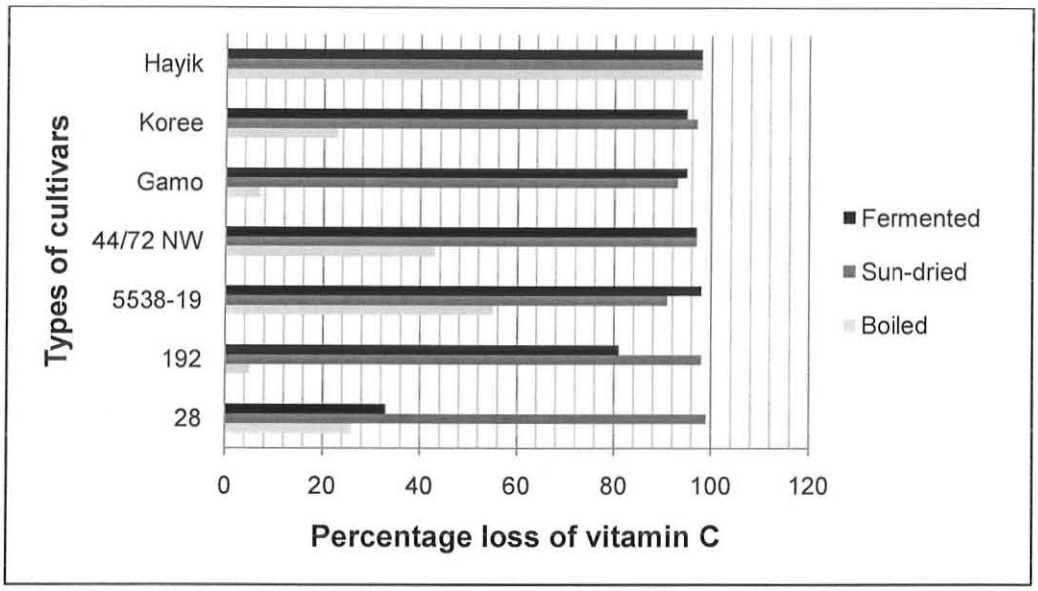


Figure 4.3. Effect of processing on percentage reduction of vitamin c content in seven cassava roots cultivars.

4.4.2 Pro-vitamin A (ß-carotene)

The Pro-vitamin A (ß-carotene) content of cassava roots is shown in Table 4.8. The beta-carotene in unprocessed cassava roots was found to be in the average of 0.14 µg/100 g. The cultivar Hayik had the highest (0.26 ± 0.00 µg/100 g) pro-vitamin A (ß-carotene), as opposed to the cultivar 28 which is showed the lowest value (0.03 ± 0.00 µg/100 g). The ß-carotene content in cultivar Hiyik was significantly higher ($P<0.05$) than other cultivars. While the result value obtained is not in agreement to the value (2.2-6.4 µg/g fwt) reported by Chavez, et al. (2005); Vimala, et al. (2008). This study result is also different from orange-fleshed sweet potato ß-carotene recorded by Esther and Ignitiatus (2010) which was observed for un blanched and blanched flour range value 3.48 - 5.48 and 1.54 - 4.24 µg/g, respectively. The seven cultivars of cassava roots were not appreciable in their β–carotene content due to lack of yellow-orange colour in their flesh portion, which

indicates the presence of carotenoids (Vimala, et al., 2008). The beta-carotene content in a flours processed by the three methods were below detectable limits. The unprocessed cassava root flours had β-carotene within detactable levels were also very low compared to those orange-fleshed root crops (β-carotene). As these processing of cassava roots results in substantial loss of ß-carotene the flour has to be supplemented by other vitamin A (retinol) rich foods like orange-fleshed root crops (Woolfe, 1992; Jalal, et al. 1998) in order to avoid deficiency. Substantial amount of vitamin A is important for the normal functioning of the visual system, normal immune functioning of the body, growth and development (FAO/WHO, 2001) and in reducing the incidence of certain cancers in humans (Gester, 1993).

Table 4.8. ß-carotene content in the raw and processed cassava cultivar roots (µg/100 g).

	Treatments[1]			
Cultivars	Raw (µg/100 g)	Sun-dried	Boiled	Fermented
28	0.03 ± 0.00a	Nd^C	Nd^C	Nd^C
192	0.06 ± 0.01b	Nd^C	Nd^C	Nd^C
5539-19	0.06 ± 0.01b	Nd^C	Nd^C	Nd^C
44/72-NW	0.13 ± 0.00c	Nd^C	Nd^C	Nd^C
Gamo (red skin)	0.21 ± 0.00d	Nd^C	Nd^C	Nd^C
Koree (white skin)	0.24 ± 0.01e	Nd^C	Nd^C	Nd^C
Hayik (red skin)	0.26 ± 0.00f	Nd^C	Nd^C	Nd^C
Means	**0.14**	-	-	-
SD	0.09	-	-	-
% cv	64.00	-	-	-
P of cultivar	*			
P of processing methods	*			
P of cultivar x Processing methods	*			

Results were mean values of triplicate determination (dwb) ± SD, [1]means with the same letters within a column are not significantly different with a<b<c<d<e<f (P>0.05), *accession number of cultivars; Nd^C - Not detectable, *- Interactions significant at P<0.05, cv-coefficient of variation.

4.5.1. Physical and functional properties of unprocessed cassava root.

Functional and physical properties of unprocessed cassava root cultivars are shown in Table 4.9a. The water binding capacity (WBC) for the seven cultivars of cassava root flours was found to be in the range of 1.21 to 2.04 mL/g. The WBC of cultivars of Koree and Hayik (2.04 mL/g) were significantly higher than other cultivars. Whereas, cultivars of 28, 192, and 44/72-NW having similar value (1.21), which were significantly ($P<0.05$) the lowest of all. The swelling power (SP) of the seven cultivars of cassava roots flour is found to be in the range of 8.95 to 22.09 g/g. The cassava flour could be attributed to the presence of more fibrous materials in the powder that posses more water holding capacity (Sandhya and Bhattacharaya, 1989). The swelling power of cassava roots flour was significantly ($P<0.05$) different from one another in all the seven cultivars. Out of the seven cultivars cassava roots flour the lowest swelling power was measured for cultivar of Gamo (8.95 g/g). While the highest SP was observed to the cultivar 28 (22.09 g/g). The swelling power of the studied cassava roots flour is slightly higher than the starches from different corn types ranged value (14.9 - 17.9) reported by Sandhu, et al. (2004). The starch granules with higher amylose content, on the other hand, being better reinforced and thus more rigid, probably swells less freely (Singh, 2000).

The percentage solubility of seven cultivars of cassava roots flour was found to be in the range of 14 to 59 %. The lowest measurement of solubility were observed for cultivar of 192 (14 %), while the highest solubility flour value was obtained for cultivar of 44/72-NW (59 %). The seven cultivars of cassava roots

flour was showed significant ($P<0.05$) variability in solubility level. The solubility of the seven cassava roots flour are significantly ($P<0.05$) different from one another except for the cultivars of Hayik and Gamo (30 %) (Table 4.9). However, the solubility value obtained in this study is not in agreement with the previously studied crops, starch value ($\sim$ 50 % solubility) for Japonica waxy rice flour reported by Singh, V., et al. (2000), and also higher than that reported for rice starch value ($\sim$10 %) by Singh (2000) and corn starch value (12.5 - 20.3 %) reported by Sandhu, et al. (2004).

The seven cultivars of cassava roots bulk density (loose and tapped) were found to be in the range of 0.39 to 0.45 and 0.46 to 0.58 g/mL, respectively. The tapped bulk density of flour of lowest and highest measurement were found to be for cultivars of Hayik (0.46 g/mL) and 44/72-NW (0.58 g/mL), respectively. The bulk density (loose) of the cultivars Hayik, 28 and 192 flour is not significantly ($P>0.05$) different from one another, similarly cultivars of Gamo, Koree, 5538-19 and 44/72-NW having the same value. The observed results of the seven cultivars of cassava roots flour bulk density is less than the bulk density of previous study of cassava flour value (0.64 - 0.76 g/mL) reported by Sanni, et al. (2006) and Udensi, et al. (2008). In most of the bakery, the highest bulk density can be chosen as good quality flour for its property to increase the rate of dispersion of the flour.

4.5.2. Effect of processing on physical and functional properties of cassava root flours.

Functional and physical properties of cassava roots cultivars are shown in Table 4.9b. The water binding capacity (WBC) for the processed cultivars of cassava root flours were found to be in marginal means of 1.37, 1.34 and 1.28

mL/g for the boiled, sun-dried and fermented flours, respectively. The interaction of cultivars and processing methods was significant ($P<0.05$) for WBC. Similarly, effects of cultivars and processing methods were significant ($P<0.05$) for WBC (see Appendix-V). The WBC of boiled flour was significantly ($P<0.05$) higher than sun-dried and fermented flours. This is probably due to attraction of water molecules to the crushed granules of starch sites. The marginal means of WBC of cultivars over processing methods Hayik cultivar (2.05 mL/g) were significantly ($P<0.05$) higher than other cultivars. The cultivars marginal means WBC values were significantly ($P<0.05$) different to each other this is due to the genotypic, environmental and soil fertility variability.

Table 4.9a. Physical and functional properties of unprocessed cassava root flours.

Cultivars *[1]	WBC (mL/g)	SP (g/g)	S (%)	LBD (g/mL)	TBD (g/mL)
28	1.21 ± 0.09	22.09 ± 0.09	24 ± 0.02	0.45 ± 0.03	0.55 ± 0.02
192	1.22 ± 0.07	18.57 ± 0.40	14 ± 0.03	0.45 ± 0.04	0.56 ± 0.04
5538-19	1.30 ± 0.10	9.34 ± 0.48	25 ± 0.06	0.42 ± 0.02	0.56 ± 0.02
44/72-NW	1.28 ± 0.08	14.05 ± 0.13	59 ± 0.02	0.42 ± 0.06	0.58 ± 0.02
Gamo (red skin)	1.43 ± 0.07	8.95 ± 0.15	30 ± 0.02	0.39 ± 0.03	0.50 ± 0.01
Koree (white skin)	2.04 ± 0.07	10.07 ± 0.17	20 ± 0.03	0.40 ± 0.02	0.47 ± 0.02
Hayik (red skin)	2.04 ± 0.06	11.07 ± 0.12	26 ± 0.03	0.44 ± 0.02	0.46 ± 0.04
Means	**1.50**	**10.29**	**28.29**	**0.42**	**0.53**
SD	0.08	0.21	0.03	0.04	0.02
% cv	5.30	2.00	0.11	9.50	4.78

[1] results were mean values of triplicate determination (dwb) ± SD, *accession number of cultivars SP-swelling power , S.- solubility, L/TBD- loose/tapped bulk density and WBC-water binding capacity, cv-coefficient of variation.

Table 4.9b. Effect of processing on physical and functional properties of seven cultivars of cassava root flours.

Pars[1]	Tres[2]	Cultivars*							Marginal			PoC	Po Pm	PoC x Pms
		28	192	5538 -19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Means	SD	% cv			
WBC (mL/g)	Boiled	1.12 ± 0.00	1.13 ± 0.00	1.13 ± 0.00	1.12 ± 0.00	1.04 ± 0.07	1.38 ± 0.04	2.00 ± 0.07	**1.37**	0.03	2.8	*	*	*
	Sun-dried	1.16 ± 0.01	1.11 ± 0.02	1.32 ± 0.01	1.36 ± 0.00	1.31 ± 0.01	1.38 ± 0.04	2.15 ± 0.01	**1.43**	0.03	2.1			
	Fermented	1.02 ± 0.01	1.05 ± 05	1.12 ± 0.00	1.05 ± 0.04	1.41 ± 0.01	1.99 ± 0.01	2.00 ± 0.01	**1.28**	0.02	1.5			
Marginal means		**1.10**	**1.10**	**1.19**	**1.17**	**1.25**	**1.58**	**2.05**						
S (%)	Boiled	18 ± 0.00	61 ± 0.00	34 ± 0.00	73 ± 0.00	31 ± 0.00	21 ± 0.00	20 ± 0.00	**37.00**	0.03	5.4	*	*	*
	Sun-dried	11 ± 0.00	60 ± 0.00	31 ± 0.00	42 ± 0.03	33 ± 0.02	21 ± 0.00	20 ± 0.13	**31.00**	0.01	3.2			
	Fermented	2 ± 0.00	20 ± 0.00	25 ± 0.01	33 ± 0.06	30 ± 0.01	20 ± 0.00	11 ± 0.01	**21.00**	0.01	4.8			
Marginal means		**10.00**	**47.00**	**30.00**	**49.00**	**32.00**	**21.00**	**17.00**						
SP (g/g)	Boiled	24 ± 0.01	21 ± 0.01	11 ± 0.03	21 ± 0.04	12 ± 0.03	14.1 ± 0.01	14.0 ± 0.04	**16.55**	3.02	18.2	*	*	*
	Sun-dried	23 ± 0.05	21 ± 0.01	13 ± 0.03	20 ± 0.04	14 ± 0.04	15.1 ± 0.03	15.0 ± 0.03	**17.00**	3.03	17.8			
	Fermented	22 ± 0.012	20 ± 0.016	10 ± 0.03	19 ± 0.04	11 ± 0.08	13.0 ± 0.04	13.0 ± 0.04	**16.00**	3.04	19.0			
Marginal means		**23.00**	**20.70**	**11.30**	**20.00**	**13.00**	**14.00**	**14.00**						
TBD (g/mL)	Boiled	0.62 ± 0.00	0.61 ± 0.00	0.63 ± 0.00	0.71 ± 0.01	0.55 ± 0.00	0.54 ± 0.00	0.61 ± 0.00	**0.61**	0.05	8.2	*	*	*
	Sun-dried	0.65 ± 0.00	0.61 ± 0.01	0.65 ± 0.00	0.65 ± 0.00	0.56 ± 0.00	0.45 ± 0.00	0.58 ± 0.00	**0.60**	0.07	11.7			
	Fermented	0.50 ± 0.00	0.53 ± 0.00	0.62 ± 0.00	0.62 ± 0.00	0.54 ± 0.03	0.42 ± 0.01	0.53 ± 0.00	**0.52**	0.07	13.5			
Marginal means		**0.59**	**0.58**	**0.63**	**0.66**	**0.55**	**0.47**	**0.58**						

[1]results were mean values of triplicate determination (dwb) ± SD, *accession number of cultivars, pars-parameters, Tres-treatments, cv- coefficient of variation, PoC-p of cultivars , [2]PoPm-p of processing, PoCxpms-p of cultivars and processing methods, SP-swelling power , S-solubility, TBD-tapped bulk density and WBC-water binding capacity.

The water binding capacity of the processed roots flours were found to be less than the value (3.52 to 10.5 mL/g) reported by Eke, et al. (2007). Water binding capacity give an advantage of being used as a thickener in liquid and semi liquids foods since the flour has the ability to absorb water and swell for improved consistency. The water binding capacity of flour has been observed to be dependent on the starch in the flour and protein concentration, and the size of the particles (Wotton and Bamunuarachchi, 1978; Sandhu, et al., 2004).

The solubility (S) for the processed cultivars of cassava root flours across the cultivars were found to be in marginal means of 37, 31 and 21 % for the boiled, sun-dried and fermented flours, respectively. The interaction of cultivars and processing methods was significantly ($P<0.05$) different for dependent variable solubility. Similarly, effects of cultivars and processing methods were significant ($P<0.05$) for solubility. The solubility of boiled flour was significantly ($P<0.05$) higher than sun-dried and fermented flours. This is might be due to the polysaccharide carbohydrates more degraded to dissolvable simplest carbohydrate. The marginal means of solubility of cultivars over processing methods 44/72-NW cultivars (49 %) were significantly ($P<0.05$) higher than other cultivars. The cultivars marginal means solubility values were significantly ($P<0.05$) different to each other. The variations were might be due to the genotypic, environmental and soil fertility difference.

The swelling power (Sp) for the processed cultivars of cassava root flours across the cultivars were found to be in marginal means of 16.55, 17.00 and 16.00 g/g for the boiled, sun-dried and fermented flours, respectively. The

interaction of cultivars and processing methods was significantly ($P<0.05$) different for dependent variable swelling power. Similarly, effects of cultivars and processing methods were significant ($P<0.05$) for swelling power. The swelling power of sun-dried flour was significantly ($P<0.05$) higher than boiled and fermented flours. This is might be due to the polysaccharide carbohydrates more degraded to dissolvable simplest carbohydrate. The cassava flour could be attributed to the presence of more fibrous materials in the powder that posses more water holding capacity (Sandhya and Bhattacharaya, 1989). The marginal means of swelling power of cultivars over processing methods 28 cultivar (23 g/g) was significantly ($P<0.05$) higher than other cultivars. The cultivars marginal means swelling power values were significantly ($P<0.05$) different to each other except for Koree and Hayik cultivars. The variations were might be due to the genotypic, environmental and soil fertility difference.

The Tapped bulk density (TBD) for the processed cultivars of cassava root flours across the cultivars were found to be in marginal means of 0.61, 0.60 and 0.52 g/mL for the boiled, sun-dried and fermented flours, respectively. The interaction of cultivars and processing methods was significantly ($P<0.05$) different for dependent variable TBD. Similarly, effects of cultivars and processing methods were significant ($P<0.05$) for TBD (see Appendix-V). The TBD of boiled flour was significantly ($P<0.05$) higher than sun-dried and fermented flours. The marginal means of TBD of cultivars over processing methods 44/72-NW cultivar (0.66 g/mL) was significantly ($P<0.05$) higher than other cultivars. The cultivars marginal means TBD values were significantly ($P<0.05$) different to each other. The variations were might be due to the

genotypic, environmental and types of soil. The observed results of the processed cultivars of cassava roots flour bulk density is less than the bulk density of previous study of cassava flour value (0.64 - 0.76 g/mL) reported by Sanni, et al. (2006) and Udensi, et al. (2008). In most of the bakery, the highest bulk density can be chosen as good quality flour for its property to increase the rate of dispersion of the flour.

4.6 CASSAVA BASED COMPOSITE FLOURS

Out of the studied seven-cassava root accession, number 28 flour was selected for composite flour preparation because of its nutrient density (fibre, protein, ash and carbohydrate contents) and physical properties (water binding capacity and bulk density) better than the others.

4.6.1 Physical and functional properties of composite flour ingredients

The functional properties of blended flour ingredients are shown in Table 4.10. Common bean had the highest water binding capacity (WBC) (4.17 ± 0.29 mL/g) followed by quality protein maize (3.00 ± 0.87), tef (2.17 ± 0.29) and cassava (1.21 ± 0.09). Similarly, common bean had the highest tapped and loose bulk density followed by quality protein maize, tef and cassava flour blend. The common bean bulk density value (0.63 ± 0.02) is significantly higher than other ingredients flour ($P<0.05$). It is observed that there was a slight difference between the loose and tapped bulk density of the individual ingredients.

Table 4.10. Physical and functional properties for flours ingredients used in the composite flours.

Parameters [B]	Cassava flour	White Tef	Maize (QPM)	Common bean
WBC (mL/g)	1.21 ± 0.09a	2.17 ± 0.29b	3.00 ± 0.87c	4.17 ± 0.29d
TBD (g/mL)	0.55 ± 0.02a	0.68 ± 0.02b	0.73 ± 0.02c	0.74 ± 0.02d
LBD (g/mL)	0.45 ± 0.03a	0.51 ± 0.01b	0.55 ± 0.02c	0.63 ± 0.02d

[B] Mean value ± SD, n=3, means with the same letters within a row are not significantly different with a<b<c<d (P>0.05), QPM-quality protein maize,
WBC-Water Binding Capacity, TBD/LBD-Tapped/Loose Bulk Density.

4.6.2 Physical and functional properties of composite flours

The bulk density (BD) and water binding capacity (WBC) of maize-tef-cassava composite flour properties are shown in Table 4.11. The tapped BD value was found to be within the range (0.31 ± 0.01 - 0.62 ± 0.02 g/mL). The smallest and highest BD values were obtained in composite flour MTC4 (0.31 ± 0.01 g/mL) and MTC1 (0.62 ± 0.02 g/mL), respectively. The BD values in MTC of composite flours were significantly (P<0.05) lower than for the control (tef = 0.68 ± 0.02). The MTC composite flours disperse in water was not as good as tef flour, but could be packed to less volume of container.

The WBC values were found to be within the range 0.83 ± 0.29 to 2.17 ± 0.29 mL/g. The highest and smallest values were obtained in composite flours MTC6 (2.17 ± 0.29 mL/g) and MTC1 (0.83 ± 0.29 mL/g), respectively. Water binding capacity of protein is important in viscous foods in order to provide body, thickening and viscosity. The result in Table 4.11 show that the water-binding capacity (WBC) of the entire composite flours were significantly (P<0.05) lower than the control tef flours except for the blend MTC6 and tef had, similar value of WBC (2.17 ± 0.29 mL/g). This study result of WBC is not

in agreement to the observed value (0.96 - 1.07) for cereal flour reported by Sandhu, et al. (2004). The MTC6 and tef flours WBC, similarity was might be due to more small size of ingredients' flours in the composite MTC6 flour. When the percentage of tef decreases in the blend it shows the increment on the water binding capacity. This might be attributed to more small grain fractions in contact with water. Among the MTC composite flours MTC6 (2.17 ± 0.29 mL/g) shows relatively the highest water binding capacity. The BD and WBC values for common bean-tef-cassava composite flours are shown in Table 4.12. The tapped and loose bulk densities ranged from 0.63 ± 0.02 to 0.75 ± 0.02 and 0.51 ± 0.01 to 0.62 ± 0.01 (g/mL), respectively. Both highest tapped and loose bulk densities found in composite BTC1, while the smallest value were obtained in composite flours BTC6 and BTC3, respectively.

Table 4.11. Bulk density and water binding capacity for composite flours from maize, tef and cassava (MTC) [b]

Blended four [F]	Bulk Density (g/mL)		Water Binding Capacity (mL/g)
	Tapped	Loose	
MTC 1	0.62 ± 0.02e	0.53 ± 0.01f	0.83 ± 0.29a
MTC 2	0.59 ± 0.02d	0.49 ± 0.01e	1.67 ± 0.29b
MTC 3	0.55 ± 0.01c	0.43 ± 0.01d	1.83 ± 0.29c
MTC 4	0.31 ± 0.01a	0.23 ± 0.00a	1.83 ± 0.29c
MTC 5	0.44 ± 0.01b	0.35 ± 0.01c	1.67 ± 0.29b
MTC 6	0.31 ± 0.01a	0.26 ± 0.01b	2.17 ± 0.29d
Tef (control)	0.68 ± 0.02f	0.55 ± 0.01g	2.17 ± 0.29d

MTC1-6 = 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9 and 1:4:5, respectively, mean value ± SD, means with the same letters within a column is not significantly different with a<b<c<d<e<f<g (P>0.05), [F] MTC- maize: tef: cassava composite flour, [b] mean value of triplicate value.

The observed values of water binding capacities for BTC composite flours were higher than those for MTC. This might be due to protein introduced by bean legume and interactions between polar amino acid residues of protein and water molecules as reported by El-Nasri, et al. (2007) and Cheng, et al. (2009). The WBC for composite flours were reportedly much lower than that of protein isolated from the flaxseed cake without detoxification (Sactae and Suntornsuk, 2011). In addition, to this, the extent of protein hydration correlates strongly with the content of polar residues as well as the interaction between water molecules and hydrophilic groups, which occurs via hydrogen bonding. Accordingly, the high protein content of the blends containing common bean flour might be responsible for high hydrogen bonding and high electrostatic repulsion, both conditions facilitating binding and entrapment of water. The high WBC values in common bean composite flours provide advantage of being used as a thickener in liquid and semi-liquids foods (Altchul and Wilcke, 1985). This is due to the huge protein content of the pulse and surface area of flour has the ability to absorb water and swell for improved consistency in food.

Table 4.12. Bulk density and water binding capacity for composite flours from bean, tef and cassava (BTC)[b]

Blended flour[F]	Bulk Density (g/mL)		Water Binding Capacity (mL/g)
	Tapped	Loose	
BTC 1	0.75 ± 0.02c	0.62 ± 0.01d	3.67 ± 0.29cde
BTC 2	0.68 ± 0.02b	0.53 ± 0.01ab	1.83 ± 0.29ab
BTC 3	0.69 ± 0.02b	0.51 ± 0.01a	3.83 ± 0.29de
BTC 4	0.66 ± 0.02ab	0.53 ± 0.01ab	3.33 ± 0.29cd
BTC 5	0.69 ± 0.02b	0.57 ± 0.01c	3.17 ± 0.29cd
BTC 6	0.63 ± 0.02a	0.53 ± 0.01ab	1.33 ± 0.29a
Tef (control)	0.68 ± 0.02b	0.55 ± 0.01bc	2.17 ± 0.29b

BTC1-6 = 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9 and 1:4:5, respectively, mean value ± SD, means with the same letters within a column is not significantly different with a<b<c<d<e (P>0.05), [F] BTC-Bean: Tef: Cassava composite flour,[b] average value (n=3).

4.7 Food product (Injera) from composite flours

The composite flours used to prepare Injera (pancake), a traditionally fermented Ethiopian staple food normally prepared from tef flour. Good quality Injera is characterized by having honeycomb-like holes in its top surface, which are produced due to the production and escape of carbon dioxide during fermentation and baking. The prepared Injera samples are shown in pictorial forms in Figure 4.4.

4.7.1 Sensory evaluation of Injera prepared from composite flours

Data on sensory evaluation of Injera made from quality protein maize, tef and cassava (MTC) flour blend are shown in Table 4.13 and Figure 4.4. The

panelist scored values of MTC1-4 flour Injera were not significantly (P>0.05) different from the tef Injera (MTC7=5.93), when overall acceptability is considered. The result of sensory evaluation revealed that the Injera prepared from MTC5 (2.40) composite flour was least accepted of all other Injera when overall acceptability is considered by panelist. The sensory attribute of appearance (back and front side) of composite flour Injera (MTC1, MTC2, MTC3 and MTC4) values were not significantly (P>0.05) different from the control (MTC7). Increasing the proportion of cassava flour from 10 to 40 % in the MTC composite flour produced acceptable Injera in terms of colour, taste, flavour and texture values were obtained to be accepted. Similarly, the sensory evaluation of BTC composite flour Injera in result (Table 4.14, Figure 4.4) shows that the overall acceptability of 15 panelists scores value is found to be significantly (P<0.05) lower than control (tef Injera). However, there was no significant (P>0.05) difference in the overall acceptability of the Injera samples (BTC1-4), compared to the control tef Injera. The Injera prepared from blends of BTC1, BTC2, BTC3 and BTC4 had mean score overall acceptability values of 5.40, 5.00, 5.67 and 4.90, respectively. The least mean score was obtained for blends of Injera BTC6 (3.20) followed by BTC5 (3.47) when overall acceptability is considered. The blend BTC1-4 Injera sensory characteristics of taste, flavour, texture and back side appearance score values were significantly (P<0.05) lower than the control tef Injera. The BTC overall acceptability for BTC Injera decreased as the cassava root flour proportion increased in the composite flour, and so did the texture acceptability. This study has shown that composite flour containing 10 % quality protein maize or common bean flour, and substituting tef flour with 10,

20, 30 and 40 % with cassava flour did not reduce the sensory quality of the Injera samples. Injera prepared from M/BTC1-4 had appropriate front side/eyes from leavening fermentation and baking (Yetneberk, et al. 2004, 2005).

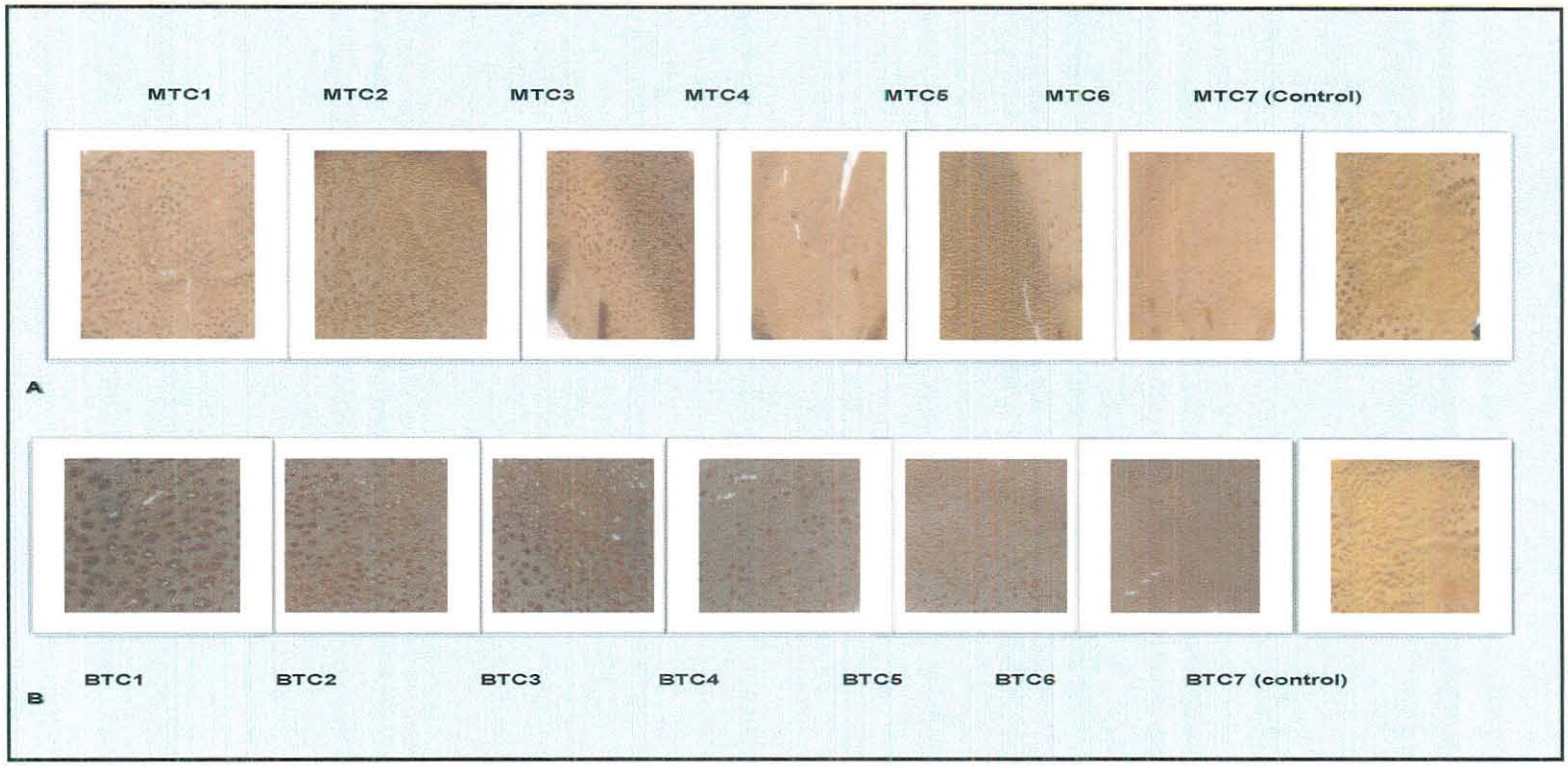


Figure 4.4. Photos for processed Injera from composite flour and whole tef as control: A = quality protein maize-tef-cassava, MTC1-7 = 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9, 1:4:5, 0:1:0, and B = Bean-tef-cassava, BTC1-7 = 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9, 1:4:5 and 0:1:0 (control).

Table 4.13. Sensory scores* of Injera made from different proportion of quality protein maize, tef and cassava flours (MTC [F]).

	Sensory attributes													
Maize blend	Colour	% cv	Taste	% cv	Flavour	% cv	Texture	% cv	Back side appearance	% cv	Front side [e]	% cv	Overall acceptability	% cv
MTC1	6.07bc	16	5.73b	19	5.53b	19	4.80c	17	5.53c	19	5.00cd	17	5.27c	20
MTC2	6.00bc	20	5.53b	18	5.40b	20	5.00cd	20	5.33c	21	4.20bc	21	5.20c	19
MTC3	6.20bc	12	5.47b	20	5.13b	20	5.33cd	8	5.27c	19	4.53cd	22	5.33c	19
MTC4	6.40c	12	5.53b	18	5.60b	19	5.73cd	19	5.93c	16	5.40d	19	6.00c	20
MTC5	5.00a	20	3.40a	24	3.47a	20	2.80a	14	2.40a	10	1.93a	7	2.40a	12
MTC6	5.40ab	22	3.80a	14	3.73a	19	3.80b	22	3.47b	19	3.27b	21	3.49b	19
Tef (control)	5.93bc	21	6.13b	14	6.00b	16	5.87cd	20	6.00c	18	5.67d	8	5.93c	13

Means ± SD with the same letters within a column are not significantly different with a<b<c<d (P>0.05),

[e] honeycomb-like holes is the structure of top surface of Injera, cv-coefficient of variation,

* On an ordinal scale of 1 to 7 (1 = dislike extremely & 7 = like extremely), N=15,

[F] Maize: tef: cassava composite flour, MTC1-7: 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9, 1:4:5 and 0:1:0 (control).

Note: If the % cv > 20 indicates there was differences in the individual genotypic makeup of panellists'.

Table 4.14. Sensory scores * of Injera made from different proportion of common bean, tef and cassava flour blend (BTC [F]).

	Sensory attributes													
Bean blend	Colour	% cv	Taste	% cv	Flavour	% Cv	Texture	% cv	Back side appearance	% cv	Front side [e]	% cv	Overall acceptability	% cv
BTC1	5.60bcd	18	5.33b	19	5.40c	18	5.00bc	20	5.33ab	20	4.93b	20	5.40b	19
BTC2	5.20abc	19	4.73ab	21	4.67bc	22	4.47abc	17	5.00ab	20	4.67b	12	5.00b	20
BTC3	5.93ab	17	5.00ab	20	5.20bc	20	5.53c	19	5.67b	18	5.67b	19	5.67b	17
BTC4	4.93ab	21	4.40ab	18	4.13b	07	3.87ab	17	4.13a	19	4.73b	21	4.90b	20
BTC5	4.67a	17	4.07a	10	3.93a	16	3.80ab	19	4.27a	14	2.87a	13	3.47a	16
BTC6	4.60a	17	4.20ab	18	4.27b	11	3.53a	20	4.33a	23	2.47a	9	3.20a	15
Tef (control)	5.93ab	21	6.13c	14	6.00d	16	5.87d	20	6.00c	18	5.67b	8	5.93c	13

Mean value ± standard deviation, with the same letters within a column are not significantly different with a<b<c<d (P>0.05),

* On an ordinal scale of 1 to 7, where 1=dislike extremely and 7= like extremely, N=15,

[e] honeycomb-like holes is the structure of top surface of Injera, cv-coefficient of variation,

[F] Bean: Tef: Cassava composite flour (BTC), BTC1-7 = 1:8:1, 1:7:2, 1:6:3, 1:5:4, 2:9:9, 1:4:5 and 0:1:0 (control).

Note: If the % cv > 20 indicates there was differences in the individual genotypic makeup of panellists'.

4.7.2 Proximate composition of Injera.

The proximate composition of blended flour Injera is shown in Table 4.15. The percentage composition analysis of composite flour Injera crude protein, crude fat, crude fibre, ash, moisture content and carbohydrate ranged from 6.37 ± 0.49 to 9.53 ± 0.11, 0.66 ± 0.13 to 1.84 ± 0.42, 3.66 ± 0.06 to 5.07 ± 0.06, 1.60 ± 0.01 to 2.15 ± 0.01, 6.99 ± 0.01 to 9.04 ± 0.05 and 78.27 ± 0.13 to 83.93 ± 1.33, respectively. The highest proximate values were found to be for crude protein, crude fat, crude fibre, ash, moisture content and carbohydrate for BTC1 (9.53 ± 0.11), MTC2 (1.84 ± 0.42), BTC3 (5.07 ± 0.06), BTC2 (2.15 ± 0.01), BTC5 (9.04 ± 0.05) and MTC5 (83.85 ± 0.76), respectively. The proximate composite values for BTC and MTC Injera were significantly ($P<0.05$) higher than for Injera from tef, except for crude fibre and carbohydrates.

The protein content of Injera baked from BTC1 (1:8:1) flour blend is significantly ($P<0.05$) higher than other (BTC 2-7, MTC1-7). This might be due to fermentation process of the protein added to it from microbiological enzymes and others. As the percentage, proportion of cassava roots flour increases the protein content of Injera decreases. This study agree with the literature reported by Eddy, et al. (2007) that whole wheat and composite bread crude protein content decreased as the amount of cassava flour increases. The protein content of all the composite flour samples was relatively reduced because of cassava, which is a poor source of protein (Okaka and Isieh, 1990; Oyenuga, 1992; Kebede, et al., 2012).

The ash content of composite flour Injera increases as level of cassava flour supplementation increases, implying that the inorganic nutrients in the composite Injera are richer than that of whole tef Injera control. The pH of the composite flour dough increases as the proportion of cassava flour increases in the composite flour. The pH value for the whole tef control (3.38) was lower than other B/MTC composite flour of Injera, which indicate whole tef Injera was more acidic. The increment of acidity might be attributed to the trace amount of cyanide present in the cassava flour that reduce the function of micro organisms in the batter at the time of fermentation. As the cassava, roots flour proportion decreases, in contrast to tef proportion the amount of protein in the Injera increases. In both the composite flours Injera carbohydrate content shows increment with the decrease of tef proportion in the blends except for composite MTC1 and BTC5. This observation might be attributed to the high content of carbohydrate in cassava. In all the solid nutrients in roots and tubers, carbohydrate content is predominating (Enwere, 1998). The composite flour Injera energy value is significantly ($P<0.05$) higher than the reference Injera value (301.84 ± 0.75 kcal/100 g). Whereas, the highest and lowest value belongs to MTC2 (373.38 kcal/100 g) and BTC5 (359.12 kcal/100 g), respectively. The studied sample energy value is comparable to the bread baked from less 50% roots and tuber (sweet potato/cassava)-maize-soy formulated mixture range value (360.07-363.13 kcal/100 g) reported by Lyimo, et al. (2007).

Table 4.15. Proximate composition (%) of Injera prepared from blended flours.

Blends [B]	pH [*]	Tem. °C	c. protein	c. fat	c. fibre	Ash	Mc	CHO	Energy, kcal/100 g
MTC 1	3.62 ± 0.00	21.70	8.67 ± 0.68	1.13 ± 0.16	4.08 ± 0.09	1.65 ± 0.01	7.03 ± 0.06	81.53 ± 0.47	370.91 ± 0.64
MTC 2	3.59 ± 0.01	22.10	8.63 ± 0.25	1.84 ± 0.04	3.87 ± 0.12	1.60 ± 0.02	7.35 ± 0.05	80.58 ± 0.44	373.38 ± 1.91
MTC 3	3.67 ± 0.01	21.90	7.73 ± 0.94	0.88 ± 0.11	3.86 ± 0.05	1.74 ± 0.01	8.08 ± 0.05	81.57 ± 0.86	365.15 ± 0.67
MTC 4	3.71 ± 0.02	21.80	6.70 ± 0.44	0.66 ± 0.03	3.90 ± 0.14	1.70 ± 0.01	7.70 ± 0.02	83.17 ± 0.33	365.38 ± 0.74
MTC 5	3.73 ± 0.00	21.70	6.37 ± 0.72	0.95 ± 0.07	3.66 ± 0.31	1.84 ± 0.01	6.99 ± 0.01	83.85 ± 0.76	369.43 ± 0.32
MTC 6	3.82 ± 0.01	22.10	6.53 ± 0.40	1.01 ± 0.18	3.96 ± 0.16	1.82 ± 0.01	7.51 ± 0.12	83.93 ± 1.33	367.71 ± 1.38
BTC 1	3.52 ± 0.01	21.20	9.53 ± 0.11	1.16 ± 0.07	4.30 ± 0.10	2.13 ± 0.01	8.91 ± 0.04	78.27 ± 0.13	361.64 ± 0.41
BTC 2	3.59 ± 0.01	22.00	9.10 ± 0.36	1.05 ± 0.06	4.65 ± 0.10	2.15 ± 0.01	8.58 ± 0.06	79.12 ± 0.35	362.30 ± 0.54
BTC 3	3.70 ± 0.01	21.80	7.87 ± 0.15	0.91 ± 0.06	5.07 ± 0.06	1.94 ± 0.05	8.95 ± 0.06	80.33 ± 0.15	360.96 ± 0.58
BTC 4	3.73 ± 0.01	22.10	6.80 ± 0.20	0.84 ± 0.17	4.52 ± 0.03	2.05 ± 0.01	8.88 ± 0.02	81.43 ± 0.06	360.48 ± 0.92
BTC 5	3.83 ± 0.00	22.11	8.23 ± 0.58	0.76 ± 0.14	3.96 ± 0.06	2.12 ± 0.02	9.04 ± 0.05	79.84 ± 0.52	359.12 ± 0.90
BTC 6	3.87 ± 0.02	21.60	6.37 ± 0.49	0.73 ± 0.13	4.60 ± 0.14	1.95 ± 0.01	8.80 ± 0.01	82.59 ± 0.60	362.44 ± 0.60
Tef (control)	3.38 ± 0.01	21.50	1.40 ± 0.03	0.33 ± 0.06	4.65 ± 0.27	1.51 ± 0.01	9.32 ± 0.20	86.75 ± 0.23	301.84 ± 0.75

[B] BTC1-6 / MTC1-6 (Maize/Bean: tef: cassava roots composite flour), MTC7-(whole tef/control), mean of triplicate value,

[*] pH fermented dough just before baking, Tem.-temperature, c-crude, CHO-carbohydrate, Mc-moisture content.

4.7.3 Mineral analysis of composite flour Injera

The mineral analysis of composite flour blend Injera (Table 4.16) shows that the Fe content of Bean-tef-cassava and Maize-tef-cassava ranged from 7.37 ± 0.46 to 9.13 ± 0.60, and 6.03 ± 0.06 to 7.28 ± 0.27 mg/100 g, respectively. The highest and lowest value were observed for BTC1 (9.13 ± 0.60), MTC1 (7.28 ± 0.27) and BTC2 (7.37 ± 0.46), MTC3 (6.03 ± 0.06), respectively. The Fe content of composite flour (BTC1- 4 and MTC1) Injera was significantly ($P<0.05$) higher than the control tef (6.76 ± 0.29 mg/100 g). The iron content of this study are indifferent to the previous report of fortified value for white (3.6 mg/100 g) and for brown (4.1 mg/100 g) wheat bread flour (Danster, et al., 2008), and cassava-tef blend Injera value (12.1 mg/100 g) (Kebede, et al., 2012).

The zinc content of Bean-tef-cassava and Maize-tef-cassava ranged from 1.31 ± 0.23 to 1.99 ± 0.11 and 0.95 ± 0.07 to 1.92 ± 0.06 mg/100 g, respectively. The highest and lowest value were observed for BTC1 (1.99 ± 0.11), MTC4 (1.92 ± 0.06) and BTC4 (1.31 ± 0.23), MTC2 (0.95 ± 0.07), respectively. Data in Table 4.16 revealed that the highest Zn content in composite flours was in BTC1 (1.99 ± 0.11) and MTC4 (1.92 ± 0.06) mg/100 g, and were significantly lower ($P<0.05$) than the control tef (3.42 ± 0.24). On the other hand the zinc content in composite flour Injera did not significantly ($P>0.05$) differ within each other. The other authors (Kebede, et al., 2012) reported similar results from cassava flour fortified composite flours Injera.

The Ca content in Injera made from Bean-tef-cassava and Maize-tef-cassava

flours ranged from 1.93 ± 0.07 to 3.88 ± 0.28 and 1.64 ± 0.45 to 2.53 ± 0.31 mg/100 g, respectively (Table 4.16). The highest and lowest ca values were observed in BTC4 (3.88 ± 0.28), MTC2 (2.53 ± 0.31) and BTC1 (1.93 ± 0.07), MTC1 (1.64 ± 0.45), respectively. The Ca content in composite flour Injera significantly ($P<0.05$) lower than the control (55.46 ± 1.01). The calcium content BTC4 (3.88 ± 0.28) is significantly ($P<0.05$) higher than other composite flour (BTC1-3, BTC5-6, MTC1-6) Injeras. The calcium content obtained is in line with the measured value of sorghum flour blend value (2.43 - 27.79 mg/100 g) reported by Awadalkareem, et al. (2008). However, Kebede, et al. (2012) reported that Injera made from tef-cassava blend was five times (275.4 mg/100 g) higher than the studied composite flour in Ca content.

The P content of Bean-tef-cassava and Maize-tef-cassava ranged from 10.27 ± 0.62 to 14.99 ± 0.10 and 9.96 ± 0.15 to 13.05 ± 0.66 mg/100 g, respectively. The highest and lowest value were observed for BTC4 (14.99 ± 0.10), MTC4 (13.05 ± 0.66) and BTC1 (10.27 ± 0.62), MTC1 (9.96 ± 0.15), respectively. The phosphorus contents in both BTC and MTC composite flour Injera were significantly ($P<0.05$) lower than control (99.58 ± 0.56). The phosphorous level in BTC4 (14.99 ± 0.10) was significantly ($P<0.05$) higher than in other composite flours (BTC1-3, BTC5-6, MTC1-6) Injera. The observed results were not in agreement with the previously reported results for tef and cassava blend Injera value of 22.1 mg/100 g (Kebede, et al., 2012); and blend of soya bean concentrate and sorghum value of 263.30 - 469.63 mg/100 g (Awadalkareem, et al., 2008).

Table 4.16. Minerals content for Injera prepared from different composite flour blends (mg/100 g).

	Minerals			
Blends	Fe	Zn	Ca	P
BTC1	9.13 ± 0.60	1.99 ± 0.11	1.93 ± 0.07	10.27 ± 0.62
BTC2	7.37 ± 0.46	1.69 ± 0.36	2.04 ± 0.06	12.57 ± 0.42
BTC3	7.51 ± 0.40	1.45 ± 0.03	2.68 ± 0.51	14.41 ± 0.38
BTC4	8.00 ± 0.11	1.31 ± 0.23	3.88 ± 0.28	14.99 ± 0.10
MTC1	7.28 ± 0.27	1.17 ± 0.07	1.64 ± 0.45	9.96 ± 0.15
MTC2	6.18 ± 0.33	0.95 ± 0.07	2.53 ± 0.31	11.55 ± 0.50
MTC3	6.03 ± 0.06	1.50 ± 0.07	1.91 ± 0.06	12.36 ± 0.55
MTC4	6.28 ± 0.62	1.92 ± 0.06	2.06 ± 0.07	13.05 ± 0.66
Tef (control)	6.76 ± 0.29	3.42 ± 0.24	55.46 ± 1.01	99.58 ± 0.56

Mean value ± SD, n=3, MTC= quality protein maize-tef-cassava,
BTC= common bean-tef-cassava composite flour and MTC7= whole tef (control).

4.8 Anti-nutritional factors in composite flour Injera

4.8.1 Phytate and tannin levels in composite flour Injera

The results of phytate and tannins in composite flour Injera are shown in (Figure 4.5). The phytate and tannin content ranged from 9.03 ± 0.04 to 16.03 ± 0.08 and 6.03 ± 0.06 to 68.08 ± 0.17 mg/100 g, respectively. Highest levels of tannin and phytate were found in Injera from the blend BTC1 (68.08 ± 0.17) and BTC4 (16.03 ± 0.08) mg/100 g, respectively. Lowest tannin and phytate values were obtained in blend MTC4 (6.03 ± 0.06) and MTC1 (9.03 ± 0.04) mg/100 g, respectively. Analysed data showed that the tannin content of BTC flour Injera was significantly ($P<0.05$) higher than for MTC. However, phytate content in the BTC Injera was slightly higher than the phytate value in the MTC

Injera. However, phytate and tannin content in both MTC and BTC Injera were significantly ($P<0.05$) lower than in the control (whole tef Injera) (Figure 4.5). The tannin content in BTC Injera was slightly higher than in MTC injera. These results are in agreement with the reported value (67.4-73.4 mg/100 g) for raw and fermented cassava flour blend foods reported by Oboh and Elusiyan (2007). The low levels of phytate content in Injera made from both BTC and MTC flours could possibly be attributed to the secretion of the enzyme phytase during fermentation. This enzyme is capable of hydrolyzing phytate thereby decreasing the phytate content of the food materials as reported by Oboh and Akindahunsi (2003); Irtwange and Achimba (2009). Furthermore, the decrease in phytate content in blended food product might be, partly, due to either the formation of insoluble complexes between phytate and other components, such as phytate-mineral or to the Inositol hexaphosphate hydrolyzed to penta- and tetra- phosphates and leached out when processed (Raules, et al., 1993). The decrease in phytate level by fermentation and boiling has been similarly observed in several plant food stuffs (Badifu, 2001). High content of phytate is tends to lower bioavailability of many essential dietary minerals in food (Siddhuraju and Becker, 2001). Therefore, reduction of phytate is expected to enhance the bioavailability of proteins and dietary minerals of the food. However, there is evidence that dietary phytate at low level may have beneficial role as an antioxidant, ant carcinogens and play an important role in controlling hypercholesterolemia and atherosclerosis (Slavin, 2004).

Results in Figure 4.5 show that tannin content of the MTC Injera (6.06 - 6.8 mg/100 g) was between 3.6 and 6.9 mg/100 g reported by Fasuyi (2005). Tannins affect nutritive value of food by forming a complex with protein there

by inhibiting protein digestion and absorption (Oboh and Elusiyan, 2007). Apart, from inhibiting effect it also impart dull colour on food stuffs and this affects the acceptance of the products, consistent with study results for BTC blend Injera. The tannin content of the BTC blend Injera was slightly lower than for the other cassava based products as reported by Oboh, et al. (2002); Oboh and Akindahunsi (2003). Tannin levels in the cassava composite flour injera were low compared with the tannin levels in leafy vegetables. Yetneberk (2005) reported that tannin free Injera could be produced with a 50:50 (w/w) composite flours from whole tannin-containing sorghum and tef.

In general it can be concluded that blending of the different cereal/legume to tuber crops followed by fermentation and baking could significantly reduce the anti-nutrients of that product.

4.8.2 Bioavailability of minerals (Fe, Zn & Ca) in Injera

Iron, zinc and calcium are essential minerals that are often lacking in human diets, either due to insufficient intake or due to malabsorption. Anti-nutrients are known to decrease the bioavailability of minerals and in particular Ca, Fe and Zn (Allen, 1982; Agte, et al., 1999; Oatway, et al., 2001).

Molar ratio of phytate to minerals (Fe, Zn & Ca) in Injera.

Results in Table 4.17 show molar ratios for phytate to Fe, Zn and Ca in composite flours Injera. The calculated molar ratio values of phytate to Fe for the bean-tef-cassava and maize-tef-cassava composite flours ranged from 0.12 ± 0.01 to 0.17 ± 0.00 and 0.10 ± 0.00 to 0.16 ± 0.01, respectively. The highest and lowest molar ratios values for phytate to iron were observed in MTC (MTC4=0.16 ± 0.01), BTC (BTC4=0.17 ± 0.00) and MTC (MTC1=0.10 ±

0.00), BTC (BTC1=0.12 ± 0.01), respectively.

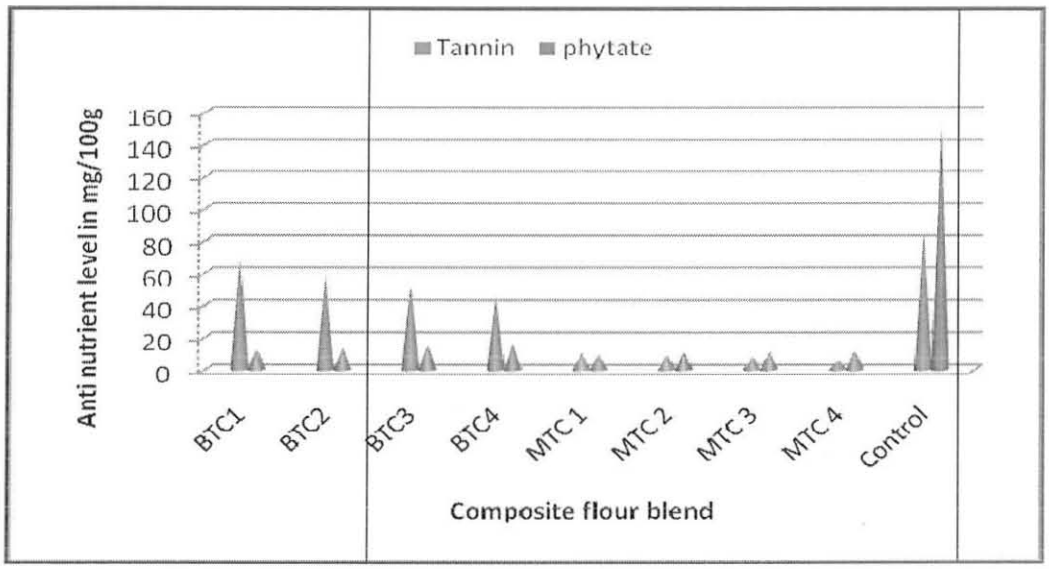


Figure 4.5. The tannin and phytate levels (mg/100g) in Injera from MTC and BTC blended flours. mean value (n=3), MTC/BTC = quality protein maize/ common bean-tef-cassava blend.

The absorption of non-haem iron is reported to be enhancing by vitamin c and inhibited by dietary factors such as phytic acid and polyphenolic compounds from tea and coffee (Hallberg, et al., 1989; Hurrell, et al., 1999). The molar ratios of phytate to iron in Table 4.17 are below the critical value of 0.40 necessary to affect bioavailability negatively (Hassen, et al., 2011). Therefore, the bioavailability of iron in all the composite flour Injera is not inhibited by phytate. Also the dietary iron intake is high and not likely to be major factor for causing iron deficiency anaemia. However, the bioavailability of iron in most foods of developing countries, is inhibited by phytate, which may play an important role in the anaemia problem (Taylor, et al., 1995).

The molar ratios of phytate to calcium values for the bean-tef-cassava and maize-tef-cassava Injera were found to be in the range of 0.25 ± 0.02 to 0.40 ±

0.01 and 0.25 ± 0.04 to 0.36 ± 0.12, respectively. The highest and lowest values were observed in BTC1 (0.40 ± 0.01), MTC1 (0.36 ± 0.12), BTC4 (0.25 ± 0.02) and MTC2 (0.25 ± 0.04), respectively. Both BTC and MTC phytate: ca molar ratios were significantly higher ($P<0.05$) than the control (MTC7) Injeras. The predicted bioavailability of calcium in Table 4.17 indicate that the phytate to calcium molar ratios in all the composite flour Injera were below the critical level of 0.5 (Anyum, et al., 2002; Hassan, et al., 2011). Therefore, the phytate levels in the cassava composite flour injeras are not likely to hinder Ca absorption.

There is no significant difference ($P>0.05$) in zinc bioavailability among the composite flour Injera. Zinc absorption is reported to be significantly affected when dietary phytate to zinc molar ratio is greater than critical level of 1.5 (Hassan, et al., 2007; Mitchikpe, et al., 2008). However, the reported phytate to zinc molar ratios in this study were as low as 10:1 and 15:1, but able to induce marginal zinc deficiency in mice, with significantly reduced plasma and hair zinc concentration as reported by Davies, et al., 1985.

The molar ratio values of [Phy.] x [Ca]: Zn were found to be in the range of 0.03 ± 0.00 to 0.13 ± 0.04 and 0.03 ± 0.01 to 0.07 ± 0.01 for Injera made from bean-tef-cassava and maize-tef-cassava composite flours, respectively. The highest and lowest values were observed in BTC4 (0.13 ± 0.04), MTC2 (0.07 ± 0.01) and BTC1 (0.03 ± 0.00), MTC1 (0.03 ± 0.01) composite flours, respectively. The molar ratios of [Phy] x [Ca]: Zn for the experimental composite flour Injera significantly ($P<0.05$) lower than the control (MTC7). The calculated calcium x phytate to zinc molar ratio was found to be below critical

value of 0.50 (Table 4.17), which is found to be better in zinc bioavailability than phytate to zinc ratio (Bindra, et al., 1986; Singh, S. et al., 2008; Obah and Amusan, 2009).

In the present study, observations indicates that is no impairment of iron, calcium and zinc in the composite flour food by phytate. However, the flour had a devoid of phytate which is used as an anti carcinogen that protects against colon cancer and it is known to be a potent antioxidant that inhibits Fenton reactions leading to lipid per oxidation and inhibition of polyphenols oxidase (Agte, et al., 1999).

4.9 Biological assays of composite flour blends

In this study eight samples (BTC and MTC each four) of test diets, a basal (N-free) diet as negative and a reference (casein) diet as positive control were further involved on the laboratory animals experiment in biological evaluation such as body growth, nutritional evaluation, protein efficiency ratio as well as biological value. They were selected to include both quality protein maize-tef-cassava (MTC1-4) and common bean-tef-cassava composite (BTC1-4) flour blend Injera having organoloptically overall acceptability on average above 4 panellists score points (see above Table 4.13 & Table 4.14).

4.9.1 Biological evaluation of formulated diets

Growth performance of mice fed on test diets (body mass & length)

The growth performance data obtained from mice fed on experimental diets is shown in Table 4.18. The biological evaluation of enhanced cassava blend composite flour showed that the group of mice maintained on the protein free control (basal) diet consumed second to the least quantity of feed when

compared with the other groups of mice kept on the experimental diets and standard casein diets (Dc). The mice on N-free (Dst) diets appeared to have lost some/erected of their furs and looked abnormal. In contrast to mice kept on the other test diets showed none of these symptoms. They looked normal, healthy and appeared agile. There were no obvious physical differences between mice kept on the reference casein (Dc) diets and those kept on the test diets. The lower feed intake of this group of mice somehow correlated with their apparent growth failure, if weight gain is used as a measure of growth. Even though, the highest weight gain was recorded for the group of mice fed on diet Dm4 and the amount of feed taken was not the highest quantity. This might be due to the quality of protein or adaptability of the diets. The higher weight gain was recorded for group of mice fed on quality protein maize blend diets, than mice fed on common bean blend test diets. This is due to the amount of feed consumed by group of mice was also higher for maize blended test diets than common bean blend group of mice test diets. The group of mice length growth rate were observed as the similar pattern as the weight gained group of mice consumed the same respective test diets (Figure 4.6).

The body weight/length gain of the mice depending on the quantity of protein quality (i.e. essential amino acids) exiting in the test diets. The feed intake and weight gain in this group was slightly closer to that observed for mice fed on the reference casein diet (Dc). The group of mice-consumed diet DB3 is found to be the least quantity of feed among the experimental diets. In this case, it seems the lower feed intake also reflected in their lower length and weight gain when compared to other experimental mice fed on test diets. The reduced feed intake by the mice could be attributed to a number of factors, which may

include a deficiency and/or imbalance of amino acids and other nutrients, adverse substances in the protein source, or reduced palatability of the diets. Jacquot and Peret (1972) reported that the nature of proteins included in diets influences the appetite of mice, and may cause significant reductions in feed intakes during *ad libitum* feeding. This reduction of feed intake indicates a reduction in the intake of other essential dietary components such as carbohydrates as it is reported by Rao and Desothale (1982). In addition, Passmore, et al. (1974) reported that if the total energy intake is inadequate, some dietary protein is used for energy, and is therefore not available to satisfy protein needs in case of basal diet fed mice (Dst). Thus, further increases in protein intake is of limited effectiveness and wasteful if energy needs are not being satisfied at the same time. Result Table 4.18 shows the body weight gain of test diets fed mice were found to be significantly ($P<0.05$) lower than the reference diet fed mice (Dc). However, the body mass gain were observed for group of mice fed on test diets Dm1 and Dm4 were not significantly different ($P>0.05$) from the mice fed on reference diet (Dc). The highest body weight gain ($P<0.05$) was recorded in the mice fed on cassava enhanced with quality protein maize test diets (Dm1 and Dm4) followed by mice fed on Dm2 and Dm3, and then DB1, DB2, DB3 and DB4. The least body weight gain was recorded for mice fed on the N-free diets (Dst). The reference diet (Dc) were measured 11.5 % increase in the body mass gain over the best composite flour based test diet (Dm4) fed mice. There was increment in body mass per 7 days in the entire mice fed on test diets Dm1-4 and DB1-4 (see Figure 4.6). The body growth performance of mice fed on test diets is significantly ($P<0.05$) lower than that of mice fed on reference diet ($Dc=8.10 \pm 0.48$).

Table 4.17. Phytate to minerals molar ratio for Injera prepared from composite flours (BTC and MTC) [b].

Blends	Phytate (mg/100 g)	Phytate: Fe [c]	Phytate: Zn[Ψ]	Phytate: Ca [Δ]	[Phy.]x[Ca]: Zn (Mol/Kg) [ω]
BTC1	12.55 ± 0.40	0.12 ± 0.01	0.61 ± 0.02	0.40 ± 0.01	0.03 ± 0.00
BTC2	13.27 ± 0.30	0.16 ± 0.01	0.80 ± 0.17	0.40 ± 0.01	0.04 ± 0.01
BTC3	14.02 ± 0.60	0.16 ± 0.01	0.95 ± 0.02	0.32 ± 0.06	0.06 ± 0.01
BTC4	16.03 ± 0.08	0.17 ± 0.00	1.36 ± 0.30	0.25 ± 0.02	0.13 ± 0.04
MTC1	9.03 ± 0.04	0.10 ± 0.00	0.76 ± 0.05	0.36 ± 0.12	0.03 ± 0.01
MTC2	10.24 ± 0.60	0.14 ± 0.00	1.07 ± 0.07	0.25 ± 0.04	0.07 ± 0.01
MTC3	11.06 ± 0.06	0.16 ± 0.00	0.90 ± 0.17	0.35 ± 0.01	0.04 ± 0.01
MTC4	11.78 ± 0.40	0.16 ± 0.01	0.60 ± 0.02	0.35 ± 0.02	0.03 ± 0.00
Tef (control)	154.00 ± 0.14	1.93 ± 0.08	4.45 ± 0.31	0.17 ± 0.00	6.16 ± 0.32
Cv	-	0.40	1.50	0.50	0.50

[b] = results were mean value ± SD, n=3, [c] = (mg of Phytate/MW of Phytate: mg of iron/MW of iron,
[Ψ] = mg of Phytate/MW of Phytate: mg of Zinc/MW of Zinc, [Δ] = mg of Phytate/MW of Phytate: mg of Calcium/MW of Calcium,
[ω] = (mol/kg Calcium) x (mol/kg Phytate)/ (mol/kg Zinc) and Cv = Critical values were sourced from Hassan, et al. (2011).

However, there was a larger significant ($P<0.05$) difference between the mice fed on the test diets and that of mice fed on the N-free (Dst) diets, which results inferior growth rate. Out of the test diets fed mice the highest body length gained ($P<0.05$) was recorded for the mice fed on cassava enhanced diets of quality protein maize (Dm1, Dm2, Dm3 and Dm4) followed by common bean composite diets (DB1, DB2, DB3 & DB4). While the least body length gain was observed for mice fed on N-free diets (Dst). The N-free diets (Dst) fed mice body weight gained was found to be significantly ($P<0.05$) lower than test diets fed mice. Feed intake and average protein intake were significantly ($P<0.05$) influenced by dietary treatment, with the same trend as average weight gain. Weight loss were observed on the group of mice fed on N-free diet (Dst), this is due to basal diets lacking protein. This is in line with reported literature (Sasaki, et al., 1982; Bronson, 1985; Cameron and Eshelman, 1996) which was stated that the deficiencies in dietary protein slow growth and delays maturation. Diet composition clearly influences both growth rates and maturation the high weight gain in the group fed with these test and standard diets can be attributed to either of dietary protein content or quality or both (McAdam and Miller, 1990).

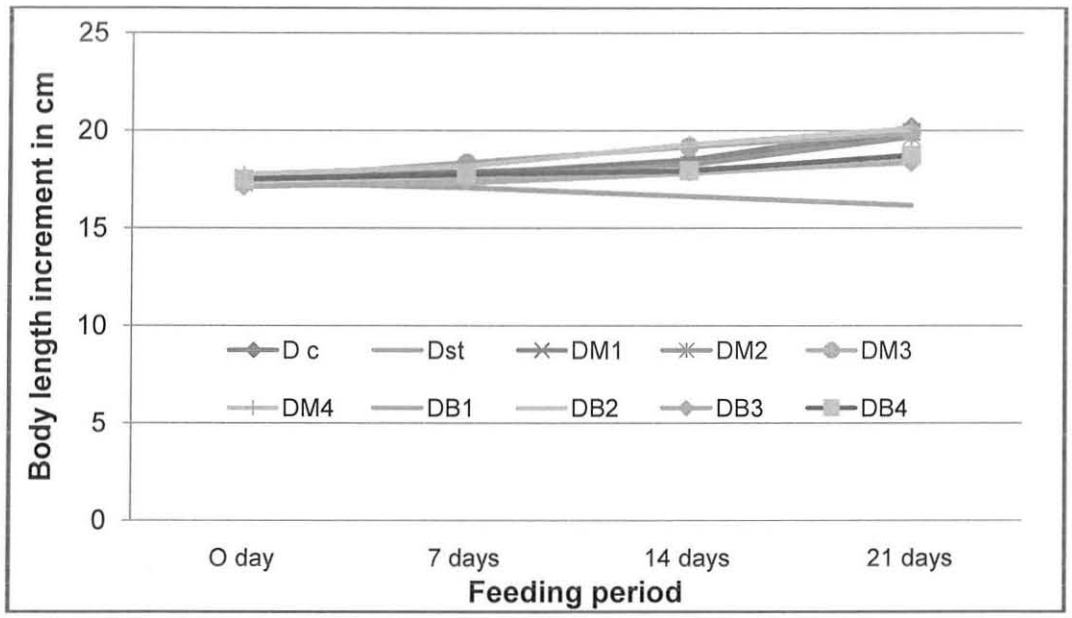
Result (Table 4.18) showed that the protein efficiency ratio (PER) value for group of mice fed on diet Dm4 was significantly ($P<0.05$) higher than the value obtained for the reference diet (Dc), while all the others were a little lower, except for the group of mice fed on N-free diet (Dst). The PER value of mice having highest significant difference ($P<0.05$) was recorded for the mice fed on test diets (Dm3 = 0.94 and Dm4 = 1.08) followed by mice fed on standard casein (Dc), DB1-4 and Dm1-2. The maximum values of PER at

lower protein concentrations for good quality proteins than for poor quality proteins. On this experiment, result is in agreement with the earlier finding of insect as a source of protein qualities, on the nutritional qualities of food products produced from cereal and legume combination (Ikujenlola and Fashakin, 2005; Ijarotimi and Olopade, 2009). The group of mice fed with N-free diet (Dst) recorded the lowest PER values which also corresponds with their lower feed intake. There was no significant difference ($P>0.05$) between the feed efficiency ratio (FER) of mice fed on the test diets (Dm1-4 and DB1-4) and that of mice fed on standard casein (Dc). While, there was a significant difference ($P<0.05$) observed between FER of mice fed on the test diet and that of mice fed with N-free diet (Dst = 0.00), which resulted in inferior feed efficiency ratio.

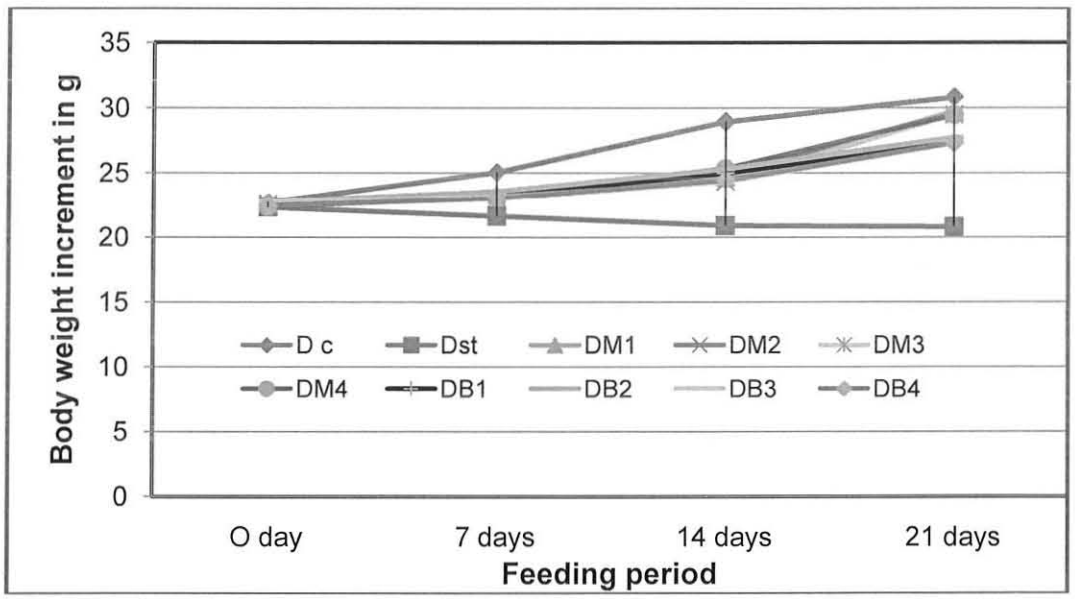
Table 4.18. Average body growth performance of mice on test diets (average value fed, 21 days) [FER and PER].

Parameters	Reference*		Test diets*							
	Dc	Dst	Dm1	Dm2	Dm3	Dm4	DB1	DB2	DB3	DB4
Length/mice										
initial, cm	17.67 ± 0.43	17.30 ± 0.10	17.56 ± 0.41	17.33 ± 0.29	17.50 ± 0.50	17.73 ± 0.46	17.12 ± 0.40	17.20 ± 0.10	17.10 ± 0.10	17.50 ± 0.30
final, cm	20.17 ± 0.32	16.16 ± 0.05	19.92 ± 0.07	19.66 ± 0.29	19.88 ± 0.10	20.08 ± 0.13	18.44 ± 0.49	18.53 ± 0.41	18.34 ± 0.25	18.72 ± 0.47
gained, cm	2.50 ± 0.30	- 1.14 ± 0.12	2.36 ± 0.46	2.33 ± 0.20	2.38 ± 0.41	2.35 ± 0.49	1.32 ± 0.60	1.33 ± 0.40	1.24 ± 0.26	1.22 ± 0.33
Weight/mice										
initial, g	22.73 ± 0.53	22.49 ± 0.31	22.70 ± 0.47	22.52 ± 0.42	22.66 ± 0.41	22.38 ± 0.52	22.71 ± 0.46	22.77 ± 0.59	22.77 ± 0.57	22.47 ± 0.46
final, g	30.83 ± 0.33	20.41 ± 0.32	29.72 ± 0.36	29.47 ± 0.30	29.53 ± 1.01	29.47 ± 1.39	27.43 ± 0.34	27.75 ± 0.34	27.35 ± 0.26	27.33 ± 0.55
gained, g	8.10 ± 0.48	- 0.08 ± 0.07	7.02 ± 0.80	6.95 ± 0.22	6.87 ± 0.86	7.09 ± 1.26	4.72 ± 0.33	4.98 ± 0.32	4.58 ± 0.41	4.86 ± 0.23
FI, g/mice	79.66	76.8	80.16	79.51	80.26	73.97	79.61	78.84	72.87	79.53
$FER	0.10 ± 0.00	0.05 ± 0.03	0.05 ± 0.03	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.00
$PER	0.87 ± 0.00b	- 0.38 ± 0.11a	0.85 ± 0.47b	0.87± 0.11b	0.94 ± 0.48b	1.08 ± 0.43b	0.55 ± 0.25ab	0.12 ± 0.11a	0.67 ± 0.13at	0.65 ± 0.34ab

Means in the same row with the same superscripts are not significantly different with a<b<c<d (P>0.05). N = 3
* Dm_1, Dm_2, Dm_3, Dm_4 (Maize: Tef: Cassava (MTC_{1-4}): 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively),
* DB1, DB2, DB3, DB4 (Bean: Tef: Cassava (BTC_{1-4}): 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively),
* Dc - diet casein, Dst- diet starch, FI-feed intake,
$ FER-feed efficiency ratio, PER-protein efficiency ratio.



A.



B.

Figure 4.6. Body weight and length gain by mice fed with test, N-free (basal) and standard (casein) diets over 21 days. (A= length; B= weight). N = 3

Dm_1 Dm_2, Dm_3 and Dm_4 (Maize: Tef: Cassava (MTC1-4): diets corresponds to ratio 1:8:1, 1:7:2, 1:6:3 and 1:5:4 respectively); DB1, DB2, DB3 and DB4 (Bean: Tef: Cassava (BTC1-4): diets corresponds to ratio 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively); Dc & Dst corresponds to diet casein & N-free, respectively.

4.9.2 Internal organ weights of mice

The result (Table 4.19, Figure 4.7) shows the ratio of internal organ to live body weight inbreed Swiss albino mice consumed test diets for 21 days. The relative ratio weight of liver having highest significant difference ($P<0.05$) was observed for the mice fed on reference casein diet (Dc) followed by mice fed Dm2, Dm3 and Dm4, then followed by mice on fed Dm1, DB3, DB1 and the least were recorded for DB4 and DB2 which was observed as having similar value with the mice fed on N-free diet (Dst). The highest spleen ratio were observed for the mice fed on test diets Dm1 and Dm2 which has no significant difference ($P>0.05$) with mice fed on standard diet (Dc) then followed by the mice fed on Dm3, Dm4, DB1, DB3 and DB4 the least value was observed for mice fed on DB2 which was recorded as similar value with mice fed on diet N-free (Dst). Whereas, the internal organ ratio of kidney weight showed significant ($P<0.05$) increment in mice fed on test diets Dm1, Dm2, Dm3 and DB1 which has the same value as mice fed on reference casein diet (Dc), followed by Dm4 then by DB3-4 the least value was found for DB2 which was the same as mice fed on N-free diet (Dst). The highest internal organ lung weight was found to be for mice fed on diets Dm1-4 and DB4, which had similar weight ratio value with mice fed on standard casein diet (Dc). The mice fed on N-free diet (Dst) significantly higher than mice fed on DB2 which is the least value of all mice fed on test diets. The relative ratio of heart weight value was found to be significantly ($P<0.05$) highest for the mice fed on Dm1-3 and DB3-4 which were the same as mice fed on control diets (Dc) followed by Dm4, DB1. The relative ratio of heart weight of mice fed on N-free diet (Dst) is significantly higher than the mice fed on test diet (DB2). The relative ratio of

pancreas weight value of the mice fed on reference diets (Dc) was significantly ($P<0.05$) different from all other relative ratio of pancreas weight values and this was the highest recorded value (0.010 ± 0.00). Whereas the least relative value of pancreas was recorded for mice fed on the test diets Dm2 and DB4, which had similar value. However, these values are significantly ($P<0.05$) lower than mice fed on N-free diet (Dst). The relative ratio of pancreas values for mice fed on test diets Dm3, Dm1, and DB3 are not significantly different ($P>0.05$) to each other. Similarly, the relative ratio of pancreas values for mice fed on test diets Dm4 and DB1 are not significantly different ($P>0.05$) from each other.

The organs of the mice placed on the protein free diet (Dst) were pale in appearance and diminished in size, as compared with those of the other mice placed on the standard casein and test diets. The liver and kidney of the animals placed on the reference casein diets were brick red in colour with no lesions on them or any sign of fatty liver. Whereas, that of the animals placed on the various test diets showed a fine brick red colouration, darker than that observed for the animals on the casein diet. The result of organ weight might indicate that there was corresponding increase or decrease in the organ weights with increased or decreased quality protein intake. In particular, the liver weight of the different group of mice fed on the various diets slightly correlated with the level and quality of the diets protein intake. The mice fed on reference casein diet (Dc) recorded the highest feed intake and the highest relative liver weight (Dc, 0.064 ± 0.00 g) closely following the mice fed on test diets (Dm3 and Dm4).

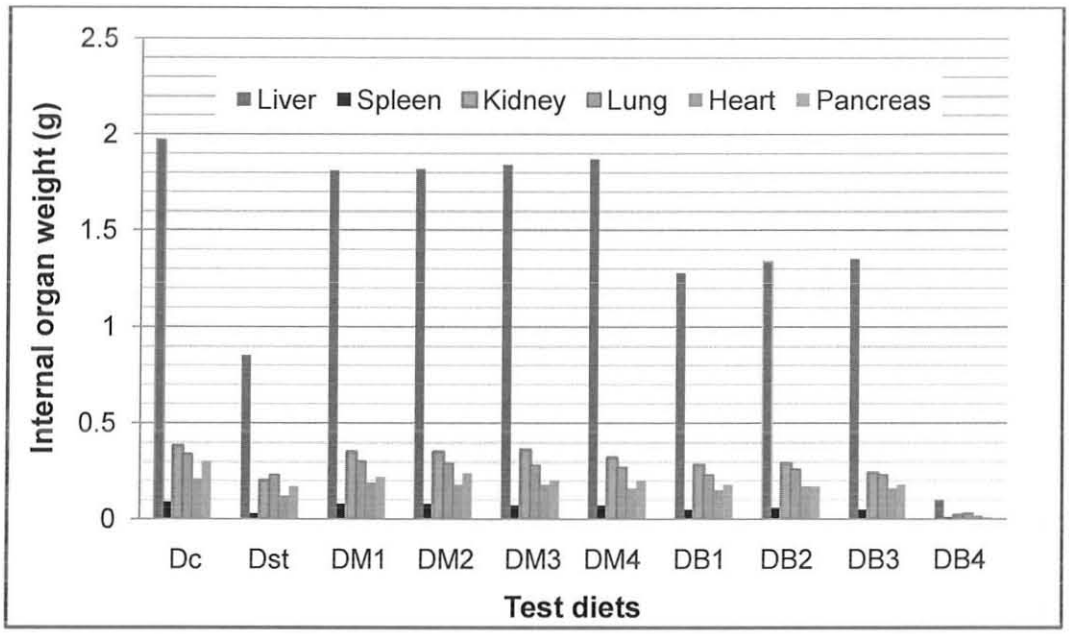


Figure 4.7. Average internal organ weight (g) of Swiss albino mice fed on experimental diets for 21 days. N=3

4.9.3 Nutritional evaluation of formulated diets

The result Table 4.20 shows the mice kept on the protein free diets (Dst) showed signs of weakness at the end of feeding period. The N-balance indices found to be for mice fed on reference diet (Dc) were significantly higher ($P<0.05$) than the mice fed on the test diets. While the least value was found to be for mice fed on the N-free diet (Dst). The consumed feed nitrogen value of the mice fed on the reference diet (Dc) is significantly different ($P<0.05$) from all other mice fed on the test diets and this is the highest (Dc, 0.75 ± 0.01) value of all others. The consumed feed nitrogen values for mice fed on maize diets Dm2 and Dm3 were not significantly different ($P>0.05$) from each other.

Table 4.19. Relative internal organ weights of mice fed on test, standard and basal diets for 21 days.

	Internal organs					
*Diets	Liver	Spleen	Kidney	Lung	Heart	Pancreas
Dc	0.064 ± 0.00de	0.003 ± 0.00c	0.012 ± 0.00c	0.011 ± 0.00b	0.007 ± 0.00b	0.010 ± 0.00d
Dst.	0.040 ± 0.00a	0.001 ± 0.00a	0.009 ± 0.00a	0.011 ± 0.00b	0.005 ± 0.00ab	0.008 ± 0.00c
Dm_1	0.061 ± 0.00bcde	0.003 ± 0.00c	0.012 ± 0.00c	0.010 ± 0.00b	0.006 ± 0.00b	0.007 ± 0.00bc
Dm_2	0.062 ± 0.00cde	0.003 ± 0.00c	0.012 ± 0.00c	0.010 ± 0.00b	0.006 ± 0.00b	0.008 ± 0.00c
Dm_3	0.063 ± 0.00cde	0.002 ± 0.00b	0.012 ± 0.00c	0.009 ± 0.00b	0.006 ± 0.00b	0.007 ± 0.00bc
Dm_4	0.063 ± 0.00cde	0.002 ± 0.00b	0.011 ± 0.00bc	0.009 ± 0.00b	0.005 ± 0.00ab	0.006 ± 0.00b
DB1	0.047 ± 0.00abc	0.002 ± 0.00b	0.010 ± 0.00b	0.008 ± 0.00ab	0.005 ± 0.00ab	0.006 ± 0.00b
DB2	0.032 ± 0.03a	0.001 ± 0.00a	0.007 ± 0.01a	0.006 ± 0.00a	0.004 ± 0.00a	0.004 ± 0.00a
DB3	0.049 ± 0.00abcd	0.002 ± 0.00b	0.009 ± 0.00b	0.008 ± 0.00ab	0.006 ± 0.00b	0.007 ± 0.00bc
DB4	0.039 ± 0.01a	0.002 ± 0.00b	0.009 ± 0.00b	0.010 ± 0.00b	0.006 ± 0.00b	0.005 ± 0.00a

Means in the same column with the same superscripts are not significantly different with a<b<c<d<e (P>0.05), N = 3

* Dm_1, Dm_2, Dm_3, Dm_4 (Maize: Tef: Cassava: 1:8:1, 1:7:2, 1:6:3 and 1:5:4 respectively),

DB1, DB2, DB3, DB4 (Bean: Tef: Cassava: BTC_{1-4}; 1:8:1, 1:7:2, 1:6:3 and 1:5:4 respectively),

Dc & Dst (Diet of casein & starch respectively).

Similarly, the consumed feed nitrogen value for mice fed on test diets DB3 and DB4 were not significantly different (P>0.05) from each others. The consumed nitrogen value of the mice fed on the diets Dm1, Dm4, DB1 and DB2 were observed to be significantly different (P<0.05) from each other's. Whereas, the nitrogen value for the mice fed on N-free diet (Dst) was significantly the lowest of all others (P< 0.05). There is significant difference in the values of nitrogen-balance of the mice fed on the reference casein-based diet (Dc) (P<0.05), which were followed by test diet Dm4 (Table 4.20). However, Nwabueze (2008) reported that low urinary and faecal nitrogen indicates high protein quality of fed diets. The result Table 4.21 shows the percentage biological value (BV) of the mice fed on test diet (DB3) was significantly (P<0.05) different from all other mice fed on the test diets.

Table 4.20. Utilization values for experimental diets by mice.

	Nutritional values			
*Diets	Faecal-N	Urine-N	N-intake	N-balance
Dc	0.33 ± 0.00h	0.15 ± 0.00e	0.75 ± 0.01i	0.27 ± 0.00i
Dst	0.23 ± 0.00a	0.06 ± 0.00b	0.00 ± 0.00a	-0.29 ± 0.00a
Dm_1	0.31 ± 0.00f	0.15 ± 0.00e	0.59 ± 0.01f	0.13 ± 0.00c
Dm_2	0.26 ± 0.00c	0.20 ± 0.00f	0.57 ± 0.00e	0.11 ± 0.00c
Dm_3	0.33 ± 0.00i	0.09 ± 0.00c	0.57 ± 0.00e	0.15 ± 0.00e
Dm_4	0.28 ± 0.00d	0.06 ± 0.00b	0.53 ± 0.00c	0.19 ± 0.00g
DB1	0.35 ± 0.00j	0.10 ± 0.00cd	0.60 ± 0.01f	0.15 ± 0.00ef
DB2	0.38 ± 0.00k	0.09 ± 0.01c	0.63 ± 0.00g	0.16 ± 0.00f
DB3	0.39 ± 0.01L	0.02 ± 0.00a	0.56 ± 0.00d	0.15 ± 0.01ef
DB4	0.32 ± 0.00g	0.10 ± 0.00d	0.56 ± 0.01d	0.14 ± 0.00d

Means in the same column with the same superscripts are not significantly different with a<b<c<d<e<f<g<h<i<j<k<L (P>0.05); *Dm_1, Dm_2, Dm_3 & Dm_4 (Maize: Tef: Cassava (MTC_{1-4}): 1:8:1, 1:7:2, 1:6:3 & 1:5:4, respectively); *DB1, DB2, DB3 & DB4 (Bean: Tef: Cassava (BTC_{1-4}):1:8:1, 1:7:2, 1:6:3 & 1:5:4, respectively); *Dc-standard casein diet & Dst- starch diet; N-nitrogen. N = 3

Table 4.21. Nutritional performance by experimental diets in mice.

Diets	Percentage nutritional values				
	NPU	BV	TD	ADP	NPR
Dc	75.14 ± 0.26d	86.00 ± 0.00d	87.30 ± 0.00g	35.77 ± 0.25g	65.00 ± 0.17b
Dst	NDa	NDa	NDa	NDa	NDa
Dm_1	71.15 ± 0.38b	82.00 ± 0.01c	86.67 ± 0.00g	21.03 ± 0.35c	62.00 ± 0.23b
Dm_2	71.86 ± 0.62b	76.00 ± 0.00b	95.23 ± 0.00i	20.20 ± 0.06c	64.00 ± 0.27b
Dm_3	77.86 ± 0.22e	94.00 ± 0.00g	83.00 ± 0.00e	26.10 ± 0.20de	69.00 ± 0.22c
Dm_4	91.64 ± 0.17h	96.1 ± 0.00h	91.43 ± 0.00h	36.40 ± 0.20g	79.00 ± 0.31c
DB1	74.56 ± 0.73d	93.00 ± 0.01fg	80.10 ± 0.00d	25.40 ± 0.70d	38.00 ± 0.25ab
DB2	72.63 ± 2.19cd	94.00 ± 0.02g	76.90 ± 0.01c	25.60 ± 2.20d	42.00 ± 0.23ab
DB3	79.82 ± 1.11f	98.11 ± 0.00i	72.10 ± 0.01b	27.43 ± 1.09e	43.00 ± 0.27ab
DB4	77.38 ± 0.09e	92.00 ± 0.00ef	84.20 ± 0.00f	24.90 ± 0.10d	46.00 ± 0.28ab

Means in the same column with the same superscripts are not significantly different with a<b<c<d<e<f<g<h<i (P>0.05); *Dm_1, Dm_2, Dm_3 & Dm_4 (Maize: Tef: Cassava (MTC_{1-4}): 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively); *DB1, DB2, DB3 & DB4 (Bean: Tef: Cassava (BTC_{1-4}): 1:8:1, 1:7:2, 1:6:3 and 1:5:4, respectively); *Dc & Dst (Diet casein and starch, respectively); NPU- Net protein utilization; BV- Biological value; TD - True digestibility and ADP- Apparent digested protein; ND - Not detected; NPR - Net protein ratio. N = 3

The BV of mice fed on test diet DB3 (98.11 ± 0.00) was the highest value than the mice fed on the test and reference casein diet (Dc). Whereas, the BV values for the mice fed on diets Dm3, DB1 and DB2 were not significantly different (P>0.05) from each other's. The BV values for mice fed on the test diets Dm3-4, DB1, DB2 & DB4 was significantly (P<0.05) different to each other's and these

BV values were higher than for those mice fed on the reference diet (Dc, 86.00). However, the BV value for the mice fed on the test diets Dm1 and Dm2 were significantly different ($P<0.05$) from each other and their values were lower than for those mice fed on the reference casein diets (Dc). While the mean BV values for mice fed on the N-free diets (Dst) was significantly lowest of all others.

The lowest BV was observed for mice fed on test diet Dm2 (76 %) which was next to mice fed on N-free diet (Dst). Biological value of a protein used as a measure of protein quality which is not related to digestibility of food protein. Furthermore, biological value (BV) measures the efficiency of utilization of absorbed nitrogen. Not all the proteins enter the body absorbed by intestine. The high BV for test diets supports the general view that the maximal utilization of good quality proteins occurs at lower levels of protein intake.

The percentage net protein utilization (NPU) value of the mice fed on the test diets (Dm4) was significantly ($P<0.05$) different from all other NPU values. The mice fed on test diet Dm4 (91.64 ± 0.17) was observed as significantly ($P<0.05$) higher value than the mice fed on reference diet (Dc). The NPU values for mice fed on diets Dm1 and Dm2 were not significantly different ($P>0.05$) from each other's. The NPU values for mice fed on the diet DB1 and reference diet (Dc=75.11±0.26) were not significantly different ($P>0.05$) from each other's. Similarly, the values for mice fed on diet Dm3 and DB4 were not significantly different ($P>0.05$) from each other's. The NPU value for mice fed on the diets DB3, DB4, Dm3 & Dm4 is significantly higher than the mice fed on standard diets

(Dc) ($P<0.05$). Whereas, the mean NPU values for mice fed on diets Dst was lowest of all others. The NPU is also another way of measuring the protein quality that takes into account also the digestibility of protein (Gaman and Sherrington, 1992). The highest quality proteins were denoted 100 %, for the value of BV and NPU (Ijarotimi and Bakare, 2006). There were significant differences in the BV and NPU values among different test and casein based diets ($P<0.05$). The value obtained for BV and NPU ranged from 76.00 - 98.11, and 71.15 - 91.64 %, respectively. The value suggested that the blend were of moderate protein quality.

The result Table 4.21 shows the true digestibility (TD) for all the diets ranged from 72.10 to 95.23 %. The percentage true digestibility (TD) value of the mice fed on diets (Dm4, 91.43 ± 0.01) and (Dm2, 95.23 ± 0.00) were significantly ($P<0.05$) higher than mice fed on the reference casein (Dc). However, the TD values for mice fed on the Dm1 and reference casein diet (Dc, 87.30 %) were not significantly different ($P>0.05$). The TD value for the mice fed on the diets DB1-4 and Dm3 were significantly different ($P<0.05$) from each other. These TD value were lower than the mice fed on the reference diets (Dc, 87.30 ± 0.00).

The mean TD values for the mice fed on the N-free diets (Dst) were observed to be least of all others. High digestibility does not always mean high protein quality. Digestibility is a measure of protein hydrolysis, whereas protein quality is a measure of the balance of acids that are absorbed and utilized for growth and other purposes (Friedman and Cuq, 1988; Ijarotimi and Bakare, 2006).

The high digestibility of the test diet may be due to the low levels of anti nutrients (tannin) found in the blend, since dietary tannins are often responsible for the poor digestibility of dietary proteins (Liener, 1980).

Result obtained for the apparent digestibility of protein (ADP) was highest for the diet Dm4 (36.40%), however, there was no significant difference ($P>0.05$) from reference casein diet (Dc). The percentage apparent digestible protein (ADP) value of the mice fed on the diets (Dc, 35.77 ± 0.25) and (Dm4, 36.40 ± 0.20) were not significantly ($P>0.05$) different from each other. The ADP values of the mice fed on the reference diets (Dc, 35.77±0.25) was significantly ($P<0.05$) higher than all others mice fed on the test diets. The ADP values for mice fed on the test diets Dm1 and Dm2 were not significantly different ($P>0.05$) to each other's. In addition, the ADP values for the mice fed on the diets DB1, DB2 and DB4 were not significantly different ($P>0.05$) from each other's. The ADP value for the mice fed on test diets Dm3 and DB3 were found to be significantly different ($P<0.05$) from each other's. The mean ADP values for mice fed on the N-free diets (Dst) were observed to be least of all others.

The percentage net protein ratio (NPR) value of mice fed on the reference casein diet (Dc= 65 ± 0.17) is significantly ($P<0.05$) higher than mice fed on test diets Dm1-2 and DB1-4. While NPR value of mice fed on the test diets Dm3-4 were significantly ($P<0.05$) higher than mice fed on reference diet (Dc). The mean NPR values for mice fed on the N-free diets (Dst) were least of all others.

The NPR value of mice fed on the test diets Dm1 and Dm2 are not significantly

(P>0.05) different from mice fed on reference diets (Dc=65). Similarly, the NPR value of mice fed on the test diets DB1, DB2, DB3 and DB4 are not significantly (P>0.05) different to each other. This study food product Injeras were observed well in its nutrient density. These were confirmed with mice fed on the composite flour diets body mass, length gained. Therefore, it indicates the availability of body maintaining nutrients in blended food products Injera.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this study, the following conclusions and recommendations can be made:

5.1 CONCLUSIONS

✓ This research has demonstrated the possibility of producing better nutritional products from cassava roots flour, which may adequately supply some of the nutrients in composite meals, rather than by using fresh bulky roots, which make processing operations inflexible and often logistically precarious.

✓ The studied cultivars were significantly varied in nutritional composition; this is due to genetic, environmental, soil pH variation.

✓ Out of the three common processing methods fermentation of the cassava roots flour sufficiently reduces toxic substance (HCN) contents to safe level of human consumption (<10 ppm, FAO) than other methods.

✓ All processing methods enhance the availability of nutrients in cassava by decreasing the anti-nutritional factors including cyanide, phytate, tannin and oxalate. Among the anti-nutritional factors analyzed, the low content of tannins in the three processing techniques of the seven cassava root cultivars is one good advantage for consumers of cassava in terms of inhibitory effect of tannin on nutrient availability and digestibility of protein.

✓ Out of the seven cultivars the crude protein and crude fibre content of cultivar with accession number 28 was significantly ($P<0.05$) higher than others.

✓ The three common processing techniques of cassava roots results in

substantial loss of vitamin c and ß-carotene levels.

✓ The panellist score point for Injera baked with BTC1 and BTC3 composite flour to be rated higher than the control (whole tef) particularly in taste, flavour, front/eyes and back side appearance and overall acceptability.

✓ The result suggested that common bean/quality protein maize (10 %) and (10, 20, 30 & 40 %) cassava flour could economically be substituted for tef flour in Injera production to improve the nutrient content without reducing the sensory quality.

✓ The blended flour food product Injera nutritional values were found to be estimated to cover energy of 14.9 % (MTC2, 373 of 2500 kcal), 61 % of Fe (BTC1, 9.13 of 15 mg) and 17 % of protein (BTC1, 9.53 of 56) daily recommended dietary allowance (RDA) value that is sufficient to meet the nutrient requirements of nearly all (97-98 %) healthy individuals in each age and gender group of adult man (age, 19-50).

✓ The enhancement of the nutritional value of food product Injera with the addition of nutrient quality protein maize/common bean flour could help to alleviate the problem of protein-energy malnutrition prevalent in this country and other developing countries of the tropics.

✓ The highest body weight gain was recorded in the mice fed on cassava enhanced with quality protein maize test diets (Dm1 and Dm4) followed by mice on Dm2 and Dm3 and then DB1, DB2, DB3 and DB4. The least body weight gain was recorded for mice fed on N-free diets (Dst).

✓ The amount of feed consumed by group of mice was higher for maize blended

test diets than group of mice fed on common bean blended diets. However, the weight gain recorded for both group of mice fed on quality protein maize and common bean blended test diets. These indicate the goodness of the produced food for children.

5.2 RECOMMENDATIONS

After thoroughly investigating the situations in this country, the following recommendations can be made:

✓ Promotion of the safe cassava processing to reduce significantly dietary exposure sufficient peeling, cutting and grating combined with drying has been proven effective in reducing cyanogens. Furthermore, organized public information should be promoted.

✓ The dynamics of fermentation process, the type of microorganisms involved in the natural fermentation of cassava should be determined, so that isolation of proper starter culture should be done. Similarly, controlled fermentation of cassava using an appropriate starter culture has to be done for comparison purpose.

✓ The effect of cassava-based foods consumer has to be well recognized and should be studied in the Ethiopian traditional recipes has to be taken into consideration.

✓ The formulation and impact of substituting cassava into bakery products particularly in reducing the cost of buying wheat from external market has to be taken into consideration and investigated.

✓ Promotion of consumption of Injera obtained from the mixing of cassava with quality protein maize and common bean should be promoted.

✓ Supplementation of protein rich crop to contribute to the detoxification of cyanide should be done in parallel with the promotion of consumption of foods rich in sulphur containing crops locally available. Study of the effect of diets administered on mice body mass performance can help to better understand the role of compositing flour to protein containing crops.

✓ Cassava is an excellent source of carbohydrate in the form of starch, thus determining the quality and quantity of starch found in the local cultivars of crops needs investigation. Hence, selecting the local cassava cultivars for industrial utilization of biodegradable packaging materials needs further research.

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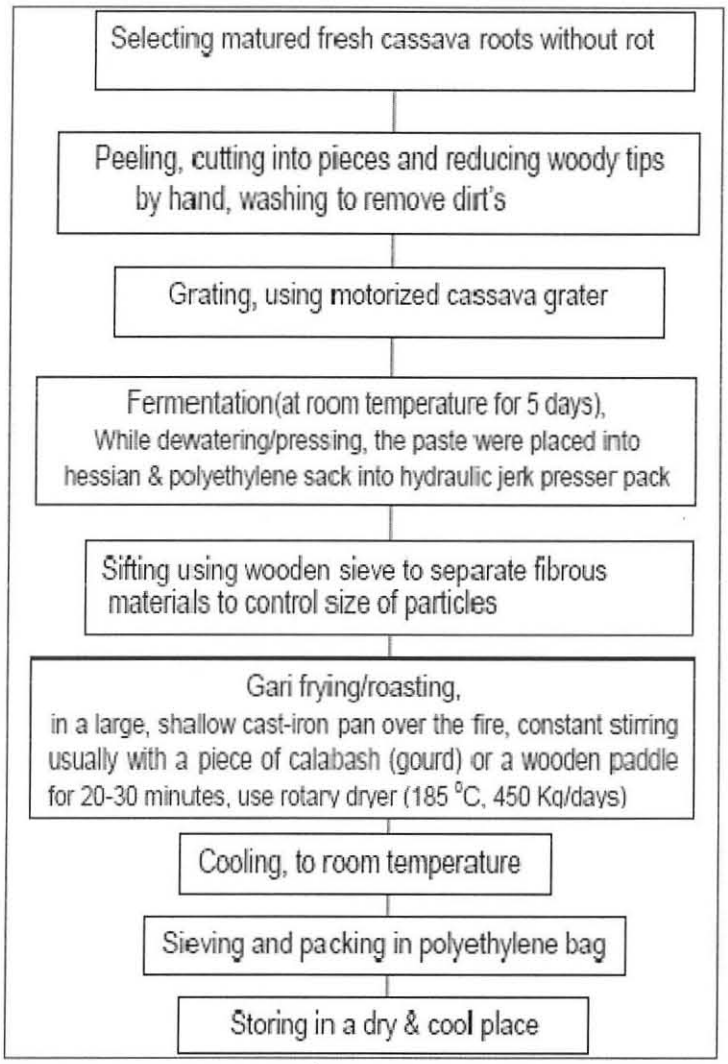
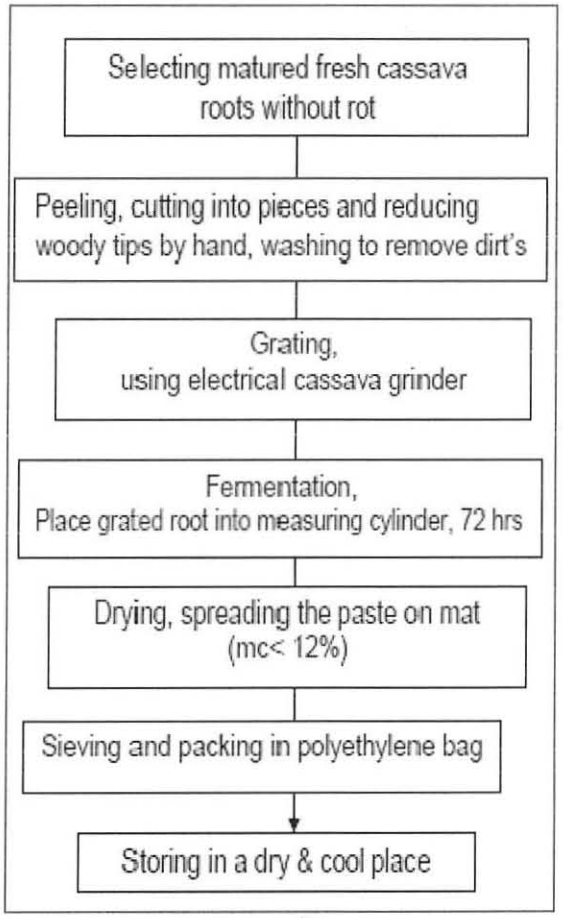
7. APPENDICES

Appendix-I. Flow chart for production of gari (A) and fermented cassava flour (B)



A.



B.

Appendix-II: Sensory evaluation score card ballot

Panellist name: ----------, sample code: ----------, date: ----------

Product type: Injera made from composite flour

INSTRUCTIONS:

Please rinse your mouth before you start to taste each sample,

Evaluate the product by looking at it for colour evaluation first and then taste it.

The judges were instructed to sip water before and after each product.

The judges recorded quality characteristics of each sample on a seven point hedonic scale where:

7 = like extremely

6 = like moderately

5 = like slightly

4 = neither like nor dislike

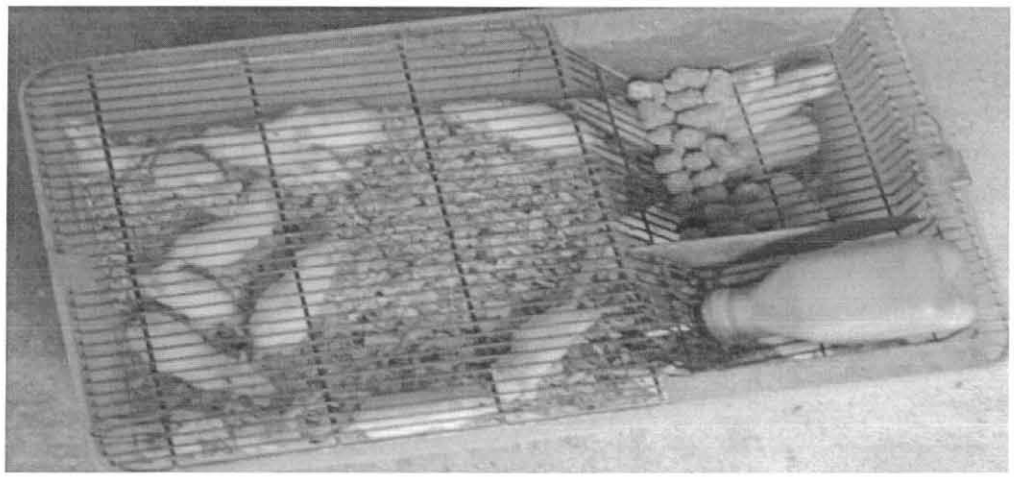
3 = dislike slightly

2 = dislike moderately

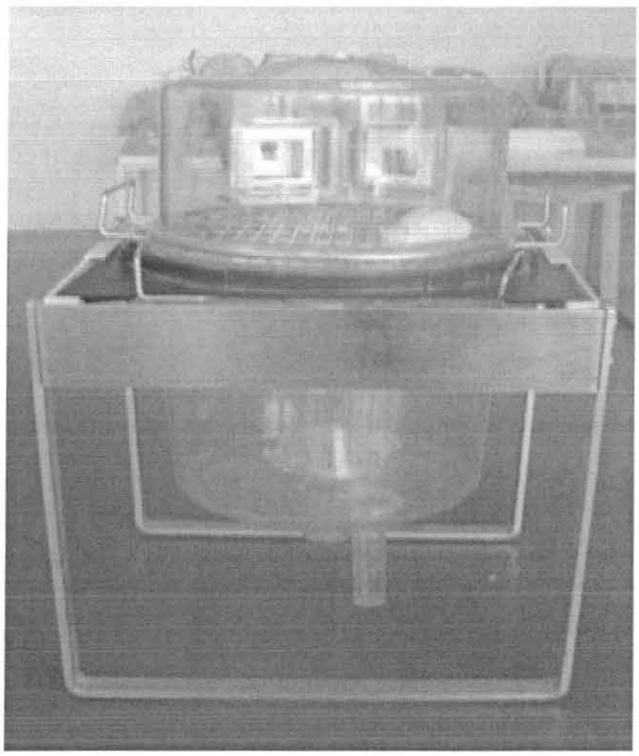
1 = dislike extremely

Hedonic scale	Sensory quality attributes						
	Colour	Taste	Flavour	Texture	Appearance (back side)	Appearance (fron/eye side)	Overall acceptability
1							
2							
3							
4							
5							
6							
7							

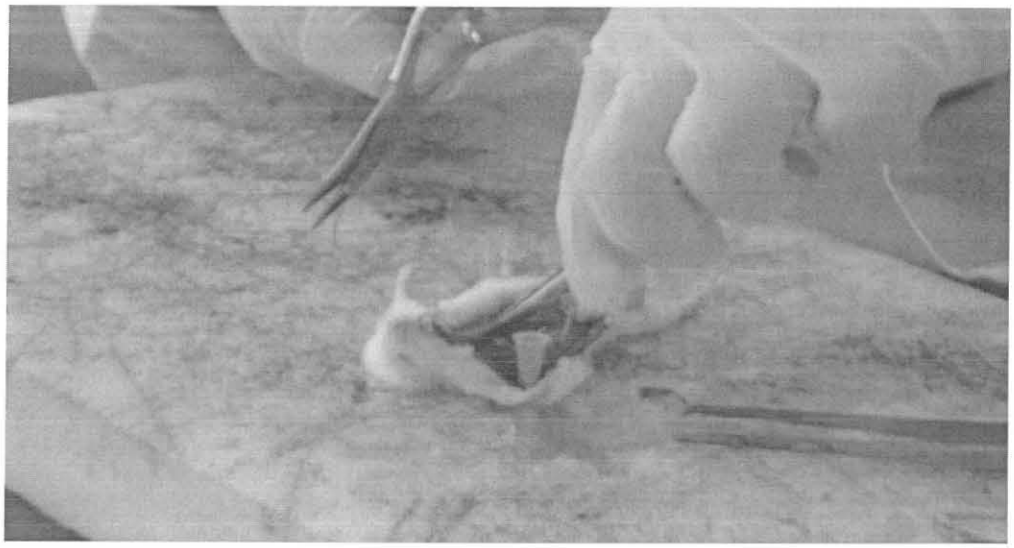
Appendix-III: Pictorial representation of animal experiment:



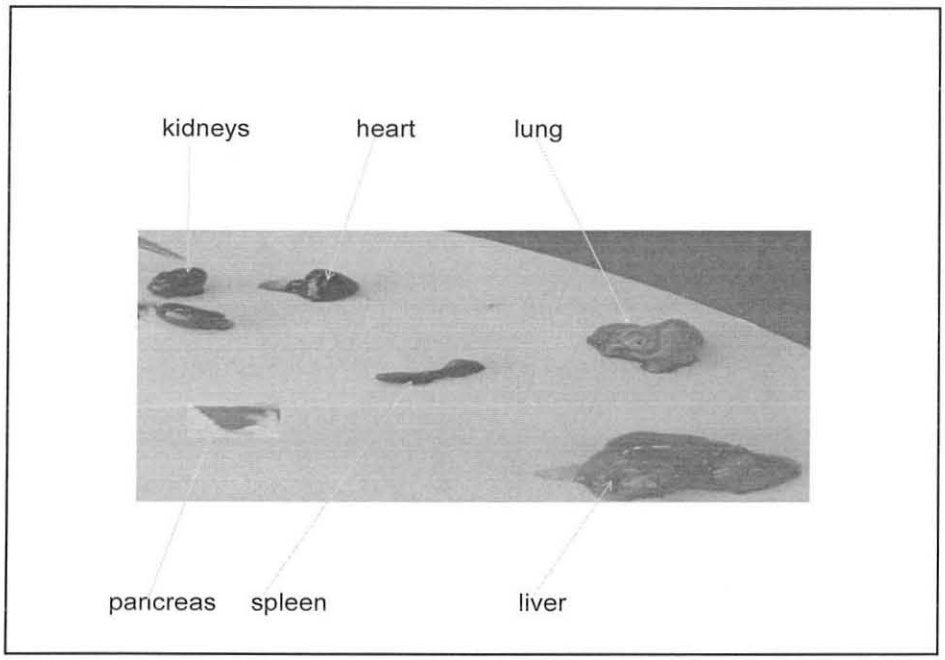
Mice in breeding house/cage with water drinking nipple



Diet testing in metabolic cage animal's house (for 21 days)



Dissection of mouse after decapsation for taking internal organ



Pictorial representation of internal organ of mouse fed on test diet.

Appendix-IV: Questionnaires for assessing of cassava-based food

(in Weredas of SNNPRS and AMARA region of Ethiopia).

Guideline for information assessment

The survey were conducted in Amaro and Thewledere weredas using household questionnaire (HH), focus group discussion (FGD) and secondary information from Weredas agriculture, NGO, health offices and related office. The HH questionnaire will be filled in households from the area where focus group discussion will be conducted to more strengthen and support the information obtained through FGD. Moreover, the HH questionnaire is used to address the differences (individualities) in indigenous food processing techniques (food preparation methods) among the HH & weredas: i.e. types of cassava based foods, processing methods, cyanide reduction/removal, and cassava based foods enrichment with other crops. The FGD will be facilitated within groups of selected community levels containing farmers in each of the two Weredas. The different raw roots and processed cassava sample was collected from HH, market place, milling house, research centre, etc.

SECTION I – Questionnaires identification

Cassava production

Zone ---------- Wereda -------- Kebele/PA ---------- Village ----------

Code ---------- Name of respondent ----------

Signature ---------- Date of Interview ----------

Estimated landholding in hectare ----------

Estimated land covered by cassava (In Hectare) ----------

Annual yield (In HH measurement) ---------- in kilogram ----------

Do the HH head like to plant more cassava in the future? 1 = Yes; 2 = No (>>23)

If yes, what is the reason? 1= for its food value; 2 = for cash; 3 = Its productivity; 4 = Require less labour; 5 = Drought resistant; 6 = Other (Specify) ----------

---

If your answer to Q.No.14 is No, what is the reason? 1 = No food value; 2 = Not productive; 3 = Due to its toxin; 4 = Other (Specify) ---------- Annual rain fall -------, average temperature ---------, soil type --------- and other related information -----

SECTION II – Cassava processing, detoxification and consumption

How often do your families consume Cassava? 1= More than once in a day; 2= Once in a day; 3 = Few times in a week; 4 = Weekly; 5 = Few times in a month; 6 = Monthly; 7 = Occasionally; 8 = none

With what food normally eat cassava? Mention the composite/ingredients or local recipe. ----------

What part of cassava do you use for meal?

1 = Roots; 2 = Leafs; 3 = Both; 4 = Other (Specify) ----------

In what form do you consume it? 1= Roasted; 2 = Cooked; 3 = Ground & mixed with other food; 4 = Sauce ("Wot"); 5 = Raw; 6 = Beverage; 7 = Others (Specify)

Do you prepare any complementary food for children from cassava?

1= Yes; 2 = No

Any event occurred by consumption of cassava in your family during the last 12 months? 1 = illness; 2 = death; 3 = other (Specify) ----------

Who was affected?: age: 1. <7; 2. 7-18; 3. >18

Did you observe any toxic effects on children below 10 years of age?

1= Yes; 2 = No

Have you heard that cassava contain toxin? 1= Yes; 2 = No

Is there a method you use to remove the toxin in cassava? 1= Yes; 2 = No

If yes to Q.no.17 what are the methods used?

1= Peel the cover; 2 = Peel cover take & out centre; 3 = Peel cover & wash; 4 = Peel cover, take out centre & wash; 5 = Peel cover, take out centre, chop & wash; 6 = Peel cover, take out centre, chop & sun dry; 7 = Peel cover, take out centre, chop, wash & sun dry; 8 = Peel cover, take out centre, chop, sun-drying & fermentation; 9 = Peel cover, take out centre, chop, sun-drying & cooking;

10 = Other (specify) ----------

SECONDARY DATA QUESTIONAIRRE (CASSAVA-BASED FOODS)

Zone: _____, Wereda: _____, Kebele/PA: _____

Name of Moderator_____, Signature_____, Date _____

SECTION I -Secondary data from Wereda health office

Was there any health problem related with consuming cassava? Mention the effect and the year? ----------

How many people are affected annually? 1 = mild; 2 = moderate; 3 = severe

What are the symptoms of disease/sickness due to cassava consumption? ------

What are the treatments given? ----------

Is there health education related to cassava consumption? By whom? ----------

From health point of view what do you recommend regarding cassava

consumption? ----------

SECTION-II Secondary data from Wereda agricultural office

Population ----------

No of Kebeles in the wereda----------

No of Kebeles cultivating cassava----------

Amount of land covered by cassava in hectare----------

Annual yield ----------

Summer season (Rainy) ---------- months

Winter season (Sunny) ---------- months

Annual rainfall ---------- mL

When is cassava introduced to the area and by whom? ----------

Is cassava recognized as a priority crop by the wereda agriculture program?

1=yes, 2=no

If no, why ? ----------

What is the main economic value of cassava? 1= For food; 2 = Additional income (Cash); 3 = For cattle; 4 = Other (Specify):----------

Are there different varieties (cultivars) of cassava in the Wereda? 1=Yes; 2 = No

Mention the different cultivars starting from the most frequent? ----------

Which cultivar has more toxicity? (Mention in order of toxicity starting from the highest)----------

Did animals (including wild) ever affected by consuming cassava? Mention the effect and the year ----------

Which agro ecological zone is favourable to cassava? ----------

Is cassava productive in your wereda? 1= Yes; 2 = No

Total production of Cassava compared to other crops (put in rank)? ----------

How do you see the acceptance of cassava by the farmer?

1=Low, 2= Moderate, 3=High, 4= Very High

How is the process (trend) in the adoption of cassava by the farmer?

1=Low, 2= Moderate, 3=High, 4= Very High

Based on the advantages & disadvantages of cassava will its cultivation be intensified in the future? ----------

What do you recommend concerning cassava? ----------

FGD QUESTIONAIRRE (CASSAVA BASED FOODS ASSESSEMENT)

SECTION I-Focus Group Discussion for Local Farmers

Zone ----------, Wereda----------, Kebele/PA ----------

Name of Moderator ----------, Signature ----------, Date ----------

To be answered by a focus group of MORE THAN FOUR community members, for instance the Kebele head, community members with special positions (head of school or clinic, extension worker etc.), elders, merchant or other knowledgeable community members. All should be residents of the community for more than a year.

When was cassava introduced into the area? ----------

Who introduced it? ----------

Planting season ----------, harvesting season ----------

High consumption season---------- why ----------

What is the cost (in Birr) of cassava/Quintal: ---------- cost/ Kg ----------

In what form do people sell cassava? (Ex: as harvested; cleaned & peeled; Cleaned not peeled; Not cleaned but peeled; Ground without peeled; Peeled and ground)? ----------

For what purpose do the purchasers use? ----------

Indicate the utilization of cassava (Ex Merchants, Hotels, Local people, Others)

How do you see the importance of cassava compared to sorghum and maize?

Is there any problem associated with cultivation of cassava? ----------

Is there storage problem associated with cassava? How do people store? --------

Are there any pest affecting cassava plant? How do people manage the pest if any? ----------

Was there education given to farmers concerning cassava (1 = Yes; 2 = No)

Plantation, ----------

Plant management, ----------

Harvest, ----------

Storage, ----------

Toxin minimization, ----------

Food preparation? ----------

Was there health problem associated with consuming cassava? Mention what & when it happened? ----------

Did animals (including wild) ever affected by consuming cassava? Mention what & when it was? ----------

Which animal is mostly affected? In which month? ----------

What are the advantages of consuming cassava? ----------

What are the disadvantages of consuming cassava? ----------

Based on the advantages and disadvantages, will cultivation of cassava be intensified or gradually eliminated? ----------

What is special about cassava? ----------

Survey about the utilization of cassava plant

Cassava based foods assessments from consumers

Survey about the utilization of cassava roots were assessed from farmers, local market places, gender office, NGO, agricultural and health centre offices using structured questionnaires and interview (above). Whereas focus group discussions were conducted with different groups of people who plant and consume cassava roots in using different processing methods. The surveys were done in two selected areas such as Amaro special Wereda and South Wello of Hayik Wereda (as shown below picture). In addition, to the survey training was given for two Weredas of selected farmers who grow plant cassava, experts of different governmental offices, religion organization and NGO whose work were related to farmers extension work. The training were creating awareness on different uses of cassava, simple detoxification process and preparing food with mixing cassava roots flour with locally grown crops.

Pictorial representation of assessing information from cassava growers in SNNPRS (Amaro) and Amara region (Hayik): group discussion, field visit and in the local market.

Appendix-V: Two-way ANOVA Summary Table (A-U)

Factors: cultivars, processing methods (treatments)

Table A. Two-way ANOVA summary table for the dependent variable mc

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	37.882	6	6.314	21.888	0.00
Treatments	42500.164	3	14166.721	4.911E4	0.00
cultivars * treatments	109.917	18	6.107	21.170	0.00
Error	16.153	56	0.288		

Table B. Two-way ANOVA Summary table for the dependent variable ash

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	3.829	6	.638	98.719	0.00
Treatments	62.989	3	20.996	3.248E3	0.00
cultivars * treatments	10.285	18	0.571	88.383	0.00
Error	0.362	56	0.006		

Table C. Two-way ANOVA Summary table for the dependent variable fibre

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	8.954	6	1.492	97.125	0.00
Treatments	10.114	3	3.371	219.424	0.00
cultivars * treatments	34.421	18	1.912	124.457	0.00
Error	0.460	56	0.015		

Table D. Two-way ANOVA summary table for the dependent variable crude fat

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	1.043	6	0.174	1.817E3	0.00
Treatments	.250	3	0.083	870.074	0.00
cultivars * treatments	1.482	18	0.082	860.352	0.00
Error	0.005	56	9.567E-5		

Table E. Two-way ANOVA Summary table for the dependent variable protein.

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	1.771	6	0.295	1.311E3	0.00
Treatments	3.523	3	1.174	5.215E3	0.00
cultivars * treatments	2.241	18	0.125	552.841	0.00
Error	.013	56	0.00		

Table F. Two-way ANOVA Summary table for the dependent variable CHO

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	45.316	6	7.553	47.187	0.00
Treatments	872.643	3	290.881	1.817E3	0.00
cultivars * treatments	91.221	18	5.068	31.662	0.00
Error	8.963	56	0.160		

Table G. Two-way ANOVA Summary table for the dependent variable energy.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	541.484	6	90.247	34.198	0.00
Treatments	17581.393	3	5860.464	2.221E3	0.00
cultivars * treatments	1233.003	18	68.500	25.957	0.00
Error	147.781	56	2.639		

Table H. Two-way ANOVA Summary table for the dependent variable calcium.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	132232.580	6	22038.763	2.169E4	0.00
Treatments	307473.677	3	102491.226	1.009E5	0.00
cultivars * treatments	145774.690	18	8098.594	7.972E3	0.00
Error	56.893	56	1.016		

Table I. Two-way ANOVA summary table for the dependent variable zinc

Source	Sum of Squares	df	Mean Square	F	Sig.
Cultivars	.709	6	0.118	10.157	0.00
Treatments	3.479	3	1.160	99.679	0.00
cultivars * treatments	2.322	18	0.129	11.091	0.00
Error	.651	56	0.012		

Table J. Two-way ANOVA summary table for the dependent variable iron.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	2.690	6	0.448	20.712	0.00
Treatments	.857	3	0.286	13.193	0.00
cultivars * treatments	5.338	18	0.297	13.701	0.00
Error	1.212	56	0.022		

Table K. Two-way ANOVA summary table for the dependent variable P

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	79.225	6	13.204	14.097	0.00
Treatments	109.944	3	36.648	39.125	0.00
cultivars * treatments	271.092	18	15.061	16.079	0.00
Error	52.455	56	0.937		

Table L. Two-way ANOVA summary table for the dependent variable HCN

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	46201.060	6	7700.177	2.752E4	0.00
Treatments	224612.955	3	74870.985	2.676E5	0.00
cultivars * treatments	67671.238	18	3759.513	1.343E4	0.00
Error	15.671	56	0.280		

Table M. Two-way ANOVA summary table for the dependent variable phytate

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	3363955.562	6	560659.260	868.274	0.00
Treatments	4038615.890	3	1346205.297	2.085E3	0.00
cultivars * treatments	4570785.791	18	253932.544	393.257	0.00
Error	36160.170	56	645.717		

Table N. Two-way ANOVA summary table for the dependent variable tannin.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	2893.205	6	482.201	1.047E3	0.00
Treatments	64864.079	3	21621.360	4.693E4	0.00
cultivars * treatments	2226.302	18	123.683	268.484	0.00
Error	25.798	56	0.461		

Table O. Two-way ANOVA summary table for the dependent variable oxalate.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	1209.038	6	201.506	1.880E3	0.00
Treatments	3007.441	3	1002.480	9.353E3	0.00
cultivars * treatments	1576.375	18	87.576	817.066	0.00
Error	6.002	56	0.107		

Table P. Two-way ANOVA summary table for the dependent variable vitamin C.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	17.737	6	2.956	173.757	0.00
Treatments	275.041	3	91.680	5.389E3	0.00
cultivars * treatments	53.334	18	2.963	174.155	0.00
Error	.953	56	0.017		

Table Q. Two-way ANOVA summary table for the dependent variable β-carotene.

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	0.040	6	0.007	685.015	0.00
Treatments	0.312	3	0.104	1.058E4	0.00
cultivars * treatments	0.121	18	0.007	685.015	0.00
Error	0.001	56	9.845E-6		

Table R. Two-way ANOVA summary table for the dependent variable of WBC

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	6.577	6	1.096	42.258	0.00
Treatments	0.186	2	0.093	3.583	0.03
cultivars * treatments	1.091	12	0.091	3.504	0.00
Error	1.089	42	0.026		

Table S. Two-way ANOVA summary table for the dependent variable of SP

Source	Sum of Squares	Df	Mean Square	F	P
Cultivars	1136.897	6	189.483	1.216E5	0.00
Treatments	23.824	2	11.912	7.642E3	0.00
cultivars * treatments	20.381	12	1.698	1.090E3	0.00
Error	0.065	42	0.002		

Table T. Two-way ANOVA summary table for the dependent variable of Solubility

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	1.181	6	0.197	1.924E3	0.00
Treatments	0.293	2	0.147	1.433E3	0.00
cultivars * treatments	0.380	12	0.032	309.289	0.00
Error	0.004	42	0.000		

Table U. Two-way ANOVA summary table for the dependent variable of BDT

Source	Sum of Squares	df	Mean Square	F	P
Cultivars	0.198	6	0.033	382.425	0.00
Treatments	0.060	2	0.030	347.109	0.00
cultivars * treatments	0.045	12	0.004	43.381	0.00
Error	0.004	42	8.619E-5		

I undersigned declare that this Thesis is my original work and has not been presented for any degree in any university and all the source of materials used for the Thesis has been duly acknowledged.

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