NUTRIENT CONSTITUENTS OF TARO (*Colocasia esculenta*) CORM AND LEAF FOR INFANT FOOD DEVELOPMENT AND CHICKEN FEED FORMULATION

Ph.D. Dissertation in Food Science and Nutrition

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DEDICATION

To my darling wife Genet Muluneh for all your help, love and endless support, always being positive and putting me and the completion of this thesis. To my candy daughters Meron and Meba for being so patient with me while I worked such long hours, you are my novel kids.

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ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemists
AARC	Areka Agricultural Research Center
ADG	Average Daily Gain
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BWC	Body Weight Change
CRD	Completely Randomized Design
CSA	Central Statistics Agency
DMCE	Dry Matter Conversion Efficiency
DMI	Dry Matter Intake
DMRT	Duncan‟s Multiple Rage Tests.
DZARC	Debrezeit Agricultural Research Centre
EAA	Essential Amino Acid
ED	Energy Density
EFA	Essential Fatty Acid
EIAR	Ethiopian Institute of Agricultural Research
FAME	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization
FMOC	Fluorenylmethyl Chloroformate
GC-FID	Gas chromatography-Flame Ionization detector
HIP	Hexane Isopropanol
IYC	Infants and Young Children
ME	Metabolizable Energy
OPA	*o*-Phthaldialdehyde
PDCASS	Protein Digestibility Corrected Amino acid score
PER	Protein Efficiency Ratio
RPM	Revolution Per Minute
SAS	Statistical Analysis System
SNNPR	South Nation and Nationality Peoples Region
TCA	Trichloroacetic Acid
TD	True Digestibility
TLC	Thin Layer Chromatography
TLM	Taro Leaf Meal
UHPLC-FD	Ultra High Pressure Liquid Chromatography-Fluorescence detector
WHC	Water Holding Capacoty
WHO	World Health Organization
WSC	Water Solubility Capacity

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Published papers and submitted manuscripts

This dissertation is based on three published papers and three submitted manuscripts. However, the published articles are not referring in the main body due to merging some of the results and additional review of related works after publishing the article. Therefore, the content of the articles was discussed in chapter four and only the first page of the article included in annex section. This study contained in the following papers:

III. Melese T, Negussie R, Etalem T, Rikard L (2015). **Effect of pre- gelatinization on nutritive and non-nutritive constituents of taro (*Colocasia esculenta*) corm.** Journal of Food Science and Nutrition. S14: 005. doi:10.4172/2155-9600.S14-005

IV. Melese, T; R. Negussie and T. Etalem, (2016a). **Effect of pre-curding on nutritional and anti-nutritional composition of taro (*Colocasia esculenta*) leaf**. International Journal of Food Science and Nutrition. www.foodsciencejournal.com. Volume 1; Issue 1; January 2016; Page No. 05-11.

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Submitted manuscripts

I. **Optimized complementary food from taro-maize-soybean**, for Journal of LWT-Food science and Technology, Received in revised form 26 Jan 2017. ELSEVIER

II. **Taro leaf as alternative feed ingredients**: In chicken feeding, for Journal of Animal Feed Food science and Technology, Received in revised form 26 Jan 2017, Received in revised form 21 Jan 2017. ELSEVIER

III. **Amino acid and fatty acid of feed and breast meat after taro leaf feedingtaro leaf in the diet** for Journal of Meat science, Received 11 February 2017. ELSEVIER

NUTRIENT CONSTITUENTS OF TARO (*Colocasia esculenta*) CORM AND LEAF FOR INFANT FOOD DEVELOPMENT AND CHICKEN FEED FORMULATION.

ABSTRACT

Ensuring food and nutrition security in Ethiopia has remained a widespread challenge. In the process of ensuring food security and better livelihood status, underutilized and less exploited food and feed resources should get a prior attention. The objective of this study is utilizing the extensive research outputs on how to use taro corm and leaves accompanied with processing methods for infant food and chicken diet formulation at a good level of nutrient sources. The study was conducted using taro corm for complementary food development and taro leaf for chicken feeding. The analyses undertaken were proximate composition mineral content, anti-nutritional content, functional properties of the food and feed, amino acid, fatty acid profile, β-carotene and other phytochemicals. Crude protein content ranged from 10.49±2.38 to 12.13±2.32 and 25.43±1.92 to 26.25 ±1.53 for taro corm and taro leaf, respectively. Similarly, crude fat in taro corm and taro leaf ranged from 2.61±1.11 to 3.42±0.65, and 5.84±0.16 to 6.37±0.95, respectively. Crude fiber content in taro corm ranged from 2.11±0.26 to 2.80±0.93, and in taro leaf from 3.19±1.98 to 4.60±1.39. The total ash, carbohydrate and energy content in corm were higher than that of taro leaves. However, amino acid composition was higher in the leaf than corm. The optimized complementary food had a proximate content of crude protein; crude fat, total ash, and carbohydrate and energy value were 22.23±0.6, 6.32±0.78, 4.8±0.32 and 56.5±0.46, respectively. The chicken feeding treatments were inclusion of taro leaf meal at 0% (T_1), 3% (T_2), 5% (T_3) and 7% (T_4).The nutrient content of chicken meat was determined and differs significantly ($P<0.05$). In breast chicken muscles the nutrient compositions (protein, fat, ash and vitamin C) were in the order of $T_4 > T_3 > T_2 > T_1$ except the crude fiber content. Therefore, through this study taro leaf and corm were found to be good dietary sources and gave higher nutritional values both for infant complementary food development and chicken feed formulation. Therefore, the findings of this study were found to be very significant for future use of taro leaf in chicken diet and it is believed that this study could give insights for use of taro flour in industry level for making complementary foods to mitigate household food insecurity and malnutrition in Ethiopia.

Key words: Chicken meat, Complementary food, Digestibility, Malnutrition and Taro.

CHAPTER ONE: INTRODUCTION

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1.1. Background

Taro (*Colocasia esculenta*) a family of Aracea, is cultivated for its edible corms used as a subsistence food by millions of people in developing countries (FAO, 1999). The corm of taro contains more than twice the carbohydrate content of potatoes and yield 135 kcals per 100 g and 11% crude protein on a dry matter (DM) basis. These reported carbohydrate and protein values are even higher than other root crops like yam, cassava or sweet potato (FAO, 1999). Though, protein and fat content of taro are low, but is high in carbohydrates, fiber and minerals (Del Rosario and Lorenz, 1999). It contains 85-87% starch on a DM basis with small granule size of 3-18 µm and other nutrients such as zinc, vitamin C, thiamine, riboflavin and niacin are higher than other root crops (Jirarat, *et al.,* 2006).

In the leaves, the rich protein content (23%) found (Annan and Plahar, 1995) might be favorably complemented the high carbohydrate contents (87%) found in the tuber part of the plant as a source of human food. In Asia, the leaves of taro have been reported to be rich in minerals like Ca, P, Fe, and vitamins. The high level of dietary fiber found in the taro leaf are also advantageous for their active role in the regulation intestinal transit, increasing dietary bulk and faeces consistency due to their ability to absorb water (Dubois and Savage, 2006; Singh, *et al.,* 2012; Miyasaka, *et al*. 2013; Abuajah *et al.,* 2015). While taro has many nutritional, economic and health attributes, it has also some anti-nutritional compounds that are harmful to health. Because of these inherent factors of anti-nutritional compounds, taro demand is often unsatisfactory in consumers.

To maximize taro utilization, application of different processing methods such as blanching, pre-curding, pre-gelatinization and flour making, which eliminate the anti-nutritional compounds, is crucial (Savage and Catherwood, 2007). The use of these preparation and processing methods can reduce dominant anti-nutritional compound when consumed as part of human diet and also important to produce a durable product that solves the problems related to post harvest loss, storage facility, food and nutritional security.

In addition, lack of knowledge on the nutritional values, product development, limited consumption, marketing aspects and the conservative eating habit of the people may be the reasons that constrained the introduction of taro into wide areas of Ethiopia. In rural areas, people are eating taro corm as boiled with peel, and there is no awareness about various processing methods that increase the quality and safety. Generally, taro is the underutilized root crop in Ethiopia (Umeta, 2000; Adane, *et al.,* 2013). Underutilized plants when properly brought into use can be good sources of nutrition (Jane, *et al.,* 1992; Obadina, *et al.,* 2013).

To fill this gap, this study has aimed to promote domestic production, maximize utilization and product development for extended use of taro products. Moreover, beyond processing and product development from taro, it is essential to obtain scientific evidence on nutritive and non-nutritive constituents including amino acid and fatty acid profiling.

1.2. Justifications and Statement of Problem

Ethiopia is one of the least developed countries in the world (UNDP, 2011) and is the second food unsecured country in the horn of Africa (de Onis *et al.,* 1997). Therefore, there are food insecure peoples in both rural and urban areas. In the process of ensuring food security and better livelihood status, and considering, underutilized and less exploited food resources should get a prior attention as they have the potential to improve the livelihoods of the community by supporting food security, improving nutrition and serve as income sources. In the country, most rural peoples suffer from malnutrition, not only because of the economic status but also because of lack of awareness to utilize the available nutritious food crops to meet their daily requirements (Annan and Plahar, 1995).

Now a day high cost of staple food crops, natural resource degradation due to climate change, high population growth in Ethiopia are the most serious problems. Therefore, characterization of such less domesticated and less exploited food sources is essential as an alternative food source that supports food and nutrition security (Yihunie, *et al.,* 2014).

Root crops are widely grown in the southern part of the country (CSA, 2013). Among the root crops, taro is one of the most important food crop as well as an income source for the poor farmers (FAO, 1999). Unfortunately, most growers and consumers are not aware of the nutritional advantages of taro and there is difficulty in storage because of spoilage property. In differnt part of the world there are different reserachs conducted on the potential use of taro (Sefa-Dedeh and Agyir-Sackey, 2004; Scheffer, *et al.,* 2005; Flyman and Afolayan, 2006; Modi, *et al.,* 2006; Obadina, 2010; Obadina, *et al.,* 2016; Obadina, 2016). However, taro has many nutritional and economic potential, in Ethiopia, the researches done so far are limited and has not contributed much to the processing and food development from taro (Edossa, 1996; Yared, 2004; Tewodros, 2012; Tewodros and Getachew, 2013; Gidmwork et al., 2016). Also, no study is done on taro leaf, which has many nutritional, economic and health potential.

Hence, the crop is often referred to as a poor man"s crop or woman"s crop because it is consumed mostly by the low income earners and the economically vulnerable groups in the society. The crop has received very little attention from agricultural researchers and research services. Maximizing profits from taro for the millions of rural households depending on the crop has therefore remained a mirage. However, taro undoubtedly can be an important food crop for developing countries, particularly Ethiopia (Smith, 2012).

The present study therefore focused at analyzing nutritional and anti-nutritional constituents of two taro varieties (local and boloso-1) followed by the amino acid and fatty acid profiling. Taro corm was used for complementary food development to improve the digestibility and nutrient contents of traditionally prepared foods from cereals and grains (WHO, 2010; Baye, *et al.,* 2013; Abebe, *et al.,* 2016). Traditional weaning food could be improved by combining locally available foods that complement each other and need to be changed or modified to improve their nutritional status (Gibson, *et al.,* 2009). In Asia, taro starch is considered to be easily digestible; hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk (Benesi, *et al.,* 2004; CSA, 2014; UNICEF/WHO/WB, 2014). Moreover, the taro starch gelatinized easily and forms a clear and soft food similar to potato starch (Olayiwola, *et al.,* 2013).

The study also utilized taro leaf, as chicken feed ingredients. Such scientific incorporation of taro leaf in chicken production is economically important to fill the gap in protein supply and amino acid of chicken feeds (Silva, 2006). Knowledge on the feeding value of taro leaf for chicken would consequently enhance not only the profitability, but also saving the use of grains, complement essential amino acid, and enrich the end products required for human beings and the affordable of products to the majority of the society (Alemu, 1995; Silva, 2006; Amire, 2010; Olugbemi, *et al.,* 2010; FAO, 2014). Harvesting taro leaves does not affect tuber yield rather frequent harvesting increases the leaf yield (An, *et al.,* 2003). Therefore, use of taroleaf in the chicken feed can supply most important amino acids such as lysine, threonine, the sulphur-bearing amino acids, comely not found in cereal based feed.

1.3. Research Questions

1. Does the two varietys of taro differ in nutritional and anti-nutritional contents?
2. Does the processing method have potential to reduce the anti-nutritional contents?
3. What level of taro corm flour can be incorporated in complementary food?
4. What level of taro leaf inclusion has effect on chicken feed formulation?
5. What are the benefits of using taro corm and leaf in food and feed formulation?
6. What effects of taro leaf on digestability and chicken product quality?

1.4. Objectives of the Study

1.4.1. General objective

The general objective of this study was to evaluate use of taro leaves and corm for infant food and chicken diet formulation and consequent effect on digestibility and product quality.

1.4.2. Specific objectives:

1. Evaluate the proximate (moisture, crude protein, crude fat, crude fiber, total ash, total carbohydrate and energy value (%DM basis) composition of taro leaf, corm, complementary food and chicken feed samples.
2. Evaluate the concentration (amylose, β-carotene, total phenols contents and minerals, Na, K, Mg, P, Ca , Zn, Fe and Cu) taro leaf, corm and weaning food samples.

3. Determine the anti-nutritional contents (tannin, phytate, mucilage, oxalate, lectin, alpha (α)-amylase and protease inhibitor (mg/100 g) in taro leaf, corm, weaning food and chicken feed samples.
4. Profile the amino acid and fatty acid composition of taro leaf, corm and chicken meat (breast and thigh) samples.
5. Determine the functional properties (absorption index, solubility index and water hydration capacity (%DM basis) of complementary food and chicken feed formula.
6. Evaluate taro leaf protein intake, digestibility, protein efficiency ratio and PEDCAAs values of chicken meat.
7. Evaluate blending ratio of corm flour and taro leaves inclusion in product development
8. Evaluate complementary food optimization
9. Evaluate effects of processing methods on anti-nutrients reduction
10. Evaluate sensory quality of taro based complementary foods

1.5. Organization of the Dissertation

This dissertation is organized into six chapters.

Chapter one included introduction, statement of the problem and objectives of the study. **Chapter two** presents the literature review on taro origin and domestication, nutrient composition and the importance of taro, the anti-nutritional contents of taro, processing of taro into different products, taro corm as complementary food ingredients and taro leaf as chicken feed ingredients. **Chapter three** presents materials and methods used in the study. Procedures for sample preparation, complementary food ingredient formulation, chicken feed formulation, determination of nutritional and anti-nutritional composition, determination of functional properties, sensory and other protein quality analysis and statistical analysis are presented in this chapter. **Chapter Four** presents the findings of the different studies in the form of the submitted manuscript, or manuscript prepared for publication. The chapter comprehends four different research findings. **Chapter five** presents" general discussions on the findings of the studies.**Chapter six** presents conclusion and recommendation of the study.

CHAPTER TWO: LITERATURE REVIEW

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2.1. Origin and Domestication of Taro

Taro originates from humid tropical rainforest regions of Southeast Asia including India. Different researchers conclude that it is not possible to determine a single centre of origin for taro (Lebot, 1999). Taro belongs to an Aroid family (Aracaceae) and it is in the genus Colocasia (Akpan, *et al.,* 2004). It is widely produced throughout the world for its underground corms (Njintang, *et al.,* 2007). Plants of the genus Colocasia are edible aroid with large leaves and one or more food storing in their underground corms (Adane, 2009).

The species is now found throughout the Pacific islands and worldwide. There are hundreds of taro cultivation in the world. Dashing taros are wetland variety, mainly cultivated in Asia and the Pacific Islands. They have a single and large mealy corm (Eka and Osagie, 2010). Eddoe taros are dry land varietys, mainly cultivated in Africa and the Caribbean. They bear numerous smaller corms (comes) around the central corm and have a firmer structure and a nutty taste (Onwueme, *et al.,* 1994; Ecoport, 2010).

As a long history of vegetative propagation, there is considerable confusion in the taxonomy of the genus *Colocasia*. Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be polymorphic. There are at least two botanical varieties; *Colocasia esculenta*) Schott var. *esculenta* and *Colocasia esculenta*) Schott var. *ant quorum* (Schott) FAO, (1999). Taro plant is best planted in soil with pH around 5.5–5.6 and in an environment that is high in humidity with rainfall level of 1000 mm each year and optimum temperature around 21–27 ^{0}C (Afoakwa and Sefa-Dedeh, 2001). Taro varietys are distinguished on the bases of corm, shoot characteristics or on the basis of agronomic and cooking behavior (Pandey, *et al.,* 1996; Onwuem, 1999) distinguished genetic variability among taro varietys for weight of mother cornels. The number and mean weight of cornels, and plant fresh weight varied according to clone, (Heredia, 1995).

Variation was found among varietys in the moisture content of corms (Strauss, *et al.,* 1980), weight of corm and secondary cornels (Takeshita, *et al.,* 1991), sugar content (Ghosho and Hasan, 1992) as well as the shape and size of corms and cormels (Kuruvilla and Singh, 1991). Some varietys were noted to be acrid even after frying (Paull, *et al.,* 2000) and the other varietys, especially long-term varieties are known to vary in response to nitrogen fertilizer with respect to corm yield as well as the taste and texture of corms (Tattiyakul, *et al.,* 2006). Taro is an ecologically unique crop because it has the ability to grow in ecological conditions, waterlogged soil, hydromorphic and saline soils and shady conditions (FAO, 1999; Arnavid-vinas and Lorenz, 1999).

The major producers in Asia are China, Japan, Philippines and Thailand; while in Oceania, production is dominated by Papua New Guinea, Samoa, Solomon Islands, Tonga and Fiji (FAO, 1999). In Africa, Nigeria, Ivory Coast, Ghana, Zaire (Congo**)** and Cameroon are the dominant producers of cocoyam (FAO, 2008a). In Eastern Africa 407,437 Mt of taro corm from 73,454 ha with an average yield of 5.5 t/ha was obtained (FAO, 2004). In Ethiopia, root crops are grown widely in the southern region. Among the root crops, taro is one of the important food items as well as an income source to the farmer. It has a great potential to supply high quality food and one of the cheapest sources of energy.

The most common and widely distributed varieties of taro are local variety (bereka) and boloso-1 under cultivation (MoARD, 2009). Most of the southwestern zones (Sheka Keffa, Bench Maji, and Dawro) and special woredas (Yem and Konta) of the SNNPR produce both local and boloso-1 taro as a staple food crop. Apart from these zones and special woredas, taro is extensively cultivated as a staple and co-staple food in most parts of the SNNPR (CSA, 2013). The total cultivation area of taro in Ethiopia is 26,506.36 hectar. In Most of the southwestern zones (Sheka Keffa, Bench Maji, Dawroand, Yem and Konta) taro is the second interterms of area coverage and production among the major root crops (Irish potato, cassava, enset (false banana). However, taro varieties are not properly disseminated to the other parts of the country and farmers have not promoted taro for maximum utilization and product development like of cassava, sweet potato and potato in the country (CSA, 2013).

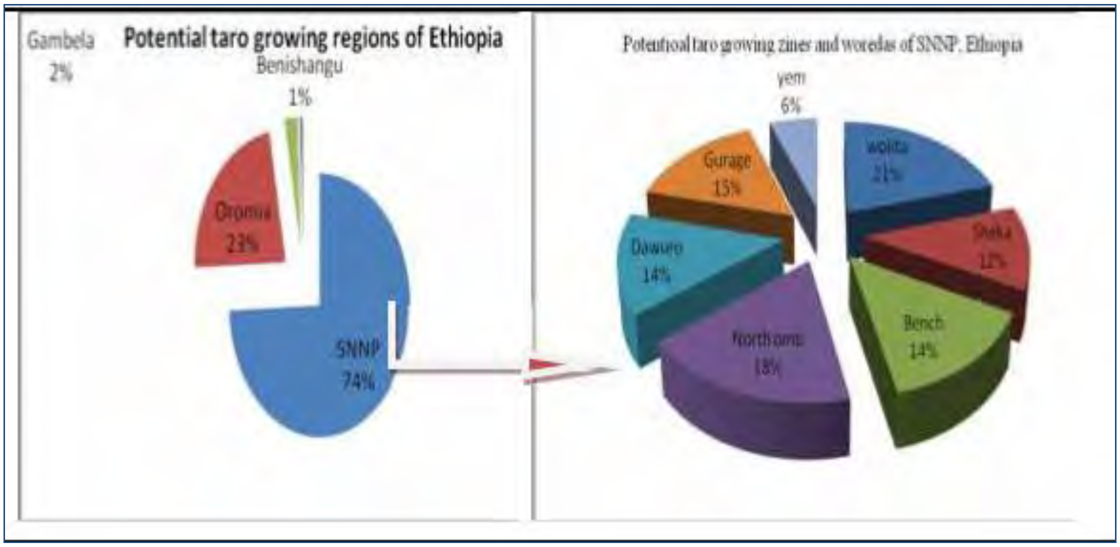

Figure 2.1: Potential taro cultivating areas of Ethiopia (CSA, 2013)

2.2. Importance of Taro

Taro has much importance in ensuring food security, in earning foreign currency as being a cash crop and also as a means for rural development. Moreover, it has been reported to have a wide range of uses in religious festivals, as mild laxative, in treatment of wounds and snake bites, reducing body temperature in a feverish patient and others (FAO, 1999). The nutritional value is the main concern when a crop is being considered as a food source. Due to the emphasis placed on the nutritional value of food by consumers, a great need exists for information on the nutritional contents of root crops (Huang, *et al.,* 2007).

Starch is the most important component (73-80%) of taro (Njintang, *et al.,* 2007). The taro proximal composition varies depending on the variety, growing conditions, kind of soil, moisture and fertilizer application, maturity at harvest, post-harvest management and storage. In general, protein and fat content at corm are low, but it is high in carbohydrates, fiber and minerals. About 11 % of the total protein in taro is albumin with high amounts of phenylalanine and leucine. The protein of taro is well supplied with hundred essential amino acids though in low histidine and lysine (Arnavid-vinas and Lorenz, 1999). Tubers and roots are important sources of energy and are used as staple foods in tropical and subtropical countries (Liu, *et al.,* 2006). Compaoré, *et al.* (2004) considered taro a good complementary crop for an orchard, ensuring a positive cash flow between fruit harvests in Australia.

Taro is highly valued and is a dominant staple food and an important source of cash income when marketed locally in North Omo in Ethiopia (Simon, 1992). Although the roots are the most widely consumed and important parts of the plant, the leaves, and rhizomes of taro are also eaten in Asia/ Pacific regions (Jianchu, *et al.,* 2001). The leaves are usually boiled and are prepared in various ways mixed with other ingredients. Taro can be consumed as boiled, baked, roasted or fried and in conjunction with fish, and coconut preparations (Nip, 1997). Taro flour in precooked forms may find good uses in pastry filling, binder in sausage and as an emulsifier in food systems (Fagbemi and Olaofe, 1998). In Ethiopia, processed forms like flour, nodule and slices of taro are uncommon except in southern part of Ethiopia especially in Wolayita zone simply boiled with the skin and consumed as similar to potatoes.

Regarding to taro corm handling and storage facilities, good cooling and good ventilation prolongs shelf life of root and tuber. Paull and Chen (2004) studied that taro should be room-cooled to 10–14 °C and recommend stored at 7–10 °C with 80–95% Relative Humidity for up to 18 weeks. After harvesting, attention on careful handling and storage can extend the shelf life of the product and corms can be stored for 4–5 months at 7–10 °C if harvested and handled carefully (Central Queensland University, 2008; Masalkar and Keskar, 1998).

Figure 2. 2: Taro crop pre-harvest and post-harvest

2.3. Nutrient Composition of Taro Leaf and Corm

The nutritional composition of taro corm like other root crops is low in protein and low in fat, but high in the carbohydrate(Table 2.1; 2.2). It is a good source of potassium and provides moderate level of phosphorus. It is low in vitamin C and deficient in the vitamins A (USDA, 2002). Taro corm is a good source of minerals and smaller granule size of its starch helps increase (Addis, *et al.,* 2005).

2.3.1. Starch

Taro corm has been reported to have 70–80% (dry weight basis) starch with small granules (Jane, *et al.,* 1992). The presence of the small sizes (1–4 μm in diameter) of its starch granules, taro is highly digestible and as such has been reported to be used for the preparation of infant foods in Hawaii and other Pacific Islands (Nip, 1997). Taro starch is considered to be easily digestible; hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk (Benesi, *et al.,* 2004). Taro starch, in view of its small size, has also been used for industrial applications (Jaenicke, *et al.,* 2006). Taro starch is easily digestible, the starch grains are fine and very small, it has hypoallergenic nature (low tendency to cause allergic reactions) (Jane, *et al.,* 1997; Kochhar, 1998 and Dawson, *et al.,* 2005) and also the starch is gluten free (Rekha and Padmaja, 2002).

Taro starch is also good for peptic ulcer patients, patients with pancreatic disease, chronic liver problems and inflammatory bowel disease and gall bladder disease (Emmanuel-Ikpeme, *et al.,* 2007). The size of the starch granules varies with the variety and ranges from 1.5 to 6.6 µm. The shape is polygonal. Taro starch contains about 50% less amylose and an amylopectin content which is higher compared to other cereals. The amylose/amylopectin ratio is 1:7. The taro starch forms a clear and soft paste similar to potato starch. Starch gelatinization temperature is dependent on the variety as well as of the maturity at harvest and is lower as the age increases, ranging from 63- 73 °C. The most important sugar in taro is sucrose, but fructose, maltose, glucose and raffinose are also present. Malic acid is the most important organic acid (60%) followed by citric acid (25%) and oxalic acid (15%) (Arnavid-vinas and Lorenz, 1999).

2.3.2. Moisture

The moisture content in taro is very high and accounts two third of the total weight of the fresh crops (FAO, 1999). Moisture content of taro varies with variety, growth condition and harvest time. In general the moisture content of taro ranges from 60-83% (Huang, *et al.,* 2007).

2.3.3. Protein and amino acid

Taro contains about 11% crude protein on a dry weight basis. This is more than yam, cassava or sweet potato which is 7.4%, 4.5%, 8.5%, respectively (Jane, *et al.,* 1992). The protein content of the corm is higher towards the corm"s periphery than towards its centre. This implies that care should be taken when peeling the corm; otherwise a significant amount of the protein could be lost in the peeling (Mbfung, *et al.,* 2006). Concerning the leaf, like higher plant, taro leaf is rich in protein. It contains about 23% protein on a dry weight basis (FAO, 1999). The protein fraction is rich in essential amino acids of threonine, leucine, arginine, valine and phnylalanine (Figure 2.3). Among the essential amino acids methionine, lysine, threonine, phenylalanine and leucine are relatively abundant respectively in the leaf than the corm. Taro corm contains a better amount of threonine, leucine, arginine, valine and phenylalanine.

Like other roots and tubers are deficient in most other vitamins, but contain significant amounts of beta carotene and ascorbic acid. For instance, cooked leaf of taro contains beta carotene (96 mg/100g), iron (12 mg/100g) and ascorbic acid (87 mg/100g) (FAO, 1990; Jirarat, *et al.,* 2006).

In general, most important minerals are contained in taro including calcium, phosphorus, magnesium, iron, copper, zinc, sodium and potassium (Abbo, *et al.,* 2000). From a nutritional point of view, the difference between peeled and non- peeled corm in mineral composition is marginal except for calcium. Most of the corm calcium is located in the skin (Abbo, *et al.,* 2000). Therefore, the consumption of non-peeled, cooked taro would be useful in calcium-deficient diets. Taro also contains higher levels of iron (10.4 mg/100g) in comparison with other root crops (Salunkhe, *et al.,* 1985; Chavan, *et al.,* 1986; FAO, 2008).

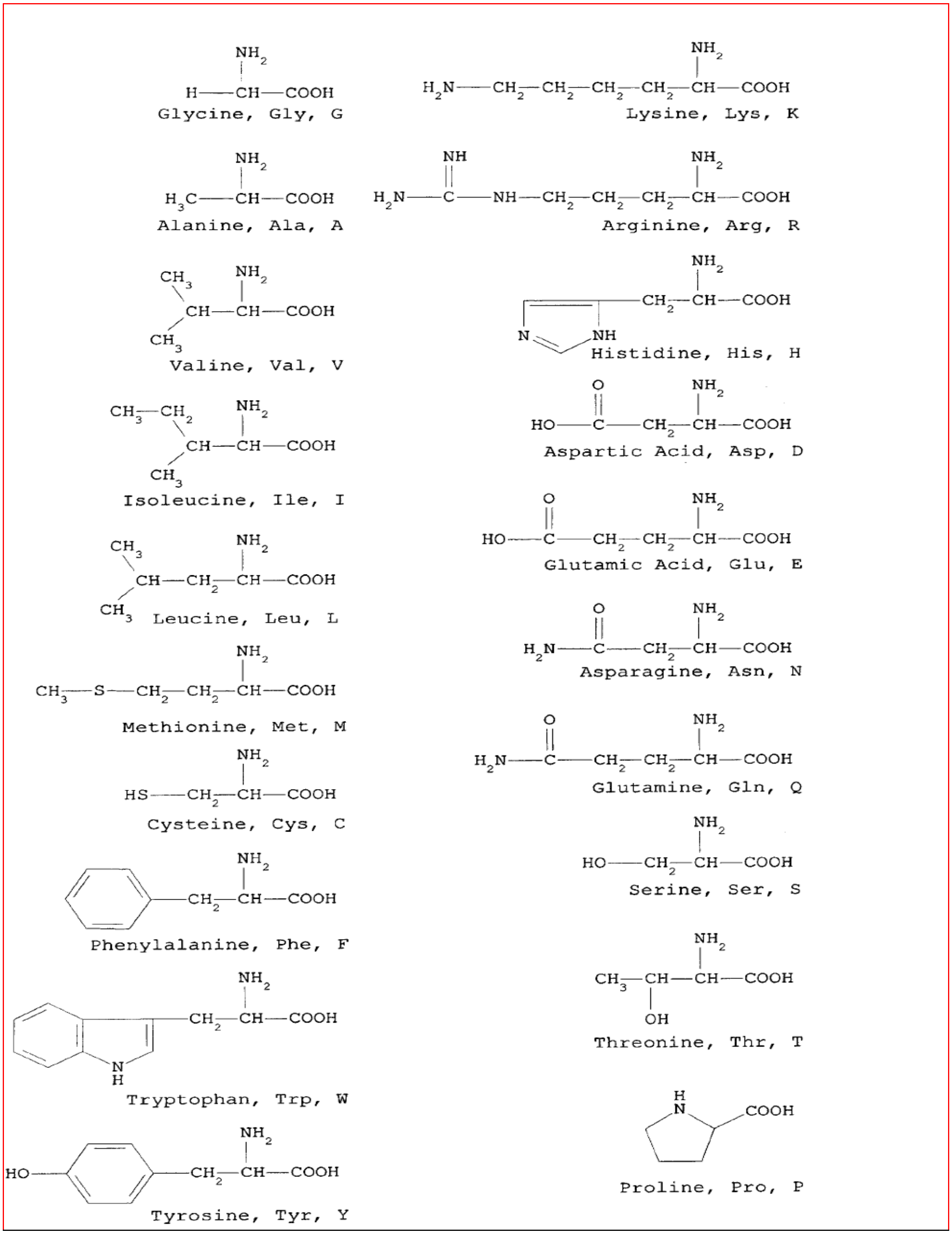
Glycine, Gly, G
Lysine, Lys, K
Alanine, Ala, A
Arginine, Arg, R
Valine, Val, V
Histidine, His, H
Isoleucine, Ile, I
Aspartic Acid, Asp, D
Glutamic Acid, Glu, E
Leucine, Leu, L
Asparagine, Asn, N
Methionine, Met, M
Glutamine, Gln, Q
Cysteine, Cys, C
Serine, Ser, S
Phenylalanine, Phe, F
Threonine, Thr, T
Tryptophan, Trp, W
Proline, Pro, P
Tyrosine, Tyr, Y

Figure 2. 3: The 20 essential and non essential amino acids (Source: Kivi, 2000)

Table 2. 1: Proximate composition of taro corm on fresh weight basis (FWB).

Composition	Content [a]	Content [b]	Content [c]
Moisture (%)	68.11	63-85	65.73
Carbohydrate (%)	26.83	13-29	26.34
Protein (%)	0.34	1.4-3.04	1.14
Fat (%)	0.11	0.16-0.36	0.46
Crud Fiber (%)	2.53	0.6-1.18	2.15
Ash (%)	1.91	0.6-1.3	2.16
Vitamin C (mg/100g)	14.33	3.43	<1
Thiamin ,B1 (mg/100g)	0.028	0.18	0.11
Riboflavin ,B2 (mg/100g)	0.029	0.04	0.02
Niacin ,B3 (mg/100g)	0.78	0.94	1.36

Source: a: Hedges and Lister (2006); b: Onwume (1999) and c: Englis *et al.* (1996).

Table 2. 2: Proximate and some mineral composition of taro leaf on fresh weight basis.

Composition	Amount	Composition	Amount
Moisture (%)	8.16	Ash (%)	10.65
Carbohydrate (%)	51.31	Energy (Kcal)	344.52
Protein (%)	25.57	Potassium (mg/100g)	94.54
Fat (%)	4.11	Sodium (mg/100g)	9.86
Crud Fiber (%)	1.46	Calcium (mg/100g)	2.15

Source: (Fai, 2013)

2.4. Anti-nutritional and Phytochemicals Substances of Taro

Anti-nutrient is a substance occurring in the diet, which acts antagonistically towards one or multiple nutrients, reducing digestibility and bioavailability. This is usually done through complex formation which reduces nutrient absorption (Dumont and Vernier, 2000). The anti-nutrients which found in taro have negative implications for taro as a food, yet they also have positive implications for taro as a crop that can be grown with minimal use of fungicides and pesticides mainly in wild taro crops. The main anti-nutrients that exist in taro are: mucilage, oxalic acid, tannins, phytate, lectins, alpha–amylase inhibitors and protease inhibitors (Ramanatha, *et al.,* 2010).

2.4.1. Taro mucilage

When raw taro corms are cut into pieces, the exposed surfaces often exude droplets of a slimy substance called mucilage. The pieces or slices of corm are washed or placed in water; a great quantity of mucilage is quickly reduced or eliminated (Nip, 1997). Mucilage can be removed by discarding the water used for cooking, or acid ingredients such as lemon juice can be used to neutralize the slimy effect, or the taro can be cooked without water (*e.g.* fried or baked) in a manner that leads to partial dehydration and a dry texture. However, the mucilage in taro corms is important for health because of digestibility and slower blood glucose, slow transit of food through the upper gastro intestinal (GI) tract, absorb water and hold moisture that soften stool (Njintang *et al.*, 2014).

2.4.2. Oxalic acid / oxalates

The presence of oxalates in taro imparts acrid taste or cause irritation and one major limiting factor in the utilization of taro. This acridity is caused by needle–like calcium oxalate crystals, raphides that can penetrate soft skin (Bradbury and Nixon, 2008). Thereafter an irritant present on the raphides, probably a protease can cause discomfort in the tissue (Bradbury and Nixon, 1998). High oxalate concentrations in the leaves and corms of plants food consumed daily are of concern because of the harmful health effects associated with the intake of high amounts of oxalates (Savage and Catherwood, 2007). The total oxalate of the taro varietys ranged from 278 to 574 mg/100 g wet matter (WM), with the edible leaves generally having lower levels of total oxalates than the leaves considered inedible.

Hang *et al.,* 2011, showed that the total oxalate of the leaves of eight different varietys of taro grown in central Vietnam ranged from 115 to 362 mg/100 g in fresh weight basis while the soluble oxalate contents were more variable (4.7 to 80 mg/100 g). The levels of oxalate and the amount of calcium bound up in the insoluble oxalate fraction will have an important effect on their efficient utilization within the body (Proieti, *et al.,* 2004; Chai, *et al.,* 2004).

In large quantities (2856 mg/100g), oxalic acid is poisonous to humans and can also reduce the nutritional value of a food by binding with calcium to form calcium oxalate (Hang, *et al.,* 2011). Bradbury and Holloway (2008) determined the amounts of total oxalate, soluble oxalate, calcium oxalate, and free calcium in taro corms and leaves, and found that the amount of free calcium is adequate for human nutrition. According to (Hedges and Lister, 2006) all parts of most varietys of taro were known to contain calcium oxalate, which is destroyed by lengthy cooking. Iwuoha and Kalu (1994) reported that boiling taro corm at the 90 ^{0}C for 30 minutes and steeping in water at 30 ^{0}C for 24 hours can reduce the oxalate-salt content of 32.7% and 56.7% of its original content, respectively.

Wild taro is abundant, particularly in wetlands and is highly resistant to pest and diseases. The wild taro leaf has a high nutritive value, with 22.5-26.3% crude protein (Malavanh, *et al.,* 2008b). However, in common with other species of the Aracae family, calcium oxalates causing irritation in the throat and mouth epithelium and indirectly affecting the digestibility. The soluble oxalate content can be reduced by soaking or cooking. Soaking for 18 hours can reduce the soluble oxalate content by 26 %. During the soaking treatment the insoluble oxalate (calcium oxalate) content of the leaves remained constant (171mg /100 g). Boiling the taro leaves resulted in a 36% loss of soluble oxalates, while the soluble oxalate content of baked tissue was very similar to the raw tissue.

The mean insoluble oxalate content of the raw, boiled and baked tissue was 226 mg oxalate/100 g fresh matter. Overall, boiling the taro leaves was shown to be an effective way

of reducing the soluble oxalate content of the tissue. Besides, the calcium oxalate content in taro leaf can also be reduced by sun-drying and storing (Emmans, 2008).
Since taro is a potential food it is important to investigate whether the oxalate content poses a risk factor and whether different methods of preparation and cooking can reduce the risk of absorbing excess soluble oxalates when consumed as part of the diet. Different methods of boiling, drying, fermentation and cooking methods used in different studies have shown effects in reducing the level of oxalate, tannin, inhibitors, phytate and enhance the nutrient content of taro (Malavanh, *et al.,* 2008b).

2.4.3. Protease (trypsin and chymotrypsin) inhibitors

According to Bradbury, *et al.,* 2008 acridity from eating raw or inadequately cooked taro is caused by protease inhibitors (protein-degrading enzyme). Acridity is found in the corms and is experienced as a severe itching, stinging or burning sensation in the mouth and throat, followed by swelling or as a less severe irritation or itching of external skin (Osisiogu, *et al.,* 1994). Acridity in taro is thought to be important as a natural defense against grazing animals (Bradbury and Holloway, 1988). Njintang *et al.* (2014) suggested several possible ways, including wash with acidic ingredients or sodium bicarbonate can help to reduce acridity. The inhibitors constitute some 1- 4% of the total crude protein in corms and are absent or inactive in leaves. When corms are cooked, trypsin inhibitor activity increases at first, but is eventually lost. Twenty minutes of boiling is sufficient to remove trypsin activity and is also sufficient to remove the acridity in many taro varietys (Bradbury and Holloway, 1988; Bradbury and Hammer, 1990; Bradbury, *et al.,* 1992).

2.4.4. Lectins

Plant lectins are an extremely heterogeneous group of proteins that have only one property in common, namely their ability to bind carbohydrates. Lectins may act as storage proteins that support new growth during the plant life cycle and also as biochemical defense molecules that anticipate attack by microorganisms or plant-eating organisms such as nematodes, insects and other higher animals (Van, *et al.,* 1995). Heating the lectin to 100 ºC for 20 minutes eliminated its ability to reduce its inhibitor effect.

2.4.5. Alpha-amylase inhibitors

Alpha-amylases are enzymes that help animals and humans to digest starch. The enzymes are found in saliva and the small intestine and can be inhibited by enzyme-specific inhibitors from many plants. The presence of alpha-amylase inhibitors in taro corms can inactivate human salivary and pancreatic amylases (Mace and Godwin, 2002). However, alpha-amylase inhibitors are not very resistant to heat, losing activity in vitro after boiling for 30 minutes. If usual cooking practices do not deactivate these inhibitors, then they are reasonably tolerated by humans and may be of little concern. This argument needs to be tested experimentally.

2.4.6. Tannins

Studies have shown that tannins interact with proteins, enzymes or no enzymes and form tannin-protein complexes, which decrease protein digestibility and protein solubility. This decrease in protein digestibility may be caused by either the inactivation of digestive enzyme or the reduction of the susceptibility of the substrate proteins after forming the complex. Tannins also reduce the bioavailability of vitamins (VA, B and C) and minerals (Fe, Zn I, P and Mg) (Chavan, *et al.,* 1986). During peeling and cooking the considerable amount of tannin is reducing.

2.4.7. Phytates

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phyate which has a high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter, *et al.,* 2002). The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Lopez, *et al.,* 2002). Therefore, removal of phosphate residues from phytate results in a reduced impairment of intestinal uptake of essential dietary minerals (Sandberg, *et al.,* 2002).

The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially non absorbable from the human gastrointestinal tract. Furthermore, the human small intestine has only a very limited capability to hydrolyze phytate due to the lack of endogenous phytate degrading enzymes and the limited microbial population in the upper part of the digestive tract. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis (breaking of one or more peptide bonds in a protein to form smaller protein). Strong evidence exists that phytate-protein interactions negatively affect protein digestibility *in vitro* and the extent of this effect depends on the protein source (Cheryan, 1980). To optimize food processing and preparation with respect to phytate degradation, it is essential to know the properties of the natural occurring phytases.

The control of anti-nutritional level in foods is an important aspect of food quality as anti-nutritional content affects the acceptability and utilization of food. In this way, heat treatment could effectively reduce the level of anti-nutritional in food. It is widely accepted that simple and inexpensive traditional processing techniques are effective methods of achieving desirable changes in food. In taro most of the anti-nutritionals are partly or totally water soluble and can be eliminated through, deep in hot or cold water, blanching, cooking and pre-drying (Frias, *et al.,* and 2000).

2.4. 8. Phytochemicals

Taro has also some health promoting substances includes antioxidants and phytochemicals which have a consequential effect on human health. Taro has phytochemicals such as high amount of β-carotene in the leaf (96.6 μg/100 g) and will impart vitamin A and antioxidant property in the body (Agwunobi, *et al.,* 2002). Beta-carotene differs only very slightly in terms of structure. They are very common carotenoids (β and α-carotene) and are antioxidants, as well as having other potential health benefits. As mentioned earlier, the carotenoids can be converted into vitamin A by the body, though β-carotene has about twice the pro-vitamin A activity as α- carotene (Adams *et al.,* 1999). Beta-carotene is most Vitamin A activity of the carotenoids and used to prevent cataracts and lung cancer (Gallicchio, *et al.,* 2008).

2.4. 9. Phenolic Compounds

These are simple phenolic acids that are widely distributed in the cell walls of plants and consequently are significant components of the human diet. They have been studied largely in relation to antioxidant activity, though these have been largely *in vitro* studies and further work regarding *in vivo* effects in humans is needed before health benefits can be claimed. Pale-fleshed variety of taro is associated with a high level of total phenolic compounds than white fleshed varietys (Wolfe, *et al.,* 2007).

2.5. Processing of Taro into Different Products

Due to oxalate content, it is recommended to soak, wash or cook taro corms and dry or ensile leaves before feeding them (Pheng *et al.,* 2008a; Babayemi *et al.,* 2009). It is possible to process taro by various methods to reduce the toxicity and improve palatability and storage characteristics. These processing techniques include peeling, boiling, steaming, flouring, and drying (Hang and Preston, 2009). Taro retains its food value if cooked without skin and it must be cooked thoroughly to prevent mouth and throat itching. In Asia and Oceanic countries, taro corms and leaves are usually consumed by humans after heat treatments, such as boiling, blanching, steaming, stewing, and frying and pressure cooking. These methods are found to be effective in improving digestibility, increasing nutrient bioavailability and also minimizing anti-nutritional factors (Tay *et al.,* 2007; Savage, *et al.,* 2009; Hang and Preston, 2009). When taro corms processed into powder and further decrease will occur when processed into taro noodles and cookies (Soudy *et al.,* 2010). Therefore the combination of cooking time, temperature program is necessary to preserve the nutrients and eliminate the anti-nutritional factors. On the other hand, cooking increases antioxidant activity, crude fat, crude protein and crude fiber contents (Soudy *et al.,* 2010).

2.5.1. Pre-gelatinization

Pre-gelatinization is one of the physical methods used to modify starch. Taro starch gelatinization temperature is dependent on the variety as well as of the maturity at harvest and is lower as the age increases, ranging from 63- 73^{0}C (Arnavid-Vinas and Lorenz, 1999; Million *et al.,* 2006).

This method affects physicochemical and functional properties of flour significantly (Alam and Hasinain, 2009) due to starch granule disruption and such disrupted and pre-gelatinized flour can absorb water. Therefore gelatinization can create binder properties to obtain a uniform matrix instantly when added to water (Doubliel *et al.,* 1986). The solubility of modified starch from taro was studied by Alam and Hasinain, (2009) and found that heat, moisture treated starch is more soluble than raw starch. In addition, it was reported that pre-gelatinized product have higher overall acceptance (Sweedman *et al.,* 2003).

Pre-gelatinized starch is an instant starch that has been gelatinized and then dried. It subsequently swells in liquid without the application of heat (Ammar, *et al.,* 2009). Some properties of a pre-gelatinized starch include the following: dispersible in cold water; it can thicken without heat being applied, can be further cooked and dried, yet is able to reabsorb a lot of water in preparation without cooking the food (instant pudding), undergoes irreversible change and cannot return to its original ungelatinize condition after treatment a greater weight of starch is required to thicken a liquid because some rupturing and loss of starch granule contents occurred during gelatinization and drying (Huang, *et al.,* 2007; Nwanekezi, *et al.,* 2010).

2.5.2. Drying

Taro can be slice dried, packed and stored for further use. Sun drying is the oldest and easiest method of preservation based on reducing the moisture content of the root and tuber to a very low level. The concentration of soluble solids becomes relatively chemically stable (Vega-Mercado, *et al.,* 2001). It is no longer a substrate for the growth of molds, yeasts and bacteria, thus preventing spoilage during storage (Anon, 2001). Even currently, drying is preferable because it lowers the cost of packaging, storing and transportation by reducing both the weight and volume of the final product such as flour chips and pellets (Mandhyan *et al.,* 1988).

2.5.3. Flouring

A major problem of taro is that the corms are susceptible to physical damage during harvesting and thus leading to high post harvest losses (Onwueme and Simha, 1991; FAO, 2006). To overcome these losses, taro may be processed into flour (Onyeike *et al.,* 1995). According to (Obadina, 2016; Oyindamola, *et al.,* 2016) processing in flour prolong the shelf life of taro. Among the many alternative sources of flour are root crops that are rich in starch. Although compared to wheat flour with having high protein of starches, root crop such as taro can be considered as an alternative ingredient for cakes and other bakery products that also have a considerable amount of nutrients and vitamins (Prajapati, *et al.,* 2011; Lim, 2015).

Several tuber products are accepted and preserved as flours after appropriate drying. Flours role in the feed and in the food industry is very important (Nip, 1997). Trying to produce alternative food such as taro products from new sources is necessary. The taro flour makes part of the new types of flour. Some recent studies establish a simple process to produce taro flour (Mitsuoka, 2014). In this process taro tuber is cooked in its skin, peeled, sun dried and ground through o.5mm sieves (Njintang and Mbofung, 2003). The taro flour can then be used in other food formulations such as taro bread, taro cakes, baby food, pasta, or other products (Nip, 1990; Subhash, *et al.,* 2012). The corm‟s flour is also a good source of carbohydrate for diabetics and production of weaning food for infants and for those with gastrointestinal disorders (Onwueme, 1978; Widowati, 2009).

2.6. Taro Corm Flour as Complementary Food Ingredients

The choices of complementary foods are affected by factors like family dietary pattern, influence of elders, cultures, customs, beliefs food taboos, previous experience of feeding patterns, agriculture, inadequate nutritional knowledge, geography and climates (Suhasini, and Malleshi, 2003). Development of complementary food is guided by high nutritional value to supplement breast feeding, acceptability, low price and use of local food items (Ferguson and Darmon, 2007**).** Traditional complementary food porridges or porridge is based on starchy staple foods, such as wheat, rice, maize, barley, oat, tef, millet or sorghum, but in

some areas also forms starchy roots or tubers that produce viscous porridges that are difficult for children to consume (Temesgen, 2013; Tessema, *et al.,* 2013).
As a result, mothers commonly dilute the porridge with water to reduce its viscosity. Such dilution, however, also reduces the energy density of the mixture. Since young children have small gastric capacities, they are unable to consume enough of the diluted porridge to meet their energy requirements and consequently may become malnourished. Generally, in Ethiopia poor feeding practices and shortfall in food intake are the most important direct factors responsible for malnutrition and illness amongst children **(**Melkam, *et al.,* 2013) and a combination of nutritionally inferior diets and improper feeding practices are major contributing factors to the development of childhood malnutrition **(**Jakobs, and Rubery, 1988)**.**

Complementary feeding improvement should be of highest priority for the nutrition of infant and young children because of its crucial role in preventing mortality and enhancing child development (Temesgen, 2013)**.** Traditional weaning food could be improved by combining locally available foods that complement each other and need to be changed or modified to improve their nutritional status (Ramakrishna, *et al.,* 2006; Baye, *et al.,* 2013; Abebe, *et al.,* 2017).

Among root crops, taro starch is considered to be easily digestible; hence it is widely used in baby foods and the diets of people and children sensitive to allergic to cereals (Mil *et al.,* 2004). Moreover, the taro starch gelatinized easily and forms a clear and soft food similar to potato starch. However, taro (*Colocasia esculenta*) is an underutilized root crop with promising economic value. It is energy-rich, available and quite affordable. The utilization of taro could be increased by developing suitable processing technology and securing consumer acceptance. In a study conducted by Ammar *et al.,* (2009) on the use of taro flour as additive showed that a maximum of its 10 % blend with wheat flour in making bread and cookies was acceptable in terms of the organoleptic properties such as color, texture, taste and overall acceptability (Mbofung, *et al.,* 2006; James, *et al.,* 2013).

Therefore, any effort to be made in producing the new infant food development without affecting the food quality is very crucial and utilization of taro (*Colocasia esculenta*) is also offering additional food and alternative food choice for rural peoples, especially for children and women to ensure food security and reduce hunger (Njintang 2003; Million, *et al.,* 2006). For this reason, taro can be used as a composite in the manufacture of infant meals, getting a better condition for patients with such problems that require carbohydrate as a source of energy which will not stress their metabolic process (Rekha and Padmaja, 2002). Therefore, it can be possible to develop nutrient dense, low cost weaning food from taro and other grain composite flour if properly processed and complemented.

2.7. Taro Leaf as Chicken Feed Ingredients

In the near future, it is predicted that there will be a greatly increased and continuing demand for protein foods for human consumption in most developing countries (Hang and Preston, 2009). However, animal protein deficiency today is one of the world's serious health problems, especially for children in developing countries, which is aggravated by rapidly increasing population growth (FAO, 2010).

Recently, chicken products become out the reach of poor people due to scarcity of conventional feedstuffs essentially grains and legumes which are also highly required for direct human consumption. Therefore, replacing cereals and expensive and less available agro-industrial by-products by unconventional source of raw materials, which are less exploited by man, is one of the solutions to reduce cost of production and contribute to increased supply of animal protein (Anaeto and Adighibe, 2010). Moreover, most of chicken feeds are made of cereals which are deficient in certain essential amino acids resulting synthetic amino acid supplementation and this is also not afforded by rural farmers to maximize chicken production. Thus, the high cost of cereals and protein supplements and uncertainty about their sustainable supply pressed the need to search for other potential unconventional feed sources which are relatively less used for human consumption.

For all above reasons, it is feasible to search for locally available and less exploited new crop for chicken feed that supplies essential amino acid and other essential nutrients. To overcome this remaining problem, taro leaf could be possible alternative feed ingredients and supplement soya bean, industrial and expensive staples. The use of such unexploited crop for alternative feed sources can reduce staple grain dependency for chicken feed and decrease cost of feeds and consequently increase affordable products (Balagoplan, 2004). There are pervious works in our country, on chicken feed formulation by cassava, *Moringa oleifera* leaves with roots and tubers to decrease grain dependency and to improve performance, productivity, fertility, hatchability and health status of animals (Meseret *et al.,* 2011; Etalem *et al.,* 2013). However, there is no study on taro based chicken diet and this study is initiated to evaluate the nutritional potential of incorporating taro leaf at different levels to improve the digestibility, protein efficiency ratio and other chemical composition of chicken feed and the product of chicken breast and thigh meat.

CHAPTER THREE : MATERIALS AND METHODS

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3.1. Sample Source and Study Areas

Two varieties of taro (*Colocasia esculenta*) corm and leaf: local and boloso-1 varieties were obtained from Areka Agricultural Research Center (AARC), grown at the same condition in the Wolayita Zone, a town located 300 km South - West of Addis Ababa, a town with a latitude and longitude of 7°4′N 37°42′E and an elevation of 1774 meters above sea level (CSA, 2009). The analytical works were done in three different places (Swedish Agricultural University, Addis Ababa University and Haramaya University).

The rational for selecting the varieties were abundant, productivity and high prospect in the area. The leaf samples were harvested at 7 months of age, while the corms were harvested at 9 months of age and then brought to Addis Ababa for preparation and analysis. For complementary food development taro flour was mixed with maize flour and soybean flour and which were obtained from Faffa Food Company, Addis Ababa. The poultry experiment was conducted at Debre Zeit Agricultural Research Center (DZARC), located at an altitude of 1900 meters above sea level, at 8^{0}44' N latitude and 38^{0} 38"E longitudes and 47 km east of Addis Ababa.

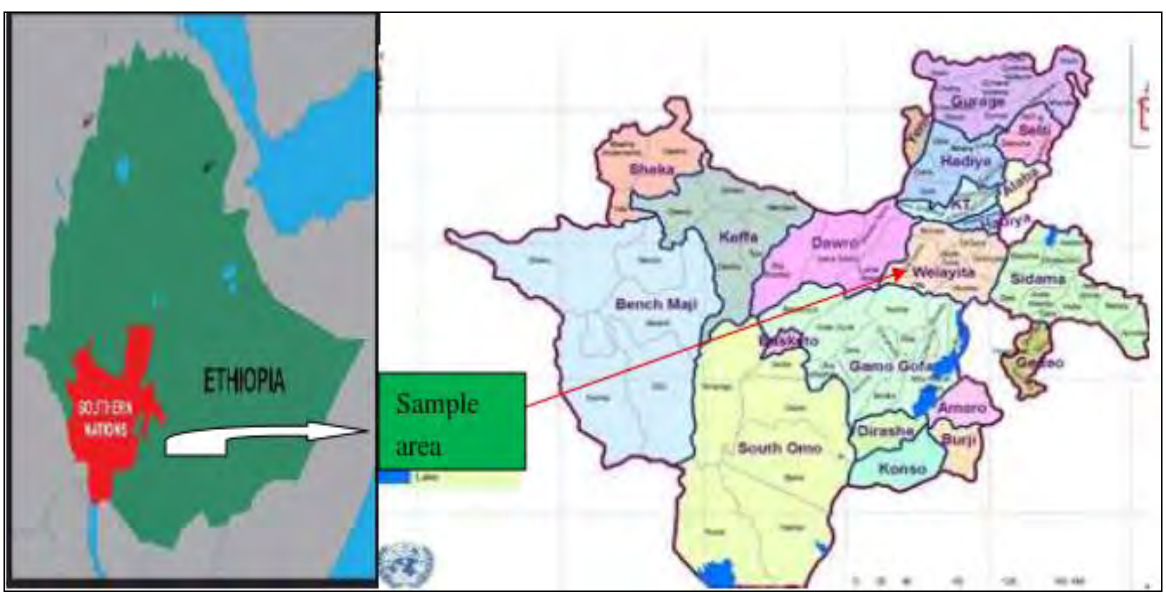

Figure 3. 1: Study sample source, Wolayita zone, SNNP, Ethiopia

3.2. Sample Preparation

3.2.1. Leaf powder and curd preparation

The fresh taro leaf has about two-thirds water (FAO, 2012). For this study, about 98 kg of fresh leaves from two varieties were divided in two equal portions. The first sub-divided (49 kg) was twigged the veins and dried in shade room by spreading the leaflets thinly on mesh tied on wood beds for 4 days according to (Cornelissen *et al*., 2003). The dried samples were powdered using stainless steel hammer mill to powder particle size of 1.6 mm, packaged in polythene bags and stored in cool, dry place until used for various chemical analyses about 16 kg. The second sub-divided (49 kg) was made into curd concentrates' fresh leaves were washed using tap water, graded based on green color, then cut into small pieces using leaf cutter, then were blanched to 65 ^{O}C for 12 minutes (He *et al.,* 2008), using a big metal pan to coagulate and separate the protein. Following this, the upper part of the boiled leaf was discarded to reduce anti-nutritional components and the extract retained was pressed using mechanical force presser to produce green curd. The curd was dried at standard dehydrating temperatures of 54 ^{O}C (Zhu *et al.,* 2007), was powdered and packaged in polythene bags about 13 kg and stored in cool, dry place until used for various chemical analyses.

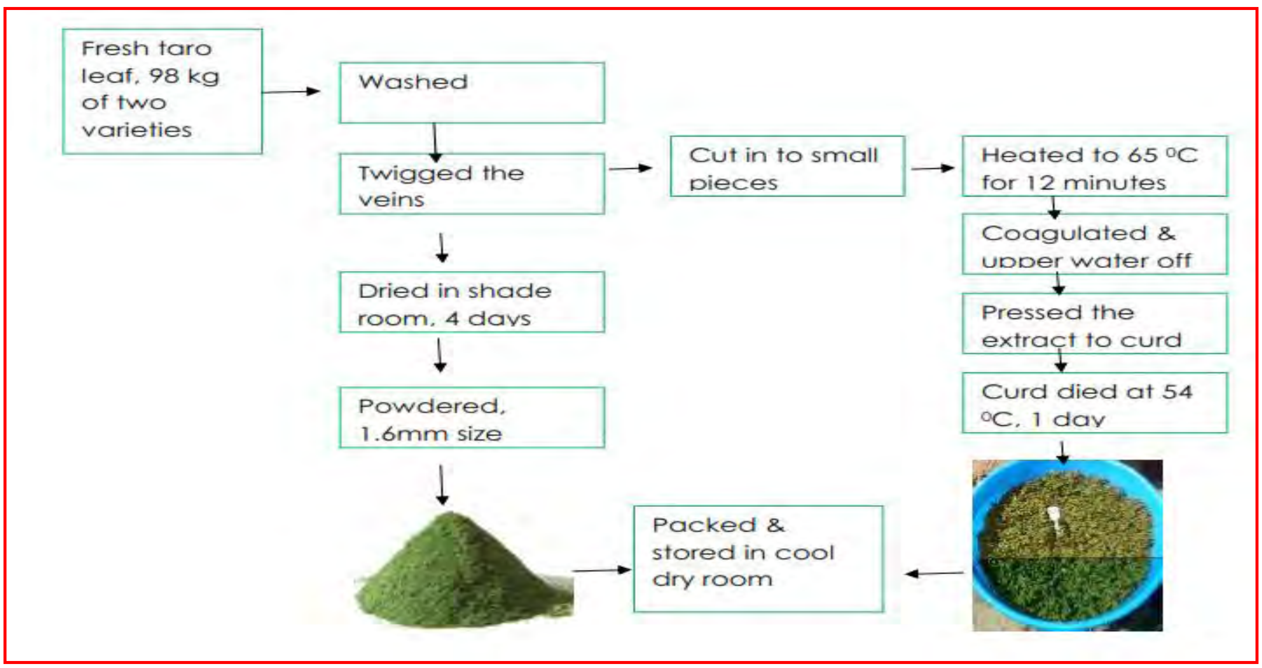

Figure 3.2a: Flow line for taro leaf sample preparation (Ikpeme-Emmanuel *et al.,* 2009)

3.2.2. Corm sample preparation

The fresh taro corm has about one-thirds water (FAO, 2012). About 72 kg of fresh leaves from two varieties were divided into two equal portions. The first sub-divided corm (36) were cleaned, peeled and steeped immediately in a portable tap water at room temperature for 30 minutes to prevent the browning of the peeled corm (Ojinaka , *et al.,* 2009; Bokanga, 1994b).Then prepared in the form of gelatinized and non-gelatinized flour. Following this the peeled corm was again held in portable tap water for 30 minutes to remove the surface contaminant, mucilage and then was sliced to 2 mm thickness using a manual kitchen slicer (Ikpeme-Emmanuel *et al.,* 2009). The sliced samples were then boiled at 95 ^{0}C for 30 minutes to reduce the trypsin and chymotrypsin inhibitors and to remove the acridity significantly (Bradbury *et al.,* 1992). After which, the water was removed by drying in an oven on aluminum foil lined trays at 105 ^{0}C for 2 hours (Chinnasarn and Rachada, 2010).

For the second pre-gelatinization method, the taro corm was selected and cleaned, then boiled, without peeling at 110 ^{0}C for 30 minutes. Following this, the boiled taro corm was air cooled and peeled. The peeled, pre-gelatinized corm was being sliced to 2 mm thickness using a manual kitchen slicer. After it was dried in an oven at 105 ^{0}C for 2 hours. Both the dried samples from the two methods were milled to flour using a hammer mill and then sieved into fine flour particles using a mesh screen sieve of 250 micron size to obtain the pre-gelatinized taro flour particle size of 1.6 mm, packaged in polythene bags (12 kg) and stored in cool dry place until used for various chemical analyses.

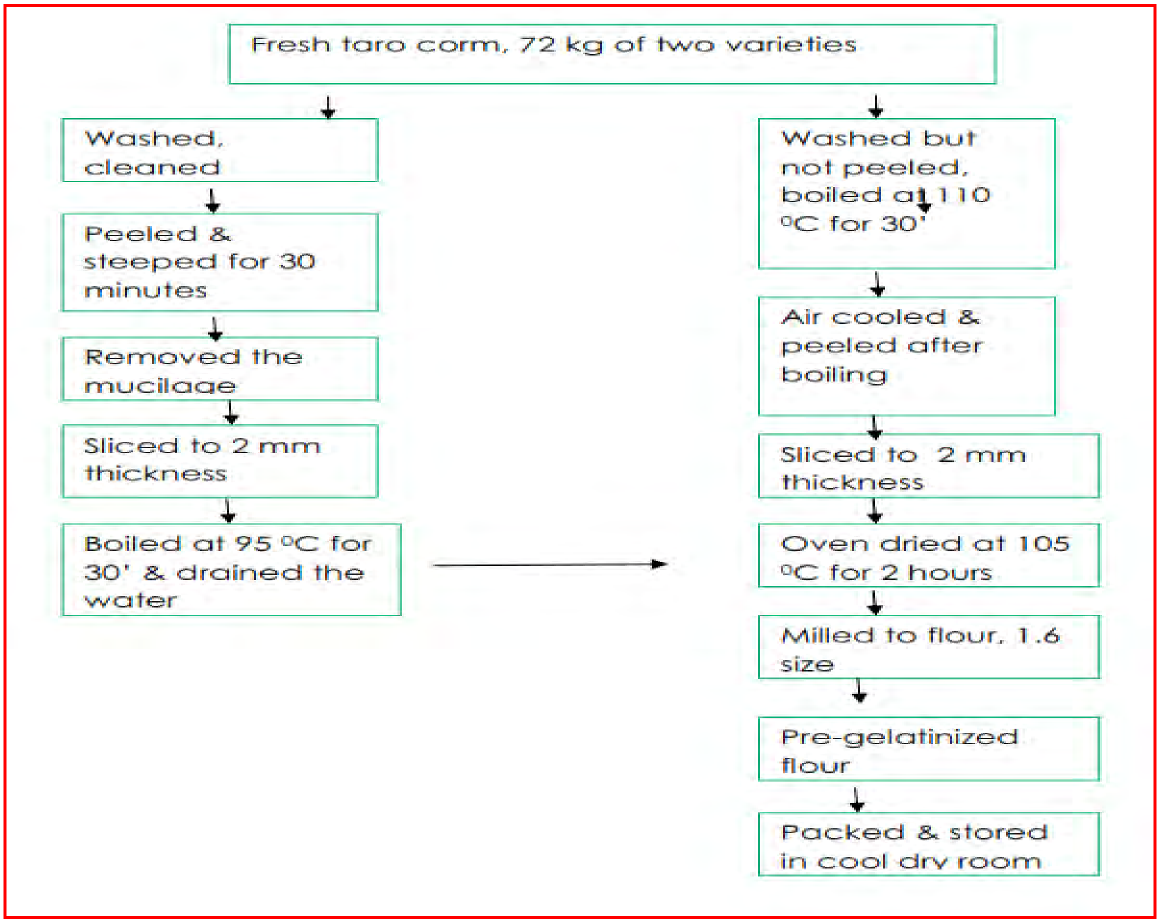

Figure 3.3b: Flow line for taro corm sample preparation (Ikpeme-Emmanuel *et al.,* 2009)

3.2.3. Complementary food formulation

The blending range was selected based upon the standard reference recommended requirement for complementary weaning food which shows energy in the range 360-400 kcal/100 g, the energy from protein not less than 10% (10 g/100 g), fat 4-6 g/100 g, iron 6-10 mg/100 g, zinc 4-9 mg/100 g, energy from carbohydrate 55-65 kcal/100 g, vitamin A 400µg retinol equivalent (RE) and calcium 300 mg/100 g (Walker, 1990; FAO/WHO, 2010).

Commercial complementary food (famix) was analyzed as a control. Taro flour from boloso-1 variety was mixed with two components, namely maize and soybean. The three components were used to optimize complementary formulation. In the blending of the components, the ingredients were constrained as taro 40-50%, maize 30- 40% and soybean 20-30%.

3.2.4.. Chicken feed ingredients formulation

The feed ingredients used in the formulation of the experimental ration for the study were taro leaf, corn grain, wheat middling, nougseed cake, soybean meal, TLM, vitamin premix, limestone, salt, lysine and methionine (Tahir and Pestig, 2012). Four treatment rations were formulated containing TLM at levels of 0% (T_1), 3% (T_2), 5% (T_3) and 7% (T_4). The formulation was using appropriate feed formulation program to be nearly isocaloric and isonitrogenous with metabolizable energy (ME) content of 3000 kcal/kg and crude protein (CP) content of 22% for the starter phase (1 to 28 days of age) and ME content of 3200 kcal/kg and CP content of 20% for the finisher phase (29 to 56 days of age) to meet the nutrient requirements. Eighty unsexed a-day old broiler chicks were randomly assigned to four dietary treatments as an observation trial in a completely randomized design experiment, thus having 20 chicks per treatment. The birds were vaccinated against Newcastle and Infectious Bursal Disease (Gumboro) at the recommended age of vaccination. Other health precautions and sanitary measures were also being taken throughout the study period.

Before the commencement of the actual experiment, the experimental watering and feeding troughs were thoroughly cleaned, disinfected and sprayed against external parasites. The chicks were brooded using 250 watt infrared electric bulbs with gradual height adjustment as sources of heat and light in a deep litter house covered with sawdust litter material. The feed was offered twice a day and clean tap water was available all the time throughout the experiment (Picard *et al.,* 2002). The experimental period lasted 56 days during which the amount of feed consumed was determined as the difference between the feed offered and refused. Feed offered and refused was sampled daily per treatment and pooled for the entire experimental period for chemical analysis.The feed protein intake was calculated as the percentage CP of experimental feed sample multiplied by feed consumed per birds to get true protein intake. For feed and fecal nitrogen mass balance, known mass of fecal samples was taken from each pen. Fecal dropped per birds were calculated as the amount of fecal dropped divided by the number of birds. The final mass of fecal dropped was calculated as percentage fecal CP multiplied by fecal dropped per birds to get true fecal dropped. Then true protein intake and fecal dropped were used to calculate feed protein digestibility.

For body weight (BW) change record, the chicks were weighed weekly and the initial and final weights were used to estimate weight gains. Average daily gain (ADG) was calculated as BW change divided by the number of experimental days (56 days). At the end of the experiment, 2 randomly selected birds from each treatment was starved for 12 hours before slaughter and slaughtered by severing the neck then dry de-feathered by hand plucking. Birds were eviscerated and carcass cuts were taken for nutrient composition and protein quality.

3.3. Methods for Proximate Analysis

All the analyses were conducted on a dry weight basis because; the biggest share of taro leaf and the corm is water. As indicated by FAO (2006), analysis of water content of fresh samples, especially leafy green crops and root were difficult. Therefore, the dry matter contents of the samples were determined after drying the samples and grinding into flours.

3.3.1. Determination of moisture content

The moisture content of the sample was determined according to the standard method of AACC (2000). A crucible was dried in an oven at 105 ^{0}C for 1 hour and placed in desiccators to cool. The weight of the crucible (W1) was determined. Samples (3g) was weighed in the crucible (W2) and dried at 130 ^{0}C for 1 hour. After cooling in a desiccators to room temperature, it was again weighed (W3). The moisture content was determined as follows.

Moisture content in percent (%) = $\left(\dfrac{W_2 - W_3}{W_2 - W_1}\right) \times 100$ (3.3.1)

3.3.2. Determination of crude protein

Total nitrogen of the sample was determined by the micro- Kjeldahl method according to AOAC (2005) standard method No. 925-09 using Automatic digestion and distillation systems (Model UDK-142, Europe). Protein nitrogen was transformed to ammonium sulfate by hot digestion of the dry sample with concentrated sulfuric acid in the presence of a catalyst. Ammonia was liberated from the sulfate by distillation after addition of sodium hydroxide (40 %) and driven into a known volume of boric acid solution. From the ammonium borate formed, the amount of ammonium ion attached to borate was titrated with standardized about 0.1 M HCl. The % of protein was estimated from % nitrogen as follows:

$$\text{Nitrogen (\%)} = \frac{V\ HCl\ in\ L x\ N\ HCl\ (ca.\ 0.1)\ x 14.0}{Sample\ weight\ on\ dry\ matter\ basis} x 100 \quad \text{------------ (3.3.2)}$$

$$\text{Protein (\%)} = \%\ N \times F$$

Where: V is volume of HCl in L consumed to the end point of the titration, N is the normality of HCl used (often 0.1N) and 14.00 is the molecular weight of nitrogen. The percent of the nitrogen is converted to % protein by using the appropriate conversion factor, Conversion factor (F=6.25). Urea was used as a control in the analysis.

3.3.3. Determination of crude fat content

About 2g of the sample was extracted with about 150 mL diethyl ether for a minimum period of 8 hours in the soxhlet extractor. The solvent was evaporated by heating on a steam bath. The flask containing the extracted fat was dried in a drying oven at 100 ^{0}C for 1h and the mass was determined (AACC, 2000 method No. 30-10).

$$\text{Crude fat, percent by weight} = \frac{W2-W1}{W} \times 100 \quad \text{------------ (3.3.3)}$$

Where: W1 = Weight of the extraction flask (g), W2 = Weight of the extraction flask plus the dried crude fat (g) and W = Weight of sample flour (g)

3.3.4. Determination of ash content

The ash content of the sample was determined according to AOAC (1995) method No. 923-09. Clean porcelain dish was ignited at about 550 ^{0}C in a muffle furnace for 3 hrs and it was cool in desiccators (Nalgene Model 5317-0120) and weighed as (M_1). Then about 3 g samples were put into the porcelain dish and weighed as (M_2). These samples were dried at 120 ^{0}C for 1h and were carbonized on a hot plate.

The dish with its contents was transferred to a muffle furnace and ignited at about 550 ^{0}C until ashing complete. The residue was weighed as (M_3). The total ash was expressed as percentages in dry matter basis as follows:

$$\text{Total ash (\%)} = \frac{M3-M1}{M2-M1} x 100 \quad \text{------------ (3.3.4)}$$

Where: M_2– M_1*is sample mass in g on dry base and* M3– M_1*is mass of ash in g*

3.3.5. Determination of crude fiber content

The crude fiber content was analyzed as described in crude fiber content Method No 32-10. About 2g sample was transferred to 600 mL beaker. After digestion with 1.25% sulfuric acid, it was washed with distilled water and then digested by 1.25% sodium hydroxide. It was then filtered in a coarse porosity 75-76 µm crucible in the apparatus at a vacuum of about 25 mm. The residue left after refluxing was washed again with 1.25% sulfuric acid near boiling point. This residue was then dried at 110 ^{0}C for one hour, cooled in a desiccators and then it was weighed (*M1*). After ashing at 550^{0}C, it was cooled in desiccators and weighed again (*M2*). The total crude fiber was expressed in percentage as:

$$\text{Total crude fiber (\%)} = \frac{M1 - M2}{M3} \, x \, 100 \quad \text{------------ (3.3.5)}$$

Where: *M1 is the dried sample, M2 is the dried ashed sample and M3 is the weight of sample*

3.3.6 Determination of total carbohydrate content

The total carbohydrate content was determined by difference from other nutrients, according to (Egan *et al.,* 1981): Carbohydrate = 100 – [%M + % P + %F +% Fb + % A]-------- (3.3.6)
Where: *C = Carbohydrate content, M = Moisture content, P= Protein, F = Fat content, Fb = Fiber content and A =Ash content.*

3.3.7 Determination of amylose content

The amyloses (%) of taro leaf and corm samples were determined by a calorimetric method (Zhu, *et al.,* 2008). The 50 mg sample was measured into a screw-capped test tube (20 mL) and then the lipid was extracted by adding 5mL methanol (85%) in a shaking water bath (100 rpm) adjusted to 70 ^{0}C for 30 minutes with an intermittent vortex mixing. The sample was separated from the supernatant (methanol + lipid) by centrifuge at 2000 rpm for 2 minutes and the supernatant (methanol + lipid) was carefully decanted off. The starch in the residue was dispersed and solublized by adding 2 mL of 2M NaOH and vortex mixing and heating in a boiling water bath. Then 6mL of distilled water was added and allowed to dissolve in a shaking (100 rpm) boiling water bath with occasional vortex mixing. Additionally, 2mL distilled water was added and heated until the starch completely dispersed. Sample (0.1 mL) was mixed with 5mL of 5% trichloroacetic acid (TCA) and 0.2mL of 0.01 M I_2-KI was added and mixed thoroughly. Then, the absorbance of the blue color developed was measured at 620 NM using UV-VIS spectrophotometer after 30 min. Against distilled water as a reference. The absorbance of the sample was determined by taking absorbance of the blank subtracted from the sample absorbance. The absorbance of the standard amylose (prepared from maize amylose and amylopectin) was taken in the range 0 – 100% amylose in 10 % intervals and the percentage of amylose was estimated from the standard calibration curve (Diego, *et al.,* 2013).

3.3.8. Determination of energy density

The energy density (DE) of taro leaf samples was calculated with 4 kcal/g for carbohydrates, 4 kcal/g for proteins and 9 kcal/g for lipids (crude fat) according to Livesey (1995). ED (energy density) = 9 x crude fat (%) + 4 x [carbohydrates (%) + proteins (%)]. However, the metabolizable energy (ME) content of the chicken experimental diets was determined according to Wiseman (1987) ME (kcal/kg DM) = 3951+54.4ether extract-88.7crude fiber-40.80ash.

3.3.9. Quality Control

To minimize the risk of extrinsic conditions, all glassware used for the analytical method was washed, and sterile disposable powder free plastic gloves were worn when handling food samples during sample preparation and analysis stages. In order to validate the accuracy, linearity and limit of quantification of the methods used for fatty acid and amino acid analysis, certified reference standards (Sigma and Fluka) were used.

3.4. Methods for Fatty acids Analysis

3.4.1. Extraction

Lipid was extracted in hexane: isopropanol (3:2 v/v) with a modified method adapted from a previous study (Ostermann, *et al.,* 2014). Approximately 1 g of each sample was used in duplicates and placed in glass tube with 10 mL hexane: isopropanol (HIP) (Sigma, USA) and homogenized for 3*30 seconds (5411 g) (ULTRA-TURRAX T25, IKA). The homogenizer was rinsed with HIP between samples. The homogenate was then quantitatively transferred to teflon centrifuge tubes using 5 mL HIP and 6.5 mL Na_2SO_4 (6.67% w/v). Samples were centrifuged at 4000 rpm, 18 °C, for 5 minutes, after which the upper phase was removed to pre-weighed evaporation tubes using glass Pasteur pipettes. One milliliter of hexane was added to the centrifuge bottle and centrifugation was repeated. The upper phase from both centrifugations were then combined and evaporated at 40°C with N_2 flushing for approximately 40 minutes until dried. Evaporation tubes were reweighed and the amounts of fat extracted were calculated. Another 0.5 mL hexane was added into the evaporation tubes, rinsed and transferred to the small glass tube. Teflon tape was used, then the samples were vortexed and stored in a freezer at -18°C.

3.4.2. Methylation

The concentration of lipids dissolved in hexane was calculated before methylation, by microbalance weighing (Metller type UMT2, Switzerland). The methylation of fatty acids was done according to (Ostermann, *et al.,* 2014). Based on the microbalance lipid concentrations, required volumes of lipid solutions with 2 mg content were transferred to glass tubes with 2 mL methanol and 15 µl standard fatty acid solution (STD) (C17:1), where STD (1.44 µg/µl) was used as an internal standard for gas chromatography. The glass tubes were vortexed and incubated in a heating block at 60°C for 10 minutes. Three milliliters of BF_3 were added to tubes and followed by incubation under the same conditions. Afterwards, the samples were cooled in ice box for 15 minutes, after which 2 mL 20% NaCl and 2 mL hexane were added. After 10 second vortexing, the tubes were stored at 4°C for 20 minutes. The upper phase was transferred to small glass vial with Pasteur pipettes and again stored at 4°C for 20 minutes. Transfer of the upper phase was repeated once more with 1 mL hexane added to the tube.

3.4.3. Thin layer chromatography (TLC) checking

The tubes were evaporated at 40°C with N_2 gas until dried (approx 20 minutes). Finally, 300 µl aliquots of lipids were transferred into test tubes and kept at -18 ^{0}C until GC analysis.

The methylation was checked on a TLC silica plate. A solvent was made of hexane: diethyl ether: acetic acid (85:15:1, v: v: v) one hour before using. Then the silica plate was prepared, by drawing a line with a lead pencil and mark out 7 dots plus a standard dot to show were to put the samples. Thereafter the methylated samples and the standard were vortexed and applied (3µl) to the silica plate. The TLC plate was placed in the chamber for one hour (with the solvent at the bottom of the chamber). After one hour the silica plate was taken up, and dried by leaning it towards the chamber for approximately 20 minutes. Thereafter the silica plate was put down into a chamber with iodine and then it was left standing there for another

20 minutes. The fatty acid methyl esters were recognized by comparison to the standard TLC mixture.

3.4.4. GC-FID instrumentation and analytical procedure

Fatty acids were analyzed with a Gas chromatography-Flame ionization detector (GC-FID) system (Varian CP-3800, Sweden) with a flame ionization detector (FID) equipped with a 50 m*0.22 mm inner diameter, 0.25 µm film DB-5 fused capillary column (Agilent Technologies, USA). The column temperature was programmed to initiate at 158 °C for 5 minutes and increased by 2 °C/ minute up to 220 °C and remained for 8 minutes. The makeup gas was nitrogen and carrier gas was helium (0.8 mL /min). The injector and detector temperatures were 230 and 250 °C, respectively. Fatty acids were analyzed by comparing with the standard fatty acid solution (STD) and retention time. Chromatograms were analyzed using Galaxie chromatography data system software version 1.9 (Varian AB, Sweden).

3.5. Methods for Amino acid Analysis

3.5.1. Digestion

Samples were digested in acid and alkaline medium to the complete hydrolysis of the protein fraction according to (Kim, *et al.,* 2013; Luyen, *et al.,* 2012). Briefly, 100 mg, of each sample, taro leaf and corm were digested with 3 mL of 6 N HCl at 200^0C in heated oven for 24 hours after sealing tubes with nitrogen gas to prevent oxidation. The digested samples were filtered with Whatman No. 6 and the filtrates were evaporated at 100^0C water bath for removing the chlorine gas. Hydrolyzed protein was completely dry with nitrogen gas and re-constituted with 200µl (0.2 mL) of 0.1 N HCl. For Trypthopan, alkaline hydrolysis was used and 50 mg, of each sample, taro leaf and corm were suspended in 20 mL of 3N-NaOH and sealed under N_2 gas and hydrolyzed for 3 hours at 110^0C heating oven. Following hydrolysis, centrifuged for 10 min at 4,000rpm and then supernatant was taken and diluted with 50 folds with water (milliq). Then the final acid and alkaline hydrolyzates were filtered (0.2 µm) and inject into UHPLC system using MPA/OPA/FMOC derivatization protocol. Mercaptopropionic acid (MPA) used as catalyst, *o*-Phthaldialdehyde (OPA) and Fluorenylmethyl chloroformate (FMOC) used as reagents for primary and secondary amines derivetization, respectively

3.5.2. UHPLC instrumentation and analytical procedure

Amino acid analysis was conducted with the Shimadzy Nexera UHPLC system (Shimadzu, Columbia, MD). Pre-column derivetaization was taken automatically by the instrument using *o*-Phthaldialdehyde (OPA) for all primary amino acids and Fluorenylmethyl chloroformate (FMOC) for secondary amino acids (Proline and Hydroxyproline). The UHPLC system consisted of a binary pumping system: pump A (LC-10AD vp) and pump B (LC-10AT vp), a degasser (DGU-14A), an Autosampler (SIL-20AC HT), column heater (Brinkmann, CH-30) and Fluorescence detector and system controller (CBM-20A). Mobile phase A was a mixture of Na_2HPO4, Na_2B4O7 NaN_3 while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v).

The separation was obtained at a flow rate of 2 mL/min with a gradient program that 0.01 min (1% B), 7.4 min (40% B), 10 min (45% B), 10.1(100% B). Then washing at 100% B and calibration at 0% B was performed in a total analysis time of 12.1 min (Carl, 2015). In order to quantify amino acids, the mix standard was used from Asparagine, Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Serine, Tyrosine, Valine, Proline, Tryptophan, Cysteine, Norleucine and Hydroxylproline were prepared and used for easy identification of peaks in the mix as well as their individual amino acid standards (Kim, *et al.,* 2013).

3.6. Methods for Mineral Analysis

3.6.1. Iron

Iron content was analyzed by atomic absorption spectrophotometer (AACC, 2000). A sample of 3 g was weighed into an ashing vessel (that has been pre-ignited at 550°C and cooled in desiccator). The sample was carbonized on a hot plate and ashed in the muffle furnace at 550 ^{0}C. And, the ash was dissolved in 10 mL dilute HCl (0.1M). The solution was boiled and evaporated nearly to dryness on the steam bath. The residue was re-dissolved quantitatively in 20 mL 1M HCl and filtered through Whatman filter paper into 50 mL volumetric flask.

The paper and the residue were held thoroughly with water and were diluted to 50 mL mark. A standard iron solution containing (1000µg Fe/ mL) was prepared by dissolving 1.00g of pure iron wire in about 30 mL of 6M HCl with boiling and diluted to 100 mL. A series of 0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ppm was prepared from the working stock solution of Fe

(10µg Fe/ mL), which was prepared from (1000µg Fe/mL) to construct the calibration curve. Both the standard solutions and the samples were analyzed using the atomic absorption spectrophotometer and absorbance was measured at 248.3nm (AACC, 2000). Concentration of samples was read from plot of absorbance against ppm Fe. Finally the ppm concentration was converted to mg/100g.

$$Iron\,content\,(mg\,/100g) = \frac{(Cs - Cb)\,V \times D}{S} \qquad \text{------------ (3.6.1)}$$

Where: *Cs and Cb is concentration in µg/* mL *of analyte and blank, respectively. V is an original volume (50* mL*), D is dilution factor (if an original solution is diluted) = dilution volume (*mL */original aliquot volume (*mL*) used for dilution. S is sample mass in g (db).*

3.6.2. Zinc

Zinc content was determined by Atomic Absorption Spectrophotometer (AACC, 2000). A sample of 3 g was taken in to the ashing vessel (that has been pre-ignited at 550 ^{0}C and cooled in desiccators). Ashing was done at 500 ^{0}C over night and the ash was dissolved in 10 mL of concentrated HCl. The solution was boiled and evaporated to dryness on a steam bath to dissolve the residue. The residue was re-dissolved quantitatively in 20 mL 2M HCl. It was filtered through Whatman filter paper into 50 mL volumetric flask. The paper and residue were held thoroughly with water and was diluted to the 50 mL mark. Standard Zn solution (10µg Zn/mL) was prepared from analytical grade ZnO by dissolving 1.3830 g in 10mL 6M HCl and diluted to 100 mL and 5mL of the solution was taken and diluted to 500mL mark with distilled water. A series of standard solutions (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0) to construct the calibration curve was prepared from stock Zn solution (10µg Zn/ mL). Both the standard solution and the sample were analyzed using the atomic absorption spectrophotometer and absorbance was measured at 213.8nm. Concentration of samples was read from plot of absorbance against µg/ mL Zn. Zinc content was calculated by the following formula:

$$\text{Zinc content (mg/100g)} = \frac{(Cs - Cb)V \times D}{S} \qquad \text{--------------- (3.6.2)}$$

Where: *Cs Concentration of sample in µg/* mL, *Cb= Concentration of blank in µg/* mL *, V= Original volume (50* mL *), D= Dilution factor (if any used) and S = Sample mass in g (db).*

3.6.3. Calcium

Calcium content was determined by atomic absorption spectrophotometer (AACC, 2000). Sample of 3g was weighed into an ashing vessel (that has been pre-ignited at 550 ^{O}C and cooled in desiccator). The sample was carbonized over a blue flame of a Bunsen burner and put in the muffle furnace at 550 ^{0}C until ashing is completed. And, the ash was dissolved in 10 mL dilute 3M HCl. The solution was boiled and evaporated nearly to dryness on the steam bath. The residue was re-dissolved quantitively in 20 mL 2M HCl and filtered through coarse, porous filter paper into 100 mL volumetric flask. The residue on the paper was held thoroughly with water and diluted to 100 mL mark. Standard solution (25µg Ca/ mL) was prepared from analytical grade $CaCO_3$ by dissolving 1.249 g in 30 mL and 50 mL distilled water and it was diluted to 1L. Finally, calcium was measured by adding enough La stock solution to make the final dilution 1% La (*i.e.,* 5 mL Ca solution to 50 mL flask) and this was added to the sample and final standard solutions (0, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 ppm). The absorbance of the sample was read with an Atomic Absorption spectrophotometer at 422.7 nm. The calcium content was calculated using the following formula:

Calcium content (mg/100g) = $\frac{(Cs - Cb)V \times D}{S}$ **---------------** (3.6.3)

Where: *Cs and Cb is concentration in µg/mL of analyte and blank, respectively. V is an original volume (50 mL), D is a dilution factor (if original solution is diluted) = dilution volume (mL /original aliquot volume (mL) used for dilution. S is sample mass in g (db).*

3.6.4. Total phosphorous

The sample solutions prepared for mineral determination was used for phosphorous determination. One mL of the clear extract was diluted in 50 mL with deionized water. Sample solution (5mL) was added into a test tube. Molybdate (0.5mL) and 0.20mL aminonaphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. The solutions were allowed to stand for 10 minutes. Then following this, the P content in the sample was determined using atomic absorption Spectrophotometer (AAS) at 320 nm. P content (mg/100g) = $\frac{(Cs - Cb)V \times D}{S}$ **---------------** (3.6.4)

3.6.5. Other major and minor minerals

Upon ashing for the determination of the total ash content 3 drops of 1M HNO3 acid was added to the sample in each of the crucibles. The ash was digested by using 6N hydrochloric acid. The digested sample was filtered into sample bottles each using the Whitman filter paper (42 mm) prior to analysis. The K, Na, Mg and cupper, content in the sample was determined using atomic absorption Spectrophotometer (AAS) at 248.3 nm, 213.9 nm, and 285.2 nm wavelengths, respectively, using an air acetylene flame.

$$\text{K, Na, Mg, or Cu content (mg/100g)} = \frac{(Cs - Cb)V \times D}{S} \quad \text{--------------- (3.6.5)}$$

Detection limit control

Different concentration of the standard mineral solution used to detect the lower limit before sample analysis and correlation coefficients of the calibration curve for standard solution was compared with the samples analysed. Dilution factor was applied to correcting the coefficients range of samples with the standard solution. The appropriate wavelength for each mineral was set. Blanks were used to stabilize the accurate and consistent reading of measurements.

3.7. Phyto-chemical and Anti-nutritional Analysis

3.7.1. Beta carotene

Beta carotene was determined according to Laurie, *et al.,* (2012). Sample (1g) was accurately weighed in a glass test tube. Then 5mL of chilled acetone was added to it. Then the tube was held for 15min with occasional shaking and finally centrifuged at 1400 × g for 10 min. Supernatant was collected into a separate test tube, and then re-extracted with 5mL of acetone followed by centrifugation once again as above. Both of the supernatants was pooled together and then passed through the Wahtman filter paper No. 42. The absorbance of the extract was determined at 449 nm wavelength in a UV-Vis-spectrophotometer (Laurie, *et al.,* 2012).

The standard stock solution (1mg/mL) was prepared by dissolving standard beta carotene in aceton and working standard (32µg/mL) was prepared from 1mg/mL stock solution. From 32µg/mL a series of five standard solutions (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ppm) were prepared. The amount of beta carotene was expressed in µg/100g after multiplying the ppm value by 0.01 using the following formulas:

$$\text{Calculation: Amount } (\mu g/g) = \frac{A \times V(ml) \times 10^4}{A \times W} \quad \text{---------------} \ (3.6.5)$$

Where: ***A:*** *Absorbance;* ***V (ml):*** *Volume of the solution that gives an absorbance of* **A** *at a specified wavelength;* ***W:*** *weight of sample in gram*

3.7.2. Total phenolic compound analysis

Total phenolic content was determined according to the Folin-Ciocalteu method (Sharma and Gupta, 2010). Samples 200 mg was extracted with 4 mL of acidified methanol (HCl/Methanol a/Water, 1: 80: 10V/V/V) at room temperature for 2 hrs. An aliquot of the extract (200µl) was added to 1.5 mL freshly diluted (10 fold) Folin-Ciocalteu reagent. The mixture was allowed to equilibrate for 5min and then mixed with 1.5 mL of (60g/L). After incubation at room temperature for 90 min, the absorbance of the mixture was read at 725 nm (6505 UV/vis spectrophotometer). Acidified methanol was used as a blank. From the stock solution of standard Gallic acid, a series of six standard solutions (0, 5, 10, 15, 20, 25, 30, 35ppm) was prepared.

The amount of total phenolic was estimated from the calibration curve as Gallic acid equivalent (GAE) in milligram per kilogram of the sample. UV/vis analysis was used for a simultaneous measurement of several phenolic compounds.

$$\text{Phenolic cpd} = \frac{mgGalacid}{gmextract} \quad \text{-----------} \ (3.7.2)$$

3.7.3. Determination of mucilage

Taro leaf and corm samples were separately mixed with distilled water in 1:20w/v ratio and were stirred for 30 minutes to complete the extraction (Khullar, *et al.,* 2013). Then the extract was separated by filtration using glass wool. And the solution then was concentrated on a rotary evaporator at 40^{o}C. Following this, the precipitate was formed from the extracted with 80% ethanol in distilled water in 1:4v/v. The precipitate was standing for 1 hour at 4^{o}C and was centrifuged at 6000rmp for 35 minutes. Finally, the centrifuged residue was dried and was measured. The measured result was compared against the crude protein contents (Yamazaki *et. al*., 2008).

3.7.4. Determination of phytic acid

Phytic acid was determined through phytate phosphorus (Ph-P) analysis according to (Guttieri *et al.,* 2004). About 0.25g of flour sample was extracted with 12.5 mL of 3% trichloroacetic acid for 45min in a water bath with vortex mixing at ambient temperature (23^0C) and centrifuged (4000rpm/10 min). The supernatant was used for phytate estimation. About 4 mL of $FeCl_3$ was added to 10mL of the sample solution and centrifuged. The clear supernatant was carefully decanted and the precipitate was held by 20mL of 3% TCA, 0.2M of HCl and 20mL distilled water. The precipitate was digested by concentrated H_2SO_4 and H_2O_2 (30%). Digestion converts the phosphorus into phosphate. The phosphate generated was analyzed by measuring the absorbance of phosphomolybdate blue generated in addition of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$]. The stock P solution was prepared by taking 0.1mg KH_2PO_4 in to 250mL H2O. From the stock solution series of solutions (0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm) were prepared for calibration curve. The absorbance at 822nm was read using a spectrophotometer.

The absorbance of the sample was subtracted from the blank and phosphorus level was estimated from the calibration curve. Then phytate was estimated as phytate phosphorus (i.e., phytate = P × 3.55). Phytate: mineral molar ratios were calculated using the molecular weight of IP6=660.

$$\text{Phytic acid in } \mu g/g = \frac{[(Ab - As)Intercept] * 10}{Slope \times w \times 3} \quad \text{-----------} \ (3.7.4)$$

Where: A_s*= sample absorbance,* A_b*= blank absorbance, d= density and W= weight of sample*

3.7.5. Phytate/mineral molar ratio

The molar ratio between phytate and mineral was obtained after dividing the mg/100g of phytate with the mg/100g of minerals (Norhaizan and Nor Faizadatul Ain, 2009). Phytate phosphorous was calculated by assuming phytate contains 28% phosphorus, i.e. [Phytate P =

phytate * 0.28]. Accordingly, non phytate phosphorous = total phosphorous - phytate phosphorous.

3.7.6. Determination of oxalate

The AOAC (1990) method was used to determine the oxalate content of the sample. The procedure involves three steps: digestion, oxalate precipitation and permanganate titration. Digestion**:** Sample, (2g db) was suspended in 190mL of distilled water contained in a 250mL volumetric flask; 10mL of 6M HCl was added and the suspension was digested at 100°c for 1hr, followed by cooling and then made up to 250mL before filtration. Oxalate precipitation**:** Duplicate portions of 125mL of the filtrate was measured into a beaker and four drops of methyl red indicator mixed, followed by the addition of concentrated NH_4OH solution (drop wise) until the test solution changed from its salmon pink color to a faint yellow color (pH 4–4.5). Each portion was 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 mixed 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 min. The supernatant was decanted and the precipitate was completely dissolved in 10mL of 20% (v/v) H_2SO_4, solution.

Permanganate titration: The total filtrate resulting from digestion of 2g of the sample was made up to 300mL. Aliquots of 125mL of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$, solution to a faint pink color which persisted for 30s. The calcium oxalate content was expressed as calcium oxalate equivalent and calculated usingtheformula:

$$\text{calcium oxalate } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{T \times (V_{me}) \times (DF) \times 10^5}{(ME) \times M_f} \quad \text{-----------} \ (3.7.6)$$

*Where: T = Titrate of $KMnO_4$ (mL);V_{me} = Volume–mass equivalent (i.e. 1cm^3 of 0.05M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid); DF = Dilution factor V_T/A (2.4, where V**T** is the total volume of filtrate (300mL) and A is the aliquot used (125 mL); ME = Molar equivalent of $KMnO_4$, in oxalate ($KMnO_4$, redox rxn+5) and M_f = Mass of sample used*

3.7.7. Determination of Tannins

Tannins were determined using the method of (AOAC, 1995). About 0.25gm of sample was weighed in a screw capped test tube and 10mL of 1% HCl in methanol was added to each

1% HCl containing the samples, then the tubes was put on a mechanical shaker for 24 hours at room temperature. After 24 hours of shaking, the tubes were centrifuged using (DYNAC II centrifuge, Clay Adams, a division of Becton and Dikinson Company, USA) at 1000xG for 5 min. One mL of the clear supernatant was taken and mixed with 5mL of vanillin-HCl reagent in another test tube and this mixture was allowed to stand for 20 min to complete the reaction. After 20 min the absorbance was read at 500 nm using a spectrophotometer (BECHMAN Du 64, Japan). The concentration of tannins was calculated using D-Catechin standard curve and results was expressed as of D- Catechin equivalent in mg per 100g dry weight. Tannins were determined by the AOAC Official method 49.49 (AOAC, 1984).

$$\text{Tannin in mg/g} = \frac{[(Ab - As)\text{Intercept}] * 10}{\text{Slope} * w * d} \quad \text{-----------} \ (3.7.7)$$

Where: *A_s= sample absorbance, A_b= blank absorbance, W= weight of sample and= density of the solution*

3.7.8. Determination of protease inhibitors

Taro leaf and corm samples were separately defatted with hexane 1:5(w/v) for 10 minutes and were filtered using filter paper (Bhattacharyya and Babu, 2009). Then, the sediments were rinsed with hexane to remove any residual fat from the samples. The rinsed residue was oven dried (105^{0}C) in 30 minutes and was extracted with0.01M/0.02M NaOH and distilled water at 1:5 w/v ratios. Then the extract was centrifuged at 8000rpm for 30 minutes and the final residue after centrifuge was taken in gram. The inhibitory activity was compared in terms of the protein content.

3.7.9. Determination of lectins

Taro leaf and corm samples were separately mixed with 0.15M Nacl (1:8w/v) for 24 hr at 4^{0}C and were homogenized with a sodium borate buffer in a shaker overnight (De Leo, *et al.,* 2001). The samples were then filtered and serial dilutions were made. The diluted series was then filtered and centrifuged at 900 rpm for 30 minutes. The supernatant was precipitated using NH4So_4 dissolved in minimal distilled water. Then the residue was compared in terms of the protein contents of the sample to see the inhibitory effect with biological protein.

3.7.10. Determination of α-amylase inhibitors

Taro leaf and corm samples were separately extracted with ethanol (99%) and the extracts was evaporated at 45^0C then was dried for 10 minutes using water bath (Noman, *et al.,* 2006). Then buffer solution pH of 6.9 containing alpha amylase solution was added. Following this 1% starch solution was added and was incubated at 25^0C. Then, 3.5 dinitrosalicuclic acid reagents were added and incubated in a water bath for 5 minutes. Then was cooled and 10mL distilled water was added. Finally, the result was measured the absorbance at 546nm and the final result was compared with the starch or total carbohydrate contents of the sample to see the alpha amylase inhibitory effect. An alpha-amylase inhibitory activity was estimated according to the formula below:

$$I\% = \frac{Ac - As \times 100}{Ac} \quad \text{-----------} \; (3.7.10)$$

Where: Ac= absorbance of control and As = absorbance of sample

3.8. Methods for Functional Property Analysis

3.8.1. Determination of water holding capacity

Water holding capacity of infants and chicken diet samples was determined according to Anderson *et al* (1969). Sample (0.63 g) was placed in 10 mL centrifuge tube and suspended in 7.5 mL distilled water. Then was be incubated in 7.5 mL distilled water. At about 25^0C for 30 minutes and was centrifuged at 3000 g for 5 minutes. The mass of the sample was determined before and after decantation of the clear supernatant of the centrifugation. The WAC was calculated as grams of adsorbed water per gram of dry sample mass (0.625 g). The clear dried supernatant of the centrifugation was transferred into pre-dried (105^0C) and the glass beaker (50 mL) was weighed for the estimation of the water solubility index (WSI).

$$WAC\ (\%) = \frac{Ws}{Wo} \quad \text{----------------------------} \; (3.8.1)$$

Where*: W_s = weight of supernatant (g) and W_o = weight of sample (g)*

3.8.2. Determination water solubility capacity

The supernatant, containing solubilized starch preserved from WAC measurement was collected, dried at 120^0C to constant weight, and the residue was weighed. The WSC was calculated as a ratio of dry residue to the original mass (about 0.625 g) used to estimate WAC. The result was expressed as a percentage (Anderson *et al.,* 1969).

WSC (%) = $\frac{Wr \times 100}{Wo}$ ---------------------------- (3.8.2)

Where: W_r = *is the weight of residual (g),* W_o = *Weight of sample (g)*

3.8.3. Determination of water hydration capacity

The water hydration capacity (WHC) was estimated according to AACC (2000) method 56-20. Prior, centrifuge tube was held, dried in an oven, cooled in the oven and weighed (W_T). Sample (0.2 g) was measured in 10mL centrifuge tube, 4mL distilled water was shaken vigorously to thoroughly suspend the sample. After sample suspension stands for 10 min, it was centrifuged at 1000 × g for 15 min. The clear supernatant was drained. The residue inside the tube was weighed (W_{SAT}) and the hydration capacity were calculated as follows:

WHC = $\frac{Wsat}{Ws}$ ---------------------------- (3.8.3)

Where: W_s = *Sample weight on dry matter basis (g)*

3.9. Methods for Protein Quality Analysis

3.9. 1. Determination digestibility

The feed protein digestibility was calculated as apparent and true protein digestibility using the following formula (FAO/WHO, 1993).

Apparent Digestibility (%) = $\frac{\mathrm{I} - F \times 100}{I}$ ----------------------- (3.9.1a)

Where, *I: Nitrogen Intake; F: Fecal Nitrogen output on test diet*

True Digestibility (%) = $\frac{\mathrm{I} - (F - Fk) \times 100}{I}$ ----------------------- (3.9.1b)

Where, F_k = *Fecal protein of non protein diet in the experiment*

3.9.2. Determination of protein efficiency ratio

Protein efficiency ratio (PER) was calculated based on the weight gain of birds divided by intake of a particular food protein during the test period (AOAC, 2000 Official Method).

$$PER = \frac{Gain\ in\ body\ mass(g)}{Protein\ intake(g)} \text{ ---------------------- (3.9.2)}$$

3.9.3. Determination of protein digestibility corrected amino acid score

Protein digestibility corrected amino acid score (PDCAAS) was determined from protein digestibility multiplied by the amino acid score (AOAC, 2000 Official Method 999.29). Where the amino acid score was measured as gram of essential amino acid in the food sample divided by a mile gram of reference essential amino acid.

The PDCAAS can be calculated as follows:

$$\text{PDCAAS (\%)} = \frac{\text{True protein digestibility} \times \text{amino acid score}}{} \text{ ------------ (3.9.3)}$$

Where: AA score = mg EAA/g food protein or *(*Limiting EAA/Reference EAA),

3.10. Sensory Analysis

An acceptance test using a seven point hedonic scale with 7 (extremely like), 6 (very much like), 5 (moderately like), 4 (neither dislike nor like) 3 (moderately dislike), 2(very much dislike) and 1 (extremely dislike) was used for the sensory evaluation of the porridge prepared by the formulations according to Okpala, *et al. (*2013). Ten duplicates of thick porridge were prepared from 14 formulations and each sample was served monadically to each of semi-trained panelist while like warm (38°C) in coded plastic containers with spoons. Bottled water was supplied to panelists for refreshing their palates before tasting subsequent samples and to rinse their mouth between each test to remove all traces of the previous sample.

The panelists instructed to taste and evaluate the samples on evaluation sheets. A total of 10 mothers were selected from health centre nurse and attendants to assess the samples. Sensory attributes assessed were mouth feel, color, taste, given as baby food and overall acceptability. Finally, the scores of all panelists were added and divided by the number of panelists to find the attribute mean score.

$$\text{Attribute mean score} = \frac{numberofscore \times headonicscale}{N(totalpanelists)} \text{ ---------------------- (3.10)}$$

3.11. Statistical analysis

The complementary food data from Design- Expert® was analyzed using mixture contour plots to determine the most desirable formulations and to determine the effect of each component. However, all other data were analyzed using the general linear model procedures of Statistical Analysis Systems software (version 9.4 SAS, 2002, Institute Inc., Cary, USA). P values < 0.05 were considered significant. The differences between the treatments were determined by analysis of variance (ANOVA) and comparison of the mean was done by Duncan‟s multiple range tests.

CHAPTER FOUR: RESEARCH FINDINGS

CHAPTER FOUR: RESEARCH FINDINGS

4.1. NUTRITIVE AND NON-NUTRITIVE CONSTITUENTS OF TARO (*Colocasia esculenta*) CORM

4.1.1. ABSTRACT

The study was conducted using a factorial combination of two independent variables (taro corm varieties and processing methods) with three replications in a completely randomized design (CRD). An UHPLC and GC-FID method was used for the determination of amino acid and fatty acid composition, respectively. The moisture content ranged from 4.23±2.16 to 6.22±1.83, crude protein from 10.49±2.38 to 12.13±2.32, crude fat from 2.61±1.1to 3.42±0.65, crude fiber from 2.11±0.26 to 2.80±0.93, total ash content ranged from 3.29±0.49 to 3.88±0.79, carbohydrate content from 72.53±2.11 to 75.49±4.43, amylose content ranged from 12.89±0.67 to 13.56±0.49, beta (β)-carotene content µg/100 g from 2.11±0.50 to 2.31±0.16. Similarly, the five macro minerals, mg/100 g of Na, K, Mg, P and Ca ranged from 9.72±4.52, 17.74±1.36, 7.68±0.18, 0.74±0.30 and 11.28±4.78 respectively to 13.44±5.70, 18.92±1.54, 8.23±0.83, 1.06±0.30, 16.28±4.68 values, respectively. The micro minerals like Zn, Fe, and Cu (mg/100 g) ranged varied from 8.53±2.41, 88.77±17.20 and 0.29±.12 to 11.31±2.67, 104.30±16.10 and 0.40±0.09 values, respectively. The leucine Leu 1.75±0.39 to 1.99±03*, lysine Lys* 1.47±0.4 to 1.86±0.32 *and tryptophan Try* 1.21±0.36 to 1.66±0.32 *are the three highest essential amino acid found in the present study, respectively. The dominant fatty acid was oleic acid ($C_{18:1,\ n\text{-}9}$) ranging from* 101.95±0.23 to 101.91±0.43 *(mg/100g). From the general trend observed in this study, boloso-1 taro variety subjected to pre-gelatinization without peel provided better proximate (except crude fiber), mineral, functional, amino acid, fatty acid and lower anti-nutritional content than that of the, local taro variety.*

Key words: anti-nutritional, starch, gelatinization and taro

4.1.2. INTRODUCTION

Taro (*Colocasia esculenta*) a family of Aracea is cultivated for its edible corms used as a subsistence food by millions of people in developing countries (FAO, 1999). Root and tuber crops are second only in importance to cereals as a global source of carbohydrates. The corm of taro contains more than twice the carbohydrate content of potatoes and yield 135 kcals per 100 g and 11% crude protein (CP) on a dry matter (DM) basis. These reported values are even higher than other root crops like yam, cassava or sweet potato that have a carbohydrate (85 %) and CP (11 %) content respectively (FAO, 1999; Patrick *et al.,* 1999). It contains 85 to 87% starch on a DM basis with small granule size of 3-18 μm and other nutrients such as zinc, vitamin C, thiamin, riboflavin and niacin better than other cereals (Jirarat et al., 2006). Thought, protein and fat content of taro are low, but it is high in carbohydrates, fiber and minerals (Del Rosario and Lorenz, 1999).

Due to lack of appropriate storage and post harvest handling technologies, preserving crops for long period is very difficult and most rural peoples suffer from malnutrition (Annan and Plahar, 1995). For instance, zinc deficiency is currently widespread and affects the health and well-being of populations of the developing countries worldwide. Since taro is one of the few non-animal sources of zinc, its utilization should therefore be pursued to help in the alleviation of zinc deficiency which is associated weight stunting with children (Walker, 1990). The characterization of such less domesticated and unexploited food sources of the rural sources is essential to increase food choices and habits that support rural food security.

In Ethiopia, root crops are widely grown in the southern part of the country (CSA, 2005). Among the root crops, taro is one of the most important food crop as well as an income source for the farmers (Patrick, *et al.,* 1999). Farmers produce taro for their own consumption and considers difficult to store and sometimes wasteful because of spoilage and the demand for taro in urban areas has not gained the attention and neglected yet. However, it has a great potential to supply high quality food and one of the cheapest sources of energy (Patrick, *et al.,* 1999).

Even though taro has many nutritional, economic and health attributes, it has also some anti-nutritional compounds that are harmful to health. Because of these inherent factors of anti-nutritional compounds, taro demand is often unsatisfied which impose serious neglecting in both rural and urban consumers. Generally, taro is the underutilized root crop in Ethiopia (Umeta, 2000 and Adane *et al.,* 2013). Underutilized plants when properly brought under use can be good to malnutrition. People are eating taro corm as boiled, but there is no awareness about various processing methods that increase the quality and safety of the corm. Njintang *et al.* (2007) stated that taro has not gained sufficient research attention with regards to its potential having nutritional, industrial and health importance. In this context, taro corm was evaluated for its nutritional and anti-nutritional components through the application of effective processing methods that eliminate the anti-nutritional compounds (Savage and Catherwood, 2007).

Therefore, this study was aimed to investigate the nutritional and anti-nutritional content of taro and whether different improved methods of preparation and processing can reduce dominant anti-nutritional compound when consumed as part of human diet. First taro was processed into a more durable and convenient product to use in rural peoples to maximize their food and nutritional security. Samples in the form of pre-gelatinized flour were analyzed for functional properties as noted by (Rosario and Lorenz, 1999; Aprianita *et al.,* 2009).

In general, this study brought extensive research outputs on how to use taro corm accompanied with appropriate processing methods for human consumption at a high level of nutrient sources which have direct impact on improvement of food security and nutrition. Therefore, in the present study, the nutritional (crude protein, crude fat, total ash, crude fiber carbohydrate, energy and minerals), anti- nutritional (phytate, tannin, oxalate, mucilage, lectin, protease and alpha-amylase inhibitors) and functional constituents (beta-carotene and total phenols) of pre-gelatinized taro corm flour were determined. Since, the amino acid and fatty acid profile analysis in food are recently developed, which have currently gained attention by researchers (AOAC, 2006). From this study taro corm samples were further analyzed for amino acid and fatty acid compositions in addition to their proximate, mineral and anti-nutritional constituents grown in Ethiopia.

4.1.3. MATERIALS AND METHODS

4.1.3.1. Sample source

Two varieties of taro (*Colocasia esculenta*) corm: local and boloso-1 were harvested at 9 months of age from the Areka Agricultural Research Center (AARC) and brought to Addis Ababa, Ethiopia. The detailed procedure of sample preparation and each analysis is presented in chapter 3.

4.1.3. 2. Experimental samples preparation

The taro corm sample was prepared and pre-gelatinized according to (Ojinaka, *et al.,* 2009; Onwuka 2005).

4.1.3. 3. Experimental analysis

The pre-gelatinized taro flour samples were analyzed for moisture, crude fiber, total ash, crude fat and crude protein using the (AOAC, 2000, and AACC, 2000) reference methods. The total carbohydrate content was determined by nutrient difference (100 – % moisture + % protein + %fat +% fiber + % ash] according to (Egan *et al.,* 1981). The energy density (ED) of taro corm samples was calculated using standard food energy conversion factors: 4 kcal/g for carbohydrates, 4 kcal/g for proteins, 9 kcal/g for crude fat, according to Livesey (1995). The amyloses (%) content was determined by a calorimetric method (Zhu, *et al.,* 2008). The five macro minerals, of Na, K, Mg, P and Ca and three micro minerals Zn, Fe and Cu were analyzed by using atomic absorption spectrophotometer (AACC, 2000). Beta-carotene and total phenolic compounds were determined according to Laurie, *et al.,* (2012) and (Sharma and Gupta, 2010). The anti-nutritional content, i.e., mucilage was determined according to methods noted by (Yamazaki *et.al*. 2008). Phytic acid was determined through phytate phosphorus (Ph-P) analysis according to (Guttieri, *et al.,* 2004). The AOAC (1990) method was used to determine the oxalate content of taro sample. Tannins were determined using the method of (AOAC, 1995). Lectins, Alpha-amylase inhibitors and protease (trypsin) inhibitors were determined according to (Leo *et al.,* 2001; Noman, *et al.,* 2006; Bhattacharyya and Babu, 2009) respectively.

For amino acid analysis, samples were hydrolyzed in acid (HCL) medium to the complete hydrolysis of the protein fraction according to (Luyen, *et al.,* 2012). UHPLC instrumentation and analytical procedure for amino acid analysis was conducted according to (Kim, *et al.,* 2013). For tryptophan analysis, samples were hydrolysed in alkaline medium (NaOH). Fatty acid analysis protocol, the lipid first extracted with hexane: isopropanol (3:2 v/v) with, methylation and thin layer chromatography (TLC) checking was conducted using the reference method according to (Ostermann, *et al.,* 2014). Finally, Fatty acids were analyzed with a Gas chromatography-Flame ionization detector (GC-FID) instrument.

4.1.3.4. Statistical analysis

The statistical analysis was conducted to examine the interaction effects of two independent variables (taro corm varieties and gelatinization methods).All data were analyzed using the general linear model procedures of Statistical Analysis Systems software (version 9.4 SAS Institute Inc., Cary, USA). P values ≤ 0.05 were considered significant. The differences between the treatments were determined by analysis of variance (ANOVA).

4.1.4. RESULTS AND DISCUSSION

Moisture content of taro corm

The moisture content (%) in different samples ranged from 4.23±2.16 to 6.22±1.83 (Table 4.1.1). Moisture content in food sample is an index of stability and determines the appearance, keeping quality and yield of the product (Ejoh *et al.,* 2006). The difference between variety and processing method was significant ($p<0.05$). The moisture difference might be due to the application of dehydration condition remove the majority of water in pre-gelatinized taro without peel than with peel and reduce the final moisture content of the sample (Vega-Mercado, *et al.,* 2001; Akanbi, *et al.,* 2006 ; Aryee , *et al.,* 2006). The fresh taro corm is the most perishable and can deteriorate within one or two weeks after harvesting. The application of processing is vital and the general trend observed in this study was that the moisture content was found to be lower in pre-gelatinized samples without the peel.

Crude Protein Content

The crude protein content (%) in different samples ranged from 10.49±2.38 to 12.13±2.32 (Table 4.1.1). The boloso-1 taro pre-gelatinized with peel contained the highest protein content followed by local taro pre-gelatinized with the peel. However, the lowest (10.49±2.38) crude protein content was obtained from boloso-1 taro pre-gelatinized with peel samples. This might be because; peeling has induced changes in the protein composition (Nip, *et al.,* 1989; Ahmed *et al.,* 2010). The result obtained from this study is consistent with the work of (Castro *et al.,* 1992, Monte *et al.,* 2005; Sirima and Rachada, 2010) who did their research on root crops such as yam and taro and come up with a similar crude protein result. From the FAO report stated (FAO, 1999) that taro corm contains about 11% CP on a DM basis which is similar to the present study. The notable findings from this study were the crude proteins value were higher than the reported value might be the age at which the corm variety and processing method applied that increase protein solubility and deactivation of protease enzyme therefore pre-gelatinization applied in the study shows better protein supply (Njintang *et al.,* 2011).

Crude Fat Content.

As indicated in Table 4.1.1 the crude fat content (%) in different samples ranged from 2.61±1.11 to 3.42±0.65. The crude fat content from this study in percentage differ significantly ($p<0.05$). The fat content of local taro pre-gelatinized without peel composed the highest and the least value was also obtained in the local taro pre-gelatinized with the peel. The flour pre-gelatinized flour without peel samples provided higher fat content than that of flour pre-gelatinized form with the peel. These, little reduction of fat content was observed in flour pre-gelatinized form with peel samples might be boiling treatment induced skipped off some fatty acid to the skin and decreased fat content (Bradbury and Holloway, 2002; Shanthakumari *et al.,* 2008). Therefore, the result of this study is similar with the result of (Ghosho and Hasan, 1999; Mbofung *et al.,* 2006; Ndabikunze *et al.,* 2011), reported chemical composition of peeled and unpeeled taro flours as affected processing methods and variety difference.

Table 4.1 1: Proximate, β-carotene and Phenols contents in taro corm

	Taro varieties (A)				
	Boloso-1 corm		Local corm		
	Processing method (B)				
Parameters	PGF1	PGF2	PGF1	PGF2	CV (%)
Moisture (%)	4.23±2.16^{d}	6.22±1.83^{a}	4.77±3.02^{c}	5.44±3.09^{b}	3.78
Protein(%)	12.13±2.32^{a}	10.49±2.38^{cd}	11.91±2.69^{ab}	10.65±2.65^{c}	5.33
Crude fat(%)	3.01±1.35^{ab}	2.67±1.21^{c}	3.42±0.65^{a}	2.61±1.11^{cd}	3.68
Fiber(%)	2.11±0.26^{c}	2.66±1.08^{ab}	2.64±1.09^{b}	2.80±0.93^{a}	1.32
Total ash(%)	3.29±0.49^{bc}	3.57±0.97^{b}	3.88±0.79^{a}	3.83±0.88^{ab}	1.35
Carbohydrate	75.49±4.43^{a}	74.26±5.10^{b}	72.53±2.11^{c}	75.47±4.45^{a}	4.85
Energy value(kcal)	376.78±9.87^{a}	363.16±14.13^{d}	369.44±20.71^{b}	368.01±20.45^{c}	2.68
Amylose (%)	13.21±1.07^{ab}	13.13±0.99^{b}	13.56±0.49^{a}	12.89±0.67^{c}	4.69
β-carotene (µg/100g)	2.01±0.42^{b}	2.31±0.16^{a}	2.04±0.46^{ab}	2.11±0.50^{ab}	6.92
Phenols (mg/100g)	6.95±2.33^{c}	8.18±2.40^{ab}	6.92±2.25^{c}	8.32±2.16^{a}	6.45

$^{a\text{-}d}$ Means±SD within a raw with different superscripts differ significantly (p<0.05); SD: standard deviation; CV: coefficient of variation; PGF1: pre-gelatinized without peel, PGF2: pre-gelatinized with peel.

Total Ash Content

As results presented in (Table 4.1.1), the total ash content (%) was highest (3.88±0.79) for local taro pre-gelatinized without peels and pre-gelatinized with peel than boloso-1 taro. The ash content of boloso-1 taro pre-gelatinized without peel was low (3.29±0.49) value of all. The ash contents from this study were in agreement with the findings reported by (Njoku and Ohia, 2007; Ologhobo and Adejumo, 2011). This implies that local taro is a better source of ash than that of boloso-1 variety and this finding was consistent with the work of Adane *et al.* (2013) who conducted a research on the mineral contents of taro and yam corm. Similar findings by Jirarart *et al.* (2006) also revealed inconsistent changes in the chemical composition of taro cornels in Thailand. The changes in the composition of flour following peeling may probably reflect the difference in the composition of the different parts that compose the cornels.

Crude Fiber Content

As it can be seen from Table 4.1.1, the crude fiber content (%) in different samples ranged from 2.11±0.26 to 2.80±0.93. The result has revealed that crude fiber content variability found among boloso-1 and local taro varieties. The crude fiber content decreased in pre-gelatinized taro flour without peel samples and boloso-1 taro had lower values than that of local variety. This might be due to variety difference and the method of processing with peel may increase extra fiber composition of the samples (Ogunlakin, *et al.,* 2012).

Similarly, other researchers suggested that the crude fiber content of different taro samples was reduced and increased because of different processing methods, processing with peel had an effect on increasing the dietary fiber contents in food (Njintang, *et al.,* 2007, 2008; Ogunlakin *et al.,* 2012). For instance, crude fiber content of taro corms subject to drying, baking and fermentation with peel was increased (Onyeike, *et al.,* 1995; Soudy, *et al., 2010*). The effect of peeling can easily be appreciated from the variation of dietary fibers, the minerals, and the phytate contents in the flours. Similar effects have been pointed out by Ologhobo and Adejumo (2011) who reported significant reduction in crude fibers and ash of taro-based foods following peeling. In fact, peels are fibrous in nature with virtually zero starch, but also rich in minerals and anti-nutrients whereas, inner part is starcheous.

Total carbohydrate

The total carbohydrate content (%) in different samples ranged from 72.53±2.11 to 75.49±4.43 (Table 4.1.1). The carbohydrate content in percentage differs significantly ($p<0.05$). The result from this study revealed that the total carbohydrate content was higher in pre-gelatinized with peel than pre-gelatinized without peel in both taro varieties. On the other hand, the highest carbohydrate value was comprised in boloso-1 taro pre-gelatinized without peel and the lowest carbohydrate value was obtained in local taro flour1. According to the work reported by Lauzon and Kawabata (1988); Jane, *et al.* (1999); Benesi *et al.* (2004). Therefore, taro can be a good source of digestible starch and would supply high carbohydrates.

Energy value

As presented in Table 4.1.1 the total energy value (kcal/100 g) in this study was ranged from 363.16±14.13 to 376.78±9.87. The energy contents in different samples differ significantly ($p<0.05$). The high energy content was existed in boloso-1 taro flour1. The energy content is associated with carbohydrate content and flour samples with high carbohydrate held higher energy content. The result of this study showed that flour pre-gelatinized without peel of both taro varieties obtained higher energy content than that of flour pre-gelatinized with peel samples. According to Olajide *et al.* (2011) taro corm and the processed products could be rich sources of energy. The same with this report, the present study was also indicated that the taro corm could supply high energy value in human diets.

Amylose Content

As presented in Table 4.1.1 the highest percentage value of taro corm amylose was 13.56±0.49 and the lowest value 12.89±0.67 between the varieties and processing methods of taro corm. Pre-gelatinized flour without peel samples in a local taro variety showed greater amylose content than that of boloso-1 variety. In other words, the taro starch amylose percentage decreased due to pre-gelatinization with peel (Tattiyakul et al., 2006; Gordon 2000). Similar findings were also reported (Jirarat, *et al.,* 2007; Ekwe, *et al.,* 2009). Taro roots are rich in a starch composed of amylose (18 %) and amylopectin (82%) (Agunbiade *et al.,* 2011). Several studies have been conducted on functional properties of taro amyloes and amylopectin that revealed that Taro starch has some preference over other starches due to high amylopectine content. The amylose content of this study was reduced and this might be due to the processing method as proved by Elmsthl (2002); Moorthy (2002); Niba (2003); Adebayo (2008).

The result shown in Table 4.1.1 also summarizes the β-carotene and phenolic compounds of the pre-gelatinized corm samples. The higher β-carotene (µg/100 g) (2.31±0.16) was detected in taro boloso-1 pre-gelatinized flour with peel and followed by lower β-carotene (2.01±0.42) in local taro pre-gelatinized flour with the peel. However, the β-carotenes in this study did not differ significantly ($p>0.05$). In other words, β-carotene content decreased slightly in pre-gelatinized corms without peel and this may be due to breakdown of carotene contents during thermal processing and loss of some amount of β-carotene with peel before cooking (Dewanto *et al.,* 2002; Jirarat *et al.,* 2006; Horvathova, *et al.,* 2007; Ezeocha, *et al.,* 2012).

Therefore, the flour samples pre-gelatinized with peels held good β-carotene content than that of flour samples pre-gelatinized without the peel. Concerning the phenolic compound composition, it was significantly different ($p<0.05$) among the varieties and processing methods of taro corm. The total phenolic compound in this study was highest for samples pre-gelatinized with peel and ranged from 6.92±2.25 to 8.32±2.16. This might be due to the presence of phenolic groups in the peel increased the total phenols in the analyzed samples, which was pre-gelatinized with peel in both varieties.

Macro and micro minerals concentration of pre-gelatinized taro corms

As indicated in Table 4.1.2, the mg/100g values in DM basis of five major minerals were presented. The mg/100 g of Na in this study ranged from 9.72±4.52 to 13.44±5.70 and differ significantly ($p<0.05$). Greater Na value was obtained from boloso-1 taro flour 1 and flour 2 while the smaller value obtained from local taro flour 1 and flour 2 respectively. The result of this study states that the Na contents differ significantly due to taro varieties ($p<0.05$). The notable findings obtained from this study were consistent with the works of Welch and Graham, 2004; Umar *et al.,* 2005; Huang *et al.,* 2007). Accordingly, the study promoted that pre-gelatinization without peel in root crop is a good way of reducing Na level from food samples and recommended for human consumption as noted by (Gordon, 2000) and (Baruah, 2002).

Table 4.1.2 also presents the mg/100 g of K for pre-gelatinized taro flour samples and based on the findings the K (mg/100 g) in this study ranged from 17.74±1.36 to 18.92±1.54. The K (mg/100g) contents differ significantly ($p<0.05$). The results founded from this study predicted that local taro flour-1 samples had high K concentration than boloso-1 taro flour 1 and flour 2. Potassium is two times higher than that of Na in the analyzed taro flour samples (Table 4.1.2). According to (Nijoku and Ohia, 2007, Huang *et al.,* 2007) greater K concentration compared with lower level is recommended for healthy diets.

Moreover, studies of the taro nutritional composition suggest that it contains a range of important macro and micronutrients (Bradbury and Holloway, 1988; Huang, *et al.,* 2007; Lewu, *et al.,* 2010a and Mwenye, *et al.,* 2011). Some studies of the mineral compositions of taro corms suggest that potassium is the most abundant mineral (Herselman and Benesi, 2011). A 1-cup serving of cooked taro contains 639 mg of potassium. Which possibly help to control blood pressure (American Heart Association January, 2011).

The Mg (mg/100 g) in this study was ranged from 7.68±0.18 to 8.23±0.83 and differ significantly ($p<0.05$). The general trend observed in this study was local taro flour samples obtained greater Mg concentration than boloso-1 taro flour samples. In other words, local taro flour-1 composed the highest mg/100 g of Mg followed by taro flour-1. Previous studies also provided evidence that thermal processing would increase the mineral composition and reduce anti-nutritional factors in different food samples (Adejumo and Bamidele, 2012). Therefore, the present study is consistent and the notable finding from the study suggests pre-gelatinization could be an appropriate method for reducing toxic compound (Umar, *et al.,* 2005). Magnesium is needed for bone, immune health, muscle and nerve function. It also keeps blood pressure normal and regulates blood sugar in the body. Adult men need 400 to 420 mg of magnesium a day and adult women need 310 to 320 mg a day. One serving of taro meets 10 percent of the daily value for magnesium (American Heart Association, 2011).

The P (mg/100 g) in this study was ranged from 0.74±0.30 to 1.06±0.30. The P (mg/100g) contents did not differ significantly ($p<0.05$). The result provided for the present study showed flour pre-gelatinized with peel prevailed greater mg/100 g of P concentration than that of flour pre-gelatinized without peel (Table 4.1.2). Previous studies also provided evidence that the mineral contents of food samples would increase due to additional minerals found in the peel to the products (Aboubakar, *et al.,* 2008; Adejumo *and* Bamidele. 2012; Subramanian, *et al.,* 2011). Therefore, the present study is in agreement with previous studies and the notable finding from the study suggests pre-gelatinization with peel was a good ways of improving the mineral content (Nip, 1997; Sefa-Dedeh *et al.,* 2004; Buléon, *et al.,* 2014).

The mg/100 g of Ca in this study was ranged from 11.28±4.78 to 16.28±4.68 for boloso-1 flour pre-gelatinized without peel and for local flour pre-gelatinized without peel respectively. The Ca (mg/100 g) contents differ significantly ($p<0.05$). The result provided for the present study showed pre-gelatinized flour without peel composed the highest mg/100 g of Ca concentration than that of pre-gelatinized flour with peel in both varieties (Table 4.1.2). The Ca concentration was increased in processed taro corms due to the pre-gelatinization effect this might be due to simultaneous reduction of anti-nutritional constituents such as oxalate in taro that inhibit the bioavailability of Ca according to (Onyeike, *et al.,*1995; Bhandari and Kawabata, 2004; Soudy, *et al.,* 2010).

Regarding to micro mineral concentration of analyzed pre-gelatinized taro samples, the Fe (mg/100 g) in different samples ranged from 88.77±17.20 to 104.30±16.10, Zn (mg/100 g) from 8.53±2.41 to 11.31±2.67 and Cu (mg/100 g) from 0.29±0.12 to 0.40±0.09 (Table 4.1.2). The Fe (mg/100 g) concentration differs significantly ($p<0.05$). The results showed that pre-gelatinized flour without peel has greater Fe and Zn concentration than that of pre-gelatinized flour with peel in both taro varieties (Table 4.1.2).

These reductions of Fe and Zn concentration in pre-gelatinized flour with peel samples might be due to the binding interaction with anti-nutritional compounds (Noonan and Savage, 1999; Enneking and Wink, 2000; Huisman, 2005; Savage, *et al.,* 2009). However, in case of pre-gelatinization without peel provided that reduced anti-nutritional factors dominantly found in the peel. The findings of this study were in agreement with the work of Kordylas (1990); Ndimantang, *et al.,* (2006); Lima, *et al.* (2009); Lewu (2010). In contrary, Cu concentration was reduced slightly in samples pre-gelatinized without peel; hence Cu seems to have a dramatic reduction character during peeling and washing as noted by Charles, *et al.* (2005). Data from the study revealed that appreciable amounts of minerals are present and variations were observed due to genotype difference and effect of peeling (Sefa-Dedeh and AgyirSackey, 2004) reported that peeling can significantly influence the concentrations of minerals accumulated in the upper, lower and marginal parts of the corm.

Table 4.1 2: Concentration of Na, K, Mg, P, Ca, Fe, Zn and Cu in taro corm

	Taro varieties (A)				
	Boloso-1 corm		Local corm		
	Processing method (B)				
Parameter	PGF1	PGF2	PGF1	PGF2	CV (%)
Na(mg/100g)	13.44±5.70^{a}	13.42±5.74^{a}	9.72±4.52^{d}	10.82±6.43^{c}	5.35
K(mg/100g)	18.06±1.91^{b}	18.75±1.83^{a}	18.92±1.54^{a}	17.74±1.36^{c}	6.77
Mg(mg/100g)	7.68±0.18^{b}	8.11±0.92^{ab}	8.23±0.83^{a}	8.20±0.86^{a}	6.48
P(mg/100g)	0.74±0.30^{bc}	0.94±0.49^{ab}	0.86±0.49^{b}	1.06±0.30^{a}	1.85
Ca(mg/100g)	16.28±4.68^{a}	13.11±7.57^{c}	14.39±7.58^{b}	11.28±4.78^{d}	3.75
Fe(mg/100g)	104.30±16.10^{a}	88.77±17.20^{d}	99.42±24.14^{b}	92.87±23.82^{c}	12.48
Zn(mg/100g)	11.31±2.67^{a}	9.72±4.21^{c}	10.27±4.18^{b}	8.53±2.41^{d}	1.28
Cu(mg/100g)	0.29±0.12^{abc}	0.36±0.16^{ab}	0.32±0.16^{abc}	0.40±0.09^{a}	1.40

$^{a\text{-}d}$ Means ±SD within a raw with different superscripts differ significantly (p<0.05); SD: standard deviation; CV: coefficient of variation; PGF1: pre-gelatinized without peel, PGF2: pre-gelatinized with peel.

Tannin, phytate, mucilage oxalate other ant-nutritional factors

The tannin level (mg/100 g) in different samples ranged from 243.61±5.87 to 353.72±18.27 and differ significantly ($p<0.05$). The high tannin content was obtained in local taro and the lower content was found in a boloso-1 variety subjected to pre-gelatinization without and with the peel, respectively (Table 4.1.3). A great reduction of tannin content in boloso-1 samples was exhibited and this might be due to variety difference and the processing effect that might reduce the concentration of the condensed tannin (Obiolephehai, 2003). According to Prajapati, *et al.* (2011), condensed tannin, phytic acid, mucilage and oxalic acid concentrations in food samples could be reduced during washing, peeling, boiling and thermal processing (Feng, *et al.,* 2003; Phillippy, *et al*., 2004; Taiwo, *et al.,* 2008). The total acceptable tannin daily intake for man is 560mg (WHO, 2003). Ugwu and Oranye (2004) obtained a tannin concentration of 640mg/100g for raw samples of taro. Thus, based on the findings obtained from this study most of the samples contained low tannin concentrations.

Table 4.1 3: Content of tannin, phytate, mucilage and oxalate (mg/100 g) in taro corm

	Taro varieties (A)				
	Boloso-1 corm		Local corm		
	Processing method (B)				
Parameters	PGF1	PGF2	PGF1	PGF2	CV (%)
Tannin(mg/100g)	243.61±5.87^{d}	247.03±11.78^{c}	342.43±17.9	353.72±18.27^{a}	60.41
Phytate(mg/100g)	0.92±0.07^{bc}	1.71±1.32^{a}	1.66±1.35^{ab}	1.71±1.30^{a}	5.96
Mucilag(mg/100g)e	0.16±0.12^{ab}	0.46±0.52^{a}	0.41±0.54^{a}	0.49±0.47^{a}	1.82
Oxalate(mg/100g)	88.00±0.19^{cd}	92.0±22.71^{b}	90.0±21.63^{c}	102.0±6.0^{a}	20.20
Lectin (mg/100g)	0.01±0.05^{ab}	0.01±0.07^{a}	0.01±0.08^{ab}	0.01±0.07^{a}	0.57
amylase(mg/100g)	0.91±0.03^{ab}	0.94±0.04^{a}	0.91±0.04^{a}	0.92±0.04^{a}	0.37
Protase in	0.01±0.04^{ab}	0.01±0.02^{ab}	0.01±0.04^{ab}	0.01±0.04^{a}	0.07

$^{a-d}$ Means±SD within a raw with different superscripts differ significantly (p<0.05); SD: standard deviation; CV: coefficient of variation; PGF1: pre-gelatinized without peel, PGF2:pre-gelatinized with peel.

The phytate (mg/100 g) content in different samples ranged from 0.92±0.07 to 1.71±1.32 (Table 4.1.3), did not differ significantly (p>0.05). Lewu *et al*. (2010), suggested that food processing such as washing, peeling, cooking, oven drying and milling could reduce or eliminate the of phytic acid. Thus, based on the findings obtained from this study most of the samples contained low phytic acid concentration when subjected to peeling and pre-gelatinization (Huisman, 2005; Greiner, *et al.,* 2006; Nguimbou, *et al.,* 2013). The mucilage content (mg/100 g) in different samples ranged from 0.16±0.12 to 0.49±0.47 (Table 4.1.3). The local taro samples pre-gelatinized with peel obtained the high mucilage content of all, while the lowest mucilage content was found in boloso-1 taro samples pre-gelatinized without the peel. Generally, reductions of mucilage content in pre-gelatinized samples without peel were observed and this reduction might be due to the leaching out of mucilage to the water which was used for soaking sliced samples (Lee, *et al.,* 2003; Siddhuraju and Becker, 2001; Soudy, *et al.,* 2010).

The oxalate content (mg/100 g) in different samples ranged from 88.00±0.19 to 102.0±6.0 and differ significantly ($p<0.05$). The present result was in agreement with the findings of Hang, *et al.* (2000); Catherwood, *et al.* (2007); Hang and Binh (2013) who noted that washing, peeling, ensiling and boiling were the most effective methods of reducing the soluble and total oxalate. Other investigations have been reported regarding to oxalate content of taro and the findings in this study were below the suggested level of (221.652±2.014 mg/100 g) (Hang *et al.,* 2000). Catherwood *et al.* (2007) reported 60.6% of soluble oxalate of the total oxalate content of raw corms. Relatively high of oxalate content was reported by Adane and Shimelis (2009) 265.88 mg/100g and 243.06 mg/100g for raw taro samples of two different varieties.

Proportion of soluble to insoluble oxalate in a food would influence the effectiveness of processing methods such as boiling, cooking, sun drying and baking because the ability of the oxalate to solubilise in solution and its heat susceptibility will influence the residual proportion (Noonan and Savage, 1999; Hang *et al.,* 2011; Ogunlakin, *et al.,* 2012; Beyer, *et al.,* 2013). The lethal level of oxalate in man is 3000-5000 mg as reported by (WHO, 2003). Also, as demonstrated in Table 4.1.3, lectin, α-amylase and protease inhibitors in pre-gelatinized starch from two taro varieties with and without peel did not differ significantly ($p>0.05$). However, slight reduction in lectin, α-amylase and protease inhibitors were observed in flour samples pre-gelatinized without the peel.

The other thing observed in different samples, boloso-1 taro obtained the lower α-amylase, protease and lectin inhibitors while the lectin, α-amylase and protease inhibitors was founded in local taro variety. This may be more due to variety differences and abundance of inhibitory levels in the peel than in the inside corm (Kiran, *et al.,* 2003). Related findings also showed that inhibitory effects could be inactivated by effective raw material preparation and processing methods (Ugwu and Oranye, (2006; Sandberg and lid, 2002; Kiran, et *al.,* 2003; Gibson, *et al.,* 2006).

Table 4.1 4: Phytate mineral molar ratio in taro corm

	Taro varieties (A)			
	Boloso-1 corm		Local corm	
	Processing method (B)			
Parameters	PGF1	PGF2	PGF1	PGF2
[Phy]:[Na]	0.25±0.12[a]	0.21±0.05[ab]	0.27±0.11[a]	0.28±0.10[a]
[Phy]:[K]	0.14±0.06[a]	0.15±0.06[a]	0.12±0.03[a]	0.16±0.05[a]
[Phy]:[Fe]	0.08±0.02[a]	0.12±0.05[a]	0.11±0.05[a]	0.12±0.04[a]
[Phy]:[Ca]	0.22±0.13[a]	0.25±0.09[a]	0.22±0.12[a]	0.26±0.10[a]
[Phy]:[Mg]	0.19±0.04[a]	0.22±0.05[a]	0.19±0.05[a]	0.23±0.04[a]
[Phy]:[Zn]	0.07±0.02[a]	0.09±0.04[a]	0.08±0.04[a]	0.09±0.04[a]
[Phy]:[Cu]	0.09±0.04[a]	0.07±0.02[a]	0.10±0.03[a]	0.10±0.04[a]
[Phy]:[P]	0.02±0.01[a]	0.03±0.02[a]	0.02±0.02[a]	0.03±0.01[a]

[a-ab] Means±SD within a raw with similar superscripts did not differ significantly (p>0.05); SD: standard deviation; PGF1: Pre-gelatinized without peel, PGF2: Pre-gelatinized with peel.

Hence, the phytate: mineral molar ratio is considered a better indicator of mineral bioavailability than total dietary phytate levels alone. The result obtained from this study was samples with skin samples prevailed slightly greater phytate mineral molar ratio than that of samples flour without skin (Table 4.1.4). As reported by (Adane and Gullelat, 2009) phytate concentrations (115.43- 135.28mg/100g) for unprocessed taro varietys. However, the phytate level of processed samples of taro in this study was very much less. Therefore mineral levels in all samples are more bio-available due to the low phytate mineral ratio (Bothwell and Chalton, 2002). It was also reported that different domestic processing, including peeling and heating maintained high mineral bioavailability and low phytate constitutes (Gibson *et al.,* 2006) because such processing have shown reduction in anti-nutritional contents (Apata and Ologbobo, 1998).

Phytate is known to be the main inhibitor of mineral absorption in plant-based diets and reduce the bioavailability of dietary minerals by forming insoluble mineral chelates in the food and within the body (Oberleas, 1983; Bothwell and Chalton, 2002; Sefa-Dedeh, *et al.,* 2004). Therefore, to reduce the adverse effect of phytates on mineral absorption, phytate contents have to be reduced to concentrations of less than 200 mg/100 g DM (Hurrell, 2004).

With this regard, most of the analyzed samples had lower phytate: mineral molar ratio and this might be due to peeling or removing the skin and soaking in cold water prior to cooking (Siddhuraju and Becker, 2001; Dedeh, *et al.,* 2004) showed that foods with a molar ratio of phytate: mineral less than 10 showed adequate availability of mineral and problems were encountered when the value was greater than 15. On average, the daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries and 150–1400 mg for mixed diets (WHO, 2003).

All the samples analyzed had a low ratio value which is less than the critical value 15 may be due to the application of thermal processing. Studies also revealed that thermal processing would reduce anti-nutritional factors in different food samples (Ezeocha *et al.,* 2012). This means that minerals obtained from these samples would be bioavailable to the human body (Gibson *et al.,* 2006; Alinnor and Akalezi, 2010).

Table 4.1 5: Amino acids composition (g/100g) of taro corm samples

Types of AAs	Treatments				
	Abbreviations	$BPGF_1$	$BPGF_2$	$LPGF_1$	$LPGF_2$
Essential amino acids(EAA)					
L-Histidine	His	1.4±0.22[a]	1.41±0.31 [a]	1.37±0.4 [ab]	1.45±0.21 [a]
L-Threonine	Thr	1.12±0.31 [ab]	1.11±0.32 [ab]	1.17±0.33 [ab]	1.34±0.3 [a]
L-Valine	Val	1.32±0.32 [ab]	1.02±0.5 [ab]	1.31±0.43 [ab]	1.54±0.35 [a]
L-Methionine + Cys	Met-Cys	1.21±0.5 [ab]	1.01±0.34 [ab]	1.14±0.24 [ab]	1.45±0.32 [a]
L-Tryptophan	Try	1.32±0.32 [ab]	1.31±0.32 [ab]	1.21±0.36 [ab]	1.66±0.32 [a]
L-Phenylalanine	Phe	1.13±0.4 [ab]	1.13±0.32 [ab]	1.13±0.43 [ab]	1.61±0.38 [ab]
L-Isoleucine	Iso	1.32±0.43 [a]	1.12±0.43 [ab]	1.02±0.3 [ab]	1.01±0.23 [ab]
L-Leucine	Leu	1.99±0.3 [a]	1.90±0.32 [a]	1.75±0.39 [ab]	1.76±0.43 [ab]
L-Lysine	Lys	1.86±0.32 [a]	1.47±0.4 [ab]	1.59±0.1 [ab]	1.54±0.41 [ab]
Non-essential amino acids (NEAA)					
L-Aspartic acid	Asp	4.12±0.43 [a]	4.13±0.43 [a]	2.13±0.09 [b]	2.12±0.10 [b]
L-Glutamic acid,	Glu	2.12±0.53 [a]	2.03±0.43 [a]	1.54±0.47 [ab]	1.43±0.48 [b]
L-Asparagines	Asp	1.45±0.3 [a]	1.04±0.12 [ab]	1.05±0.48 [ab]	1.33±0.49 [a]
L-Serine	Ser	1.33±0.43 [a]	1.03±0.18 [ab]	1.06±0.19 [ab]	1.23±0.20 [a]
L-Glutamine	Glu	1.43±0.4 [a]	1.04±0.43 [ab]	1.03±0.20 [ab]	1.31±0.21 [a]
L-Glycine	Gly	4.22±0.34 [a]	4.04±0.11 [a]	3.54±0.12 [ab]	3.24±0.13 [ab]
L-Alanine	Ala	1.33±0.21 [a]	1.05±0.17 [ab]	1.11±0.18 [ab]	1.32±0.19 [a]
L-Arginine	Arg	3.33±0.43 [a]	3.07±0.71 [a]	3.32±0.72 [a]	3.32±0.73 [a]
L-Tyrosine	Tyro	1.44±0.3 [a]	1.16±0.15 [ab]	1.32±0.16 [a]	1.32±0.17 [a]
L-Norleucine	Nor	0.32±0.34 [a]	0.39±0.22 [a]	0.56±0.23 [a]	0.43±0.24 [a]
L-Hydroxyproline	Hyd	1.33±0.43 [a]	1.04±0.17 [a]	1.2±0.18 [a]	1.17±0.19 [a]
L-Proline	Pro	0.32±0.53 [a]	0.03±0.54 [ab]	0.13±0.55 [a]	0.05±0.56 [ab]

[a-ab] Means ± SD within a raw with similar superscripts did not differ significantly ($p>0.05$); SD: standard deviation; AAs: Amino acids; $BPGF_1$: boloso-1 taro pre-gelatinized flour with peel; $BPGF_2$: boloso-1 taro pre-gelatinized flour without peel; $LPGF_1$: local taro pre-gelatinized flour with peel; $LPGF_2$: local taro pre-gelatinized flour without peel

The essential amino acids for taro corm are ranging His (1.37±0.4 to 1.45±0.21), Thr (1.11±0.32 to 1.34±0.3), Val (1.02±0.5 to 1.54±0.35), Met & Cys (1.01±0.34 to 1.45±0.32), Try (1.21±0.36 to 1.66±0.32), Phe (1.13±0.32 to1.61±0.38), Iso (1.01±0.23 to 1.32±0.43), Leu (1.75±0.39 to 1.99±0.3) and Lys (1.47±0.4 to1.86±0.32). The leucine, lysine and tryptophan are the three highest essential amino acid found in the present study, respectively.

Similarly, the non-essential amino acids are ranging for Asp (2.12±0.10 to 4.12±0.43), Glu (1.43±0.48 to 2.12±0.53), Asp (1.04±0.12 to 1.45±0.3), Ser (1.03±0.18 to 1.33±0.43), Glu (1.03±0.20 to 1.43±0.4), Gly (3.24±0.13 to 4.22±0.34), Ala (1.05±0.17 to 1.33±0.21), Arg

(3.07±0.71 3.33±0.43), Tyr (1.16±0.15 to 1.44±0.3), Nor (0.32±0.34 to 0.56±0.23), Hyd (1.04±0.17 to 1.33±0.43) and Pro (0.03±0.54 to 0.32±0.53).The aspartic acid, glycine and arginine are the three dominant non-essential amino acids, respectively. The amino acid compositions of many root crops such as sweet potato, cassava and yam root also were reported by different studies and these results are in accordance with (Rogers, *et al.,* 1993; Hou, *et al.,* 2001; Bhandari, *et al.,* 2003; Ahmed, *et al.,* 2010). Essential amino acids are not synthesized in the body and must be obtained from food.

The calculated values are high because the concentrations were multiplied by their crude protein contents of the corm samples (Table 4.1.1). The essential amino acid composition was slightly higher in local taro pre-gelatinized flour with the peel and the second high essential amino acid compositions were found in boloso-1 taro pre-gelatinized flour without the peel. Further, boloso-1 taro pre-gelatinized flour was found to be higher in non-essential amino acid than its counter variety. Most of root crops contain a reasonable amount of lysine, though less than in legumes, but the sulphur amino-acids are limiting. For example, taro is rich in phenylalanine and threonine, but limiting in the sulphur amino-acids, cystine and methionine and in tryptophan. In order to maximize their protein contribution to the diet, roots and tubers should be supplemented with a wide variety of other foods, including legumes asnd animal source foods (Gorinstein, *et al.,* 2001; WHO/FAO/UNU, 2007; FAO, 2013).

Table 4.1 6: Fatty acid profile (g/100 g) of pre-gelatinized taro corm samples

FAs	Treatments			
	BPGF1	BPGF2	LPGF1	LPGF2
$C_{14:0}$	1.46±0.23 [a]	1.44±0.32 [a]	1.43±0.34 [a]	1.42±0.43 [a]
$C_{16:0}$	100.09±0.32 [a]	100.07±0.23 [a]	100.06±0.33 [a]	100.05±0.44 [a]
$C_{16:1(n-7)}$	7.52±0.34 [a]	7.50±0.22 [a]	7.49±0.43 [a]	7.48±0.43 [ab]
$C_{17:0}$	0.42±0.23 [a]	0.41±0.23 [a]	0.39±0.45 [a]	0.38±0.44 [a]
$C_{17:1}$	1.92±0.31 [a]	1.90±0.32 [a]	1.88±0.33 [a]	1.88±0.34 [a]
$C_{18:0}$	50.45±0.23 [a]	50.44±0.54 [a]	50.42±0.34 [a]	50.41±0.34 [a]
$C_{18:1(n-9)}$	101.95±0.23 [a]	101.93±0.32 [a]	101.92±0.54 [a]	101.91±0.43 [a]
$C_{18:2(n-6)}$	26.42±0.23 [a]	26.40±0.32 [a]	26.38±0.23 [a]	26.38±0.34 [a]
$C_{18:3(n-3)}$	0.42±0.56 [a]	0.41±0.12 [a]	0.39±0.43 [a]	0.38±0.43 [a]
$C_{20:0}$	0.42±0.23 [a]	0.41±0.32 [a]	0.39±0.34 [a]	0.38±0.34 [a]
$C_{20:1(n-9)}$	0.42±0.23 [a]	0.41±0.34 [a]	0.39±0.45 [a]	0.38±0.34 [a]
$C_{20:2(n-6)}$	0.42±0.45 [a]	0.41±0.23 [a]	0.39±0.45 [a]	0.38±0.45 [a]
$C_{20:3(n-6)}$	0.42±0.34 [a]	0.41±0.12 [a]	0.39±0.45 [a]	0.38±0.33 [a]
$C_{20:4(n-6)}$	12.75±0.43 [a]	12.73±0.32 [a]	12.72±0.46 [a]	12.71±0.32 [a]
$C_{24:1(n-9)}$	0.42±0.12 [a]	0.41±0.34 [a]	0.39±0.33 [a]	0.38±0.43 [a]
C22:6(n-3)	3.04±0.34 [a]	3.03±0.23 [a]	3.01±0.45 [a]	3.00±0.54 [a]
Total	308.56	308.28	308.01	307.93
Proportion (mg/100 g), SFA, MUFA, PUFA and UFA:SFA				
SFAs	152.85	152.76	152.68	152.66
MUFAs	113.70	113.59	113.49	113.46
PUFAs	43.48	43.37	43.27	43.24
UFAS	157.17	156.96	156.76	156.70
UFA:SFA	1.03	1.03	1.03	1.03

[a-ab] Means ± SD within a raw with same superscripts did not differ significantly (p>0.05); SD: standard deviation; FAs: Fatty acids; BPGF1: boloso-1 taro pre-gelatinized flour without peel; BPGF2: boloso-1 taro pre-gelatinized flour with peel ; LPGF1: local taro pre-gelatinized flour without peel ; LPGF2: local taro pre-gelatinized flour with peel

The dominant fatty acid was oleic acid ($C_{18:1,\ n-9}$) ranging from (101.95±0.23 to 101.91±0.43) g/100 g. The next three dominant fatty acids were palmitic acid ($C_{16:0}$), oleic acid ($C_{18:0}$) and linoleic acid ($C_{18:2,\ n-6}$) ranging from (100.09±0.32 to 100.05±0.44), (50.45±0.23 to 50.41±0.34) and (26.42±0.23 to 26.38±0.23) g/100 g values respectively.

Similar findings have been made that the predominant fatty acids in root crops like taro and yam were oleic, linoleic, palmitic and linolenic acids orderly (Katiyar *et al.,* 2005; Islam *et al*, 2012). From taro pre-gelatinized corm flour, boloso-1 taro had relatively high levels of fatty acid fractions and low levels were obtained in local taro pre-gelatinized flour (Table 4.1.6). This might be due to variety differences Valasco *et al.* (2008).

From the proportion (g/100 g) of saturated, monounsaturated and polyunsaturated fatty acids, unsaturated fatty acids in corm flour were very little higher than saturated ones (Table 4.1.6). This is further verified by calculating the ratio of unsaturated: saturated fatty acids, which all are greater than one. In taro and other root crop, unsaturated fatty acids predominate over the saturated, in the total fatty acid content (Bhandari *et al.,* 2003; Brown *et al.,* 2008; Alozie *et al.,* 2010). Although, the overall amount of crude fat in the corm was very low (3.421±0.659), the fatty acid profile of the tuber was very good, considering that linoleic acid (an essential fatty acid) was found in high level. This means that this tuber would not be considered a good source of essential fatty acid, but it is a very good source of carbohydrate.

4.1.5. CONCLUSION

To conclude this section, the notable findings were the crude protein, crude fat, crude fiber and mineral contents improved in the samples peeled before cooking than that of samples peeled after cooking. Nutrient compositions of peeled and unpeeled taro flours were affected by processing methods applied in the study (peeling, washing, drying and milling) and variety difference. Moreover, this study revealed that taro pre-gelatinized corm samples contain a range of important macro and micronutrients with a minimum level of anti-nutritional factors. Hence, the phytate: mineral molar ratio is considered a better indicator of mineral bioavailability than total dietary phytate levels alone. In this regard, all of the analyzed samples had lower phytate: mineral molar ratio less than 10 and below the critical value. This means that minerals obtained from these samples would be bioavailable to the human body. The amino acid and fatty acid compositions were affected mainly by taro varieties. The essential fatty acids $C_{18:2}$ and $C_{18:3}$ are found in low concentration as compared to oleic acid. Therefore, this study investigated the effect of peeling, washing and pre-gelatinization in order to reduce inhibitory effects and enhance the nutritional composition.

4.2: DEVELOPMENT OF OPTIMIZED COMPLEMENTARY FOOD FROM TARO-MAIZE-SOYBEAN BLENDS

4.2.1. ABSTRACT

A statistical software package (Mixture Design- Expert ®, version 10.0, Stat-Ease, SaMeep104 Inc.) mixture design was used for the generation of test formulations and analysis of the results. An attempt was made to maximize the responses for CP, fat, carbohydrate and minerals (Fe, Zn, Ca, Mg and P) and minimize dietary fiber, oxalate and phytate in the complementary food. For optimized complementary food had a proximate content of crude protein, crude fat, total ash, carbohydrate and energy value were 22.23±0.6, 6.32±0.78, 4.8±0.32 and 56.5±0.46, respectively. The development of complementary food showed that blending of taro (50 %) with maize (30%) and soybean (20%) improve the nutritional density of complementary foods.

Keywords: Complementary food, nutrient density, optimization and panelists

4.2.2. INTRODUCTION

In nutritional point of view complementary food is a semi-solid food that is used in addition to breast milk and not only replaces it (Asma *et al.,* 2012). It is prepared in the form of thick porridges. Legumes in the diet are found to improve the nutrient density of food and improve nutrient intake, which results in the prevention of malnutrition problems (Fekede, 2009). The transition from milk to solid or adult food is a critical period in the life of a child as weaning practices by the mother profoundly determines child growth and development (Solomon, 2005; Amuna, *et al.,* 2009). In developing countries a combination of nutritionally inferior diets and improper feeding practices are major contributing factors to the development of childhood malnutrition (Rubery, 1988; Jakobs, 1991). Traditional infant foods made of grains, cereals are bulky, low in energy density and low in several nutrients, including protein, vitamin A, zinc and iron and they also contain high amount of factors reducing mineral bioavailability, such as phytates and aflatoxins, which is the potential for stunting in children (Suhasini and Malleshi, 2003; Melaku, *et al.,* 2005; Dror and Allen, 2011).

Due to these all factors complementary food and feeding are the key areas to improve. Complementary feeding improvement should be of highest priority for the nutrition of infant and young children containing all essential nutrients at required amount (Dewey and Brown, 2003). In Ethiopia, the concept of improved feeding of infant and young children is not well understood by most families and there is no ingredient optimization method during infant food formulation and this could be also other gap to solve (WHO/UNICEF, 2005 and Ali *et al.,* 2011). Therefore, there must be proper complementary food formulation design using acceptable formula in order to solve problems related to mixing of complementary food items. Again, the point at which infants begin the actual weaning process, i.e. the introduction of grain based solid foods is not the same throughout the country (Ramakrishna, *et al.,* 2006). In general, complementary feeding in Ethiopia varies considerably with the ethnic make-up of the population, the degree of urbanization and the socioeconomic status of the families (Umeta, *et al.,* 2005; FARO, 2008; CSA, 2012 and Shumey, *et al.,* 2013).

As a result, mothers commonly dilute the porridge with water to reduce its viscosity. Since young children have small gastric capacity, they are unable to consume enough of the diluted porridge to meet their energy requirements and consequently may become malnourished (Yewlsew, *et al.,* 2006; Shumey, *et al.,* 2013). Such dilution; however, also reduces the energy density of the mixture. The cheapest and fastest way of meeting the growing demand for nutrient dense infant food is through increasing the development of low-cost and locally available infant ingredients and availing the products at affordable prices by lowering the cost of production to bring processed infant foods within the reach of poor people (Bhandari, *et al.,* 2004; Silva *et al.,* 2006 and Fahmida, *et al.,* 2014). Such an approach would increase the nutrient density of complementary foods. Therefore, this study is initiated to produce optimized complementary food from pre-gelatinized taro flour, mixing with maize and soybean in order to develop low-cost and easily available complementary food (WHO, 2003; Gibson, *et al.,* 2015). For problems associated with ingredients, mixing, design expert was used for an optimized formula to be recommended for complementary food development.

Taro starch is considered to be easily digestible; hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk (Million, *et al.,* 2006). Moreover, taro starch gelatinized easily and forms a clear and soft food similar to potato starch. Due to all these, taro has been used for industrial applications in many Asian countries (Wang, 1983). Taro also has low and soluble dietary fiber that has no bulkiness and satiety properties and fairly high amount of minerals; i.e. excellent source of potassium, phosphorus and zinc. In general, all nutrients important constituents of infant diet, are present in appreciable quantity in taro and scientific incorporation of taro in infant food has a great potential to supply high quality food and one of the cheapest source of energy for the child (Egounlety, *et al.,* 2002).

Any effort to be made in producing the new infant food development without affecting the food quality is very crucial and providing alternative food choice for rural peoples, especially for children to insure food security and reduce hunger at household level (Million, *et al.,* 2006 and WHO, 2008; Abebe, *et al.,* 2017). With this context, taro flour was used as energy sources to make proper complementary food rich in energy and in micronutrients, especially iron, zinc, and calcium and beta carotene. The importance of this new complementary food development was to identify locally available food sources with high digestible starch, low dietary fiber, low phytate and tannin based on optimally mixed ingredients. Such approach would be distinctively different from the previous studies and would have benefited the society to strengthen new optimized complementary food development and characterization of locally available food sources (Briend, *et al.,* 2001; Daelmans and Saadeh, 2003; Dewey and Brow, 2003).

4.2.3. MATERIALS AND METHODS

4.2.3.1. Sample source

Taro flour from boloso-1 variety was used together with soybean and maize to develop the optimized complementary food. Both maize and soya bean were obtained from the Oromia commodity exchange in Addis Ababa. The detailed procedure of each analysis is presented in chapter 3

4.2.3.2. Preparation of the ingredients

Taro, maize and soy flour were prepared according to (Hegstad, 2008; Ojinaka, *et al.,* 2009) respectively.

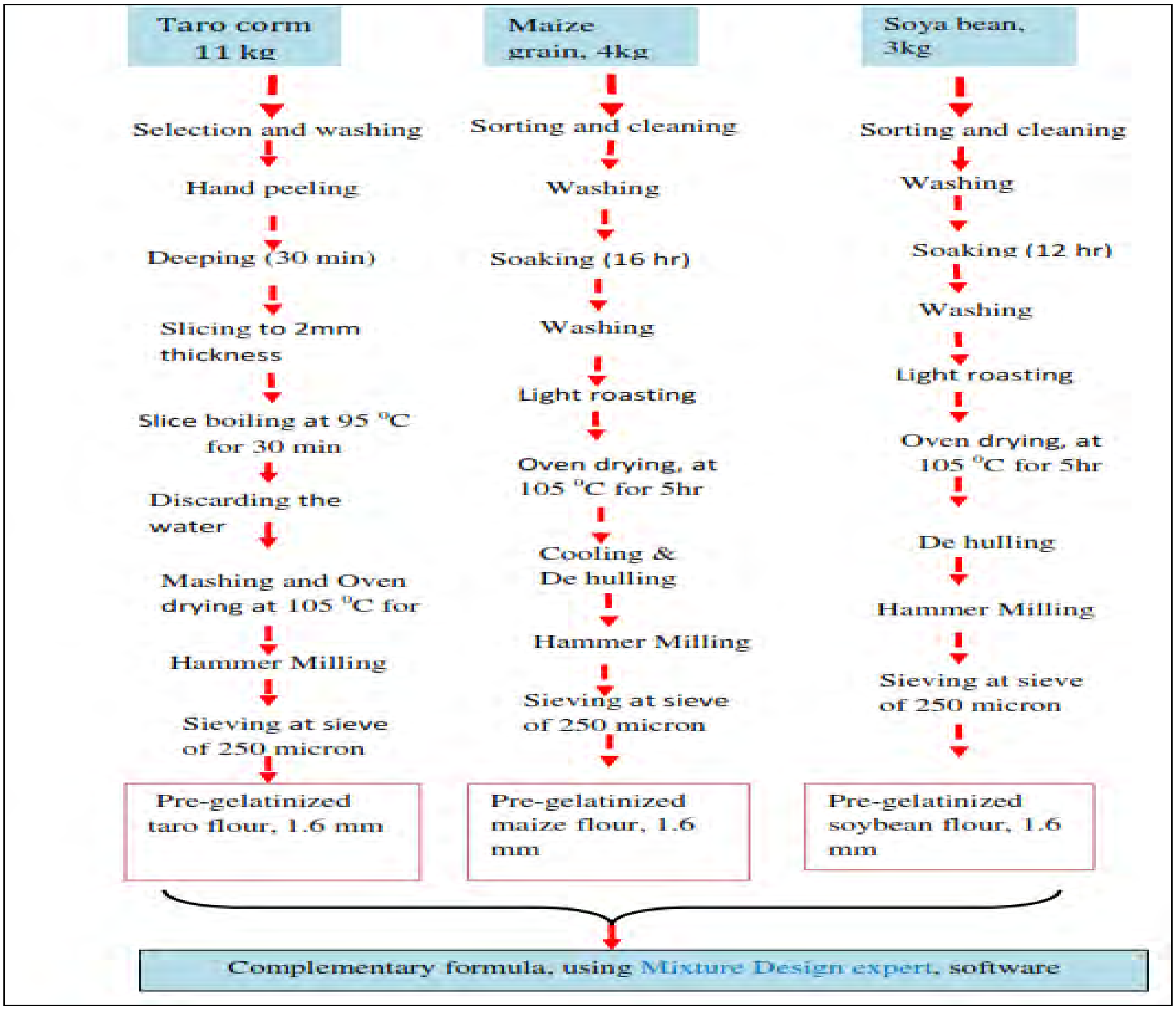

Figure 4.2.1: Flow diagram for the preparation of complementary food ingredients

Table 4.2. 1: Simplex lattice design for complementary food formulation.

	Components (%)			Proximate composition							Mineral contents			Other contents					sensory attributes			
No of formulation	Taro flour)	Maize flour (sprouted)	Soya bean flour (sprouted)	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Energy (kcal)	Fe (mg/100g)	Zn (mg/100g)	Ca (mg/100g)	Beta-carotene (ug/100g)	Vitamin A (RE)	Phenols (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Color	Flavor	Taste	Overall acceptability
1	47	32	22																			
2	43	33	23																			
3	40	40	20																			
4	40	30	30																			
5	40	40	20																			
6	40	30	30																			
7	50	30	20																			
8	45	30	25																			
9	40	35	25																			
10	42	32	27																			
11	45	35	20																			
12	42	37	22																			
13	45	35	20																			
14	50	30	20																			

The lattice design method was employed, to formulate recipes, study the main effect of parameters, create models between the variables, and determine the effect of these variables to optimize the levels of ingredients. Fourteen treatments in random order were created using simplex lattice design. The use of this mixture design expert was very useful to solve mixture problem and to get the best optimized mixture (Table 4.2.1).

4.2.3.3. Experimental design

A statistical software package (Design- Expert ®, version 10.0, Stat-Ease, SaMeep104 Inc.) mixture design was used for the generation of test formulations and analysis of the results. In this study, the effect of three components, namely taro (A), maize (B) and soybean (C) were used in optimize complementary formulation.

4.2.3.4 Experimental optimization and constraints

Both numerical as well as graphical optimization techniques were employed using the Design- Expert®, version 10.0, Stat-Ease, SaMeep104 Inc. The desired goal for each of the response attributes and the ingredients were chosen. The optimization criteria were applied for high CP, crude fat, total carbohydrate, β-carotene, Fe, Zn, Ca, energy, and low CF, phytic acid and oxalic acid in the complementary food processed.

Table 4.2. 2: Ingredient constraints design for complementary food developed

Low	Constraint	High
40.000	≤ A:taro flour (pre-gelatinized)	≤ 50.000
30.000	≤ B:maize flour(pre-gelatinized)	≤ 40.000
20.000	≤ C:soya bean flour(pre-gelatinized)	≤ 30.000
	A+B+C	100.000*

*: sum of the upper level of one component and the lower level of two components

The experimental data for each response variable were fitted to the quadratic model as: $\mathbf{Y= ß + X_1 + X_2 + X_3 + X^2{}_1 + X^2{}_2 + X^2{}_3 + X_1\ X_2 + X_1\ X_2 + X_2X_3}$............. Y: response; ß: constant; X_1, X_2, X_3: independent components; $X^2{}_1$, $X^2{}_2$ and $X^2{}_3$: quadratic regression and $X_1\ X_2$, $X_1\ X_2$ and X_2X_3: interaction regression.

4.2.3.5. Experimental analysis

Taro blended complementary food samples were analyzed for moisture, CP, CF, total ash, crude fat and using the (AACC, 2000; AOAC, 2000) reference methods. The total carbohydrate content was determined by nutrient difference (100 – % moisture + % protein + %fat +%fiber + % ash] according to Egan *et al.* (2005). The energy density (ED) was calculated using standard food energy conversion factors: 4 kcal/g for carbohydrates; 4 kcal/g for proteins and 9 kcal/g for crude fat according to Livesey (1995). The minerals, Na, Ca, Zn and Fe were analyzed by using atomic absorption spectrophotometer (AACC, 2000).

Beta-carotene and total phenolic compounds were determined according to Laurie, *et al.,* (2012) and (Sharma and Gupta, 2010). The anti-nutritional content, phytic acid was determined through phytate phosphorus (Ph-P) analysis according to Guttieri *et al.,* 2004. The AOAC (1990) method was used to determine the oxalate content of the sample. The food, functional properties of, water absorption index (WAI) was determined according to Anderson *et al.* (1992). Tannins were determined using the method of (AOAC, 1995). Lectins, Alpha-amylase inhibitors and protease (trypsin) inhibitors were determined according to e Leo et al. 2001; Noman, *et al.,* 2006; Bhattacharyya and Babu, 2009) respectively. Water holding capacity of infants and chicken diet samples was determined according to Anderson *et al* (1969). The water solubility capacity (WSC) in percentage was calculated as a ratio of dry residue from water holding capacity of the original mass and the water hydration capacity (WHC) of weaning food samples were estimated (AACC, 2000, method 56-20).

4.2.3.6. Sensory quality analysis

An acceptance test using a seven point hedonic scale with 7 (extremely like), 6 (very muchlike), 5 (moderately like), 4 (neither dislike nor like) 3 (moderately dislike), 2(very much dislike) and 1 (extremely dislike) was used for the sensory evaluation of the porridge prepared by the formulations according to (Okpala, *et al.,* 2013). The panelists instructed to taste and evaluate the samples on evaluation sheets. A total of 10 mothers were selected from health centre nurse and attendants to assess the samples. Sensory attributes assessed were mouth feel, color, taste, given as baby food and overall acceptability. Finally, the scores of all panelists were added and divided by the number of panelists to find the attribute mean score. Attribute mean score = $\frac{numberofscore \times headonicscale}{N(totalpanelists)}$

4.2.3.7. Statistical analysis

All data from Design- Expert® were analyzed using Mixture contour plots to determine the most desirable formulations and to determine the effect of each component.

4.2.3. RESULTS AND DISCUSSIONS

Moisture content of complementary foods

As summarized in Table 4.2.3, the moisture content (%) in different complementary food samples ranged from 6.13±0.4 to 6.98±0.2. The general trend observed in this study was that the moisture content was not differing as a function of the three components. Moisture content in food samples is an index of stability and determines the appearance, keeping quality and yield of the product (Ejoh *et al.,* 2006). Knowledge of the moisture content in food sample is necessary to predict the behavior of foods during processing, e.g. mixing, drying, storage and packaging. The moisture content of the food material is important to consider the food is suitable before the consumption. Moisture content affects the physical and chemical aspects of food which relates to the freshness and stability. For the storage of the food for a long period of time and the moisture content determines the actual quality of the food before consumption and to the subsequent processing in the food sector by the food producers. In this study the moisture content of the formulated samples is at low level and this might be due to the drying method (Isengard, 2001). Low moisture foods are not easily susceptible to the microbial attack and have a longer shelf life.

Crude Protein Content

The CP (%) in this study ranged from 21.18±0.4 to 26.21±0.87 (Table 4.2.3). The CP in different complementary blended products differ significantly ($p<0.05$). The protein content of blended products prepared from high soybean flour contained the highest. However, the lowest CP was obtained from the taro flour followed by maize. The CP in the complementary food formulated in this study was in the recommended ranges by WHO/UNICEF protein requirement for complementary feeding. According to the WHO/UNICEF recommendation, the minimum protein requirement is 14% (WHO/UNICEF, 2005). The CP content found in this study was higher than the one reported (17.7%) by Yewelsew *et al.,* (2006) and 8.56% of Amankwah *et al.,* (2009) and Rodriguez (2005) for complementary food processed from selected cereals and legumes. The CP% for commercial famix from this study was 23.617±0.23 and in the range of the complementary food samples. All the CP results are greater than the minimum requirement (14%) of WHO/UN specification.

The higher values of protein content of the current study might be due to the high protein content in soybean than staple cereals and grains commonly used for complementary food formulation. This result agrees with Nnam (2002) Badifu (2004) and Okoye *et al.,* (2010) who reported that the addition of legumes such as soybean in complementary food preparation would have been improved the protein content as a result of higher protein content in the soybean.

Table 4.2. 3: Moisture, Protein, Fat and Fiber composition of complementary food samples

	Components			Proximate composition (%)			
Formulation	A	B	C	Moisture	Protein	Fat	Fiber
1.	47	32	22	6.32 ± 0.4^{ab}	21.18 ± 0.4^{ab}	6.16 ± 0.57^{ab}	3.48 ± 0.45^{ab}
2.	43	33	23	6.43 ± 1.4^{ab}	26.09 ± 0.45^{a}	6.23 ± 0.67^{ab}	4.42 ± 0.46^{a}
3.	40	40	20	6.13 ± 0.4^{ab}	25.54 ± 0.45^{a}	6.12 ± 0.78^{ab}	4.16 ± 0.46^{a}
4.	40	30	30	6.54 ± 0.4^{ab}	25.76 ± 0.56^{a}	6.66 ± 0.86^{a}	4.56 ± 0.43^{a}
5.	40	40	20	6.89 ± 0.1^{a}	25.32 ± 0.65^{a}	5.98 ± 0.78^{ab}	4.18 ± 0.65^{a}
6.	40	30	30	6.53 ± 0.1^{ab}	25.87 ± 0.76^{a}	6.58 ± 0.86^{a}	4.54 ± 0.56^{a}
7.	50	30	20	6.57 ± 0.1^{ab}	22.23 ± 0.60^{ab}	6.32 ± 0.78^{ab}	3.57 ± 0.67^{ab}
8.	45	30	25	6.43 ± 0.2^{ab}	22.89 ± 0.68^{ab}	6.43 ± 0.65^{ab}	3.65 ± 0.76^{ab}
9.	40	35	25	6.18 ± 0.3^{ab}	26.17 ± 0.76^{a}	6.36 ± 0.65^{ab}	4.53 ± 0.78^{a}
10.	42	32	27	6.48 ± 0.1^{ab}	26.06 ± 0.67^{a}	6.55 ± 0.45^{a}	4.52 ± 0.32^{a}
11.	45	35	20	6.88 ± 0.2^{a}	22.16 ± 0.60^{ab}	6.25 ± 0.32^{ab}	3.21 ± 0.23^{bc}
12.	42	37	22	6.98 ± 0.65^{a}	26.21 ± 0.87^{a}	6.17 ± 0.54^{ab}	3.87 ± 0.32^{ab}
13.	45	35	20	6.23 ± 0.7^{ab}	22.67 ± 0.78^{ab}	6.16 ± 0.76^{ab}	3.33 ± 0.34^{ab}
14.	50	30	20	6.28 ± 0.6^{ab}	22.14 ± 0.78^{ab}	6.33 ± 0.45^{ab}	3.8 ± 0.43^{b}
15.	Control			7.15 ± 0.56	23.617 ± 0.23	7.445 ± 0.54	5.06 ± 0.46

$^{a-d}$ Means±SD within a column with different superscripts differ significantly ($p<0.05$); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.

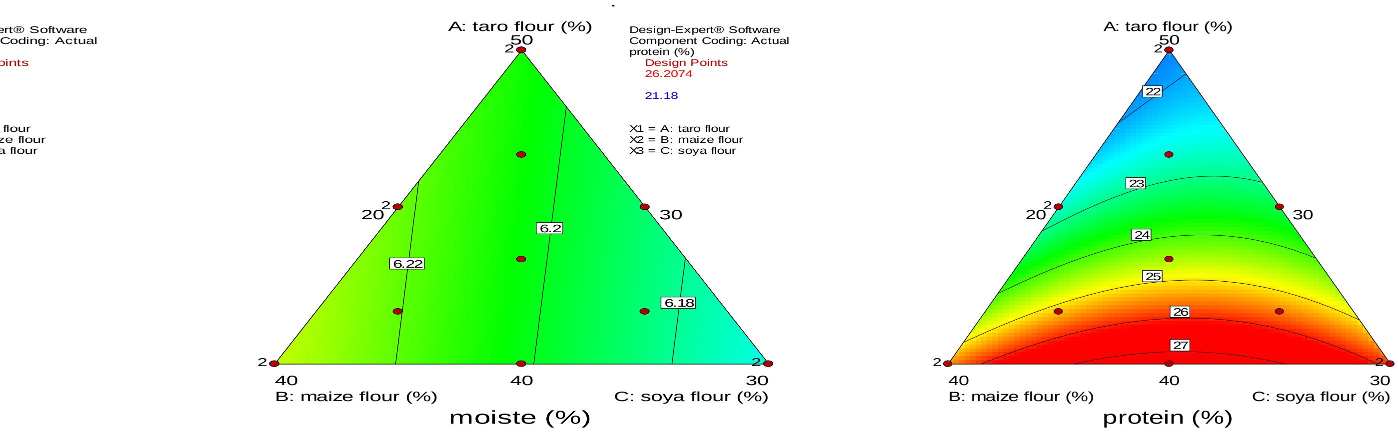

Figure 4.2.2: Contour plots for moisture and protein contents

The model equation for the moisture content was: Y= 6.21*A + 6.24 *B + 6.17*C lack of fit (P=0.231). The model equation for the crude protein content was: Y= 22.16*A + 25.46 *B + 25.79*C -5.67*AB – 4.76*AC + 1.88 BC. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

Crude Fat Content

The crude fat (%) in this study ranged from 5.98±0.78 to 6.66±0.86 (Table 4.2.3). The crude fat of blended products did not significantly differ (p>0.05). The blended products in this study had high crude fat content. This result agrees with Mosha *et al*. (2003) and Treche and Mbome (1999) who reported the crude fat in the range of 2.22-3.13g/100 g (DM basis) for homemade complementary foods. However, lower than the WHO/FAO recommendation (13.33 to 20.00 g/100 g) on crude fat (30%) requirements for complimentary food. The crude fat % of famix (7.445 ±0.54) was slightly high as compared to blended products. This might be due to the increased level of soybean in the famix (40 %) and increased the fat content. This is in agreement with the results of the other studies (Awasthi *et al.*, 2012; Ayo *et al.,* 2014; Banureka & Mahendran, 2011; Jimoh & Olatidoye, 2009; Olatidoye & Sobowale, 2011).

If a diet has a very low fat content, children may not be able to get an adequate amount of energy because of the bulkiness of the diet (Livesey, 1995). Recommended Daily Allowances of fat content for infants and young children has been suggested to be 30-45% (13.33 to 20.00 g/100 g) of energy in the complementary food (WHO/FAO, 2010). To achieve the required calories from crude fat of any complementary food, the addition of fat is preferable during the formulation (Klim, 2001; Lutter and Dewey, 2003; Akalu, *et al.,* 2010; Yusufu, 2013).

Crude Fiber Content

As the results showed in Table 4.2.3, the CF content in different complementary food samples ranged from 3.21±0.23 to 4.56±0.43 on a DM basis. The CF content of blended products prepared from soy bean flour contained slightly higher values and followed by maize flour. These values matched with the minimum recommended daily allowance of CF for infant complementary food as reported by WHO/FAO, 2010; Mariam, 2005, that the fiber content to be 2.17 g/100 g, which was also in agreement with this work. The crude fiber content of famix (5.06±0.46) was high value than that of blended products. This high crude fiber content in commercial famix could be due to use of high amount soybean (40 %) in the formulations.

If the fiber content of the complementary food is low they have to consume more to get satisfied for their daily requirement (Eka and Edijala, 1999). On the contrary, high dietary fiber in complementary food is bad, this is because, dietary fiber increases bulkiness and satiety that reduce the energy density of infant foods (Santika *et al.,* 2009; Onoja, 2011). The CF content of the complementary food is required not to exceed 5g per 100g (WHO/FAO, 1995; WHO/FAO, 2010 and Compaoré *et al.,* 2011). Therefore, all products from this study were not beyond the maximum recommended daily allowance, RDA, of crude fiber.

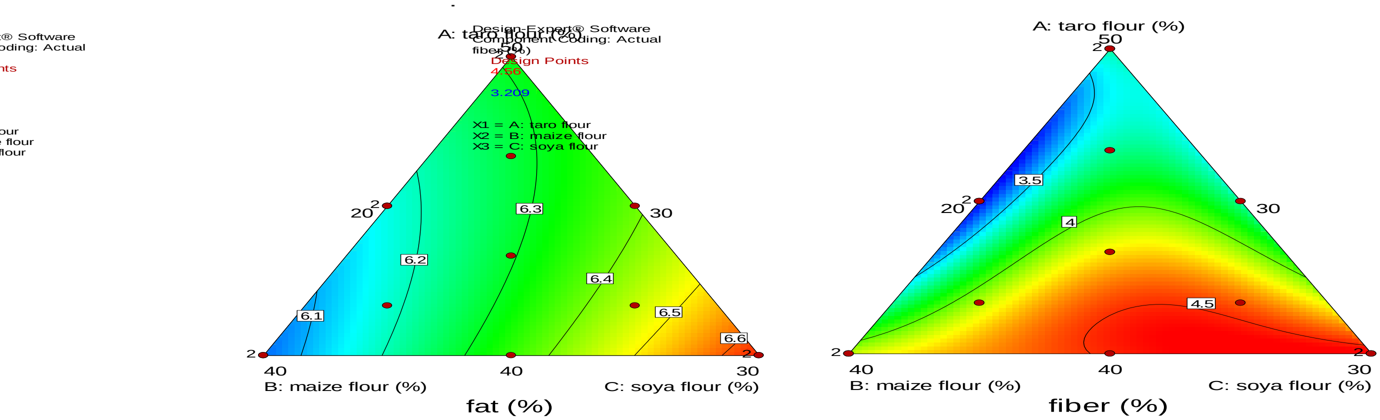

Figure 4.2. 3: Contour plots for crude fat and fiber contents

The model equation for the crude fat was: Y= 6.31*A + 6.05 *B + 6.64*C -0.052*AB – 0.34*AC + 0.033*BC. The model equation for the crude fiber was: Y= 3.66*A + 4.14 *B + 4.58*C -2.74*AB – 1.85*AC + 0.64*BC. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

Table 4.2. 4: Ash, Carbohydrate and Energy composition of complementary food samples.

	Components			Proximate composition		
Formulation	A	B	C	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)
1.	47	32	22	4.8±0.45 [a]	58.1±0.45 [a]	372±0.54 [ab]
2.	43	33	23	4.5±0.45 [a]	52.3±0.45 [b]	370±0.45 [bc]
3.	40	40	20	3.8±0.54 [ab]	54.3±0.43 [b]	374±0.43 [ab]
4.	40	30	30	4.8±0.46 [a]	51.7±0.65 [cd]	370±0.32 [bc]
5.	40	40	20	4.1±0.45 [ab]	53.5±0.76 [b]	369±0.34 [c]
6.	40	30	30	4.5±0.54 [a]	52.00±0.44 [b]	371±0.45 [b]
7.	50	30	20	4.8±0.32 [a]	56.5±0.46 [ab]	372±0.43 [ab]
8.	45	30	25	4.7±0.43 [a]	55.9±0.54 [ab]	373±0.45 [ab]
9.	40	35	25	4.3±0.45 [ab]	52.40±0.45 [c]	372±0.45 [ab]
10.	42	32	27	4.5±0.45 [a]	51.9±0.46 [cd]	371±0.65 [b]
11.	45	35	20	4.3±0.34 [ab]	57.2±0.54 [a]	374±0.54 [ab]
12.	42	37	22	3.7±0.45 [b]	53.1±0.45 [b]	373±0.45 [ab]
13.	45	35	20	4.1±0.65 [ab]	57.5±0.54 [a]	376±0.55 [a]
14.	50	30	20	4.6±0.43 [a]	56.8±0.54 [a]	373±0.54 [ab]
15.	Control			5.26±0.65	51.421±0.3	367.56±0.6

[a-d] Means±SD within a column with different superscripts differ significantly ($p<0.05$); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.

Total Ash Content

The total ash content (%) in different complementary food samples ranged from 3.7±0.45 to 4.8±0.46 as shown in Table 4.2.4. The result seen from this study revealed that total ash content was high in blended products prepared from taro flour. This might be due to taro has a high ash content (Kelbesa, 1998), which contributes a significant role in high ash content of the taro flour blended products. However, the ash content of the famix (5.26±0.65) was slightly higher as compared to blended products. This indicates that substitution of taro to maize can increase the ash (mineral) content of infant food. Some of the previous studies (Kelbesa, 1998; Ammar *et al.,* 2009; Sanful, 2011) were supported that the ash content of blended infant food increased as taro flour increased. In general, the ash content of homemade complementary foods might depend on the ash content of blended ingredients (WHO, 2003). To meet the mineral requirements of infants, a variety of mineral-rich complementary foods should be offered (Perez *et al.,* 2007; Njintang *et al.,* 2008 and WHO, 2015).

Total Carbohydrate and Energy value

The total carbohydrate content (%) in different samples ranged from 51.7±0.65 to 58.1±0.45 (Table 4.2.4). The total carbohydrate content of blended product prepared from the taro flour contained the highest carbohydrate followed by maize. The lowest total carbohydrate was obtained from soybean flour blended samples. This might be soybean is rich protein sources and low in carbohydrate. In most developing countries root, tuber and cereal grains are the major carbohydrate contributors to the diet. The carbohydrate content of the famix (51.421±0.3) was similar to some of the blended products made from greater soybean and smaller taro-maize levels in the formulation. Also the results agree with the estimated intake of 50g of carbohydrate in complementary food by an infant per day (Fernandez *et al.,* 2002). According to the work reported by Lauzon and Kawabata (1988); Jane *et al.* (1999) and Benesi *et al.* (2004), taro is a good source of small size digestible starch and would supply high carbohydrates than grain-cereals. Digestible carbohydrates are one of the main sources of dietary energy in infancy and childhood is essential for growth and development (Butte, *et al.,* 2000; Sepp, 2001).

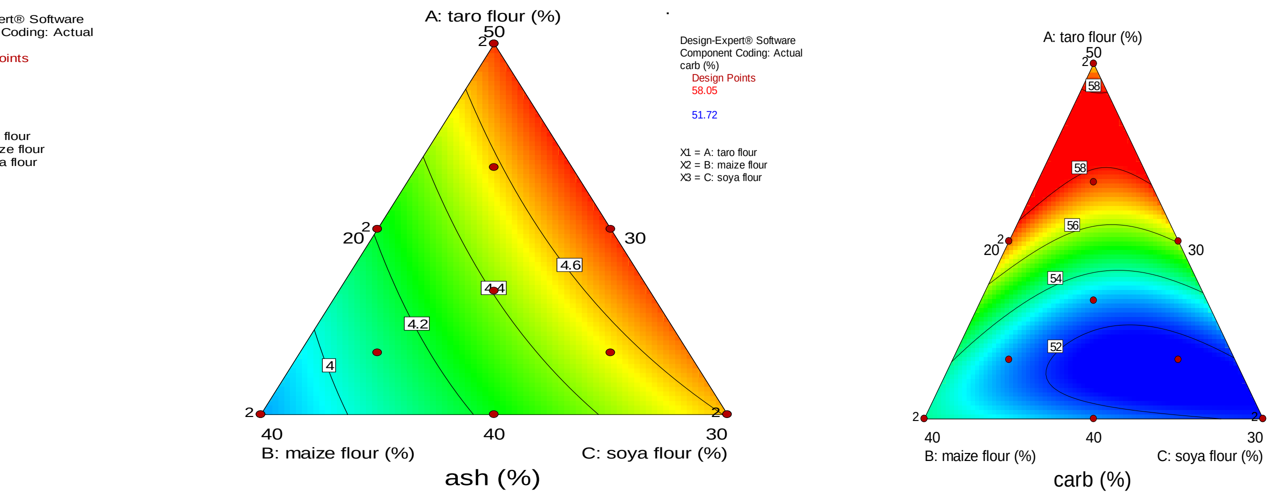

Figure 4.2. 4: Contour plots for total ash and carbohydrate

The model equation for total ash was: Y= 4.75*A + 3.86 *B + 4.61*C -0.37*AB + 0.38*AC + 0.8*BC. The model equation for total carbohydrate was: Y= 56.71*A + 53.94 *B + 51.91*C + 8.26*AB + 7.07*AC -1.48*BC. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

Energy Value

The total energy value (kcal/100 g) was ranged from 369±0.34 to 376±0.55. The energy content in different samples differ significantly ($p<0.05$). Walker (1990) suggested an energy density of 370 kcal/100 g as the minimum desirable level for infant weaning foods. Thus, the weaning foods by this work satisfied the optimum desirable level. Olajide *et al.* (2011) concluded in his research that the taro flour and taro products could be rich sources of energy food stuffs.

Table 4.2.5: Fe, Zn and Ca contents of taro based complementary food samples.

	Components			Mineral contents				
Formulation	A	B	C	Fe	Zn	Mg	P	Ca
1.	47	32	22	4.00±0.43 [a]	12.21±0.21 [a]	11.65±0.53[a]	8.43±0.54 [a]	27.65±0.45 [a]
2.	43	33	23	3.60±0.31 [a]	10.36±0.21 [ab]	9.54±0.23 [ab]	7.32±0.65 [ab]	22.34±0.45 [c]
3.	40	40	20	3.15±0.43 [ab]	8.38±0.21 [c]	9.78±0.43 [ab]	7.43±0.55 [ab]	20.56±0.45 [c]
4.	40	30	30	3.25±0.43 [ab]	9.48±0.12 [ab]	11.24±0.32 [a]	8.32±0.43 [a]	20.43±0.55 [c]
5.	40	40	20	3.05±0.32 [ab]	8.46±0.32 [c]	9.54±0.34 [b]	7.12±0.43 [ab]	27.45±0.67 [ab]
6.	40	30	30	3.35±0.23 [ab]	10.26±0.21 [ab]	11.32±0.34 [a]	7.89±0.34 [ab]	26.87±0.56 [ab]
7.	50	30	20	3.90±0.21 [a]	11.17±0.54 [ab]	11.43±0.43 [a]	8.32±0.34 [a]	29.43±0.65 [a]
8.	45	30	25	3.65±0.21 [a]	10.25±0.34 [ab]	11.98±0.43 [a]	8.61±0.34 [a]	27.87±0.45 [a]
9.	40	35	25	2.90±0.23 [ab]	8.47±0.54 [c]	10.43±0.54 [ab]	7.34±0.45 [ab]	26.87±0.56 [ab]
10.	42	32	27	2.90±0.32 [ab]	9.02±0.34 [ab]	9.56±0.45 [b]	7.12±0.32 [ab]	26.06±0.76 [ab]
11.	45	35	20	3.55±0.23 [a]	11.04±0.56 [ab]	11.43±0.54 [a]	8.32±0.34 [a]	26.16±0.65 [ab]
12.	42	37	22	3.70±0.43 [a]	10.30±0.45 [ab]	10.53±0.45 [ab]	7.76±0.34 [ab]	26.21±0.76 [ab]
13.	45	35	20	3.71±0.32 [a]	11.17±0.45 [ab]	10.76±0.54 [ab]	7.86±0.45 [ab]	26.67±0.56 [ab]
14.	50	30	20	4.00±0.32 [a]	12.04±0.45 [a]	11.43±0.45 [a]	8.32±0.34 [a]	27.14±0.67 [ab]
15.	Control			3.49	6.86	23.29	9.63	11.18

[a-c] Means±SD within a column with different superscripts differ significantly (p<0.05); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.

Mineral contents

As indicated in Table 4.2.5, the mg/100 g values in dry weight basis of Fe, Zn, Ca, Mg and P in this study was ranged from 2.9±0.23 to 4.00±0.43; 8.38±0.21 to 12.21±0.21; 20.43±0.55 to 29.43±0.65; 9.54±0.34 to 11.98±0.43 and 7.12±0.32 to 8.61±0.34 values, respectively and differs significantly ($p<0.05$). Except the P contents (7.12), the other mineral contents of famix Fe (3.49), Zn (6.86), Ca (23.29) and Mg (9.56) were smaller than that of the blended products. WHO recommended the major criteria for a good-quality complementary food to be adequate protein content, high energy value per unit of food volume, soft texture, low fiber content, adequate vitamins and minerals and absence of anti-nutritional factors (WHO, 1998, 2000; 2001).

The result of this study shows that the mineral content differ significantly due to blending levels of the components ($p<0.05$). Complementary foods prepared from the taro flour obtained greater mineral values while the smaller value contained in blended products prepared from maize flour. This might be taro is rich in mineral and as the blending level increase consequentially increase the mineral level in the blended formula. This observation is in agreement with the reports of Gordon (2000); Baruah (2002); Nijoku and Ohia (2007); Poiana *et al.* (2009) who reported that taro had higher mineral values than cereals and grains. In complementary feeding, adequate intakes of minerals are important for ensuring optimal health, growth and development of infants and young children (WHO, 2013).

In particular to Ca, the WHO (2013) had also reported the recommended Ca concentration of complementary food to be 400 - 500 mg/day and none of the complementary food prepared met this demand. This is because low Ca concentration of cereals, root crops and legumes compared with animal foods and the implication from this is that complementary food should be fortified mainly with high Ca and Fe rich food types such as milk and other animal food sources (Bultosa and Taylor, 2004). For the better sources of Mg, P and Zn taro flour obtained adequate amount and could be preferable ingredients for complementary food development. At present, in some developing countries and West Africa taro flour is industrially applied as energy and mineral source for infant food (WHO, 2013; UNICEF, 2001).

Now a day, zinc deficiency affects the health and well-being of populations worldwide. The higher Zn concentration of the complementary foods prepared for this study might be taro as a good source of non animal origin Zn in infant food (Onoja and Obizoba, 2009; Yusufu *et al.,* 2013; Alcantara, *et al.,* 2013). Zinc also enhances the body"s immune system, thus, protecting children from infections (Moshat *et al.,* 2000). Therefore, the addition of taro in the present study brought promising out put on improving the mineral content of complementary food mainly with Zn, P and Mg. Previous studies had also provided evidence that the mineral concentration of complementary food samples was increased due to the addition of taro and other root food added to the complementary products (Sefa-Dedeh *et al.,* 2004; Soudy, *et al.,* 2010; Adejumo and Bamidele, 2012).

The WHO and FAO recommendations, to meet the nutritional mineral requirements of infants, a variety of mineral-rich complementary foods should be offered, since the consumption of these foods is relatively small among infants/children aged between six and

24 months. From 9 to 11 months of life, the amount of minerals that should be provided by complementary foods is high: 97% for iron, 86% for zinc, 81% for phosphorus, 76% of magnesium and 72% for calcium (WHO /FAO, 2008).

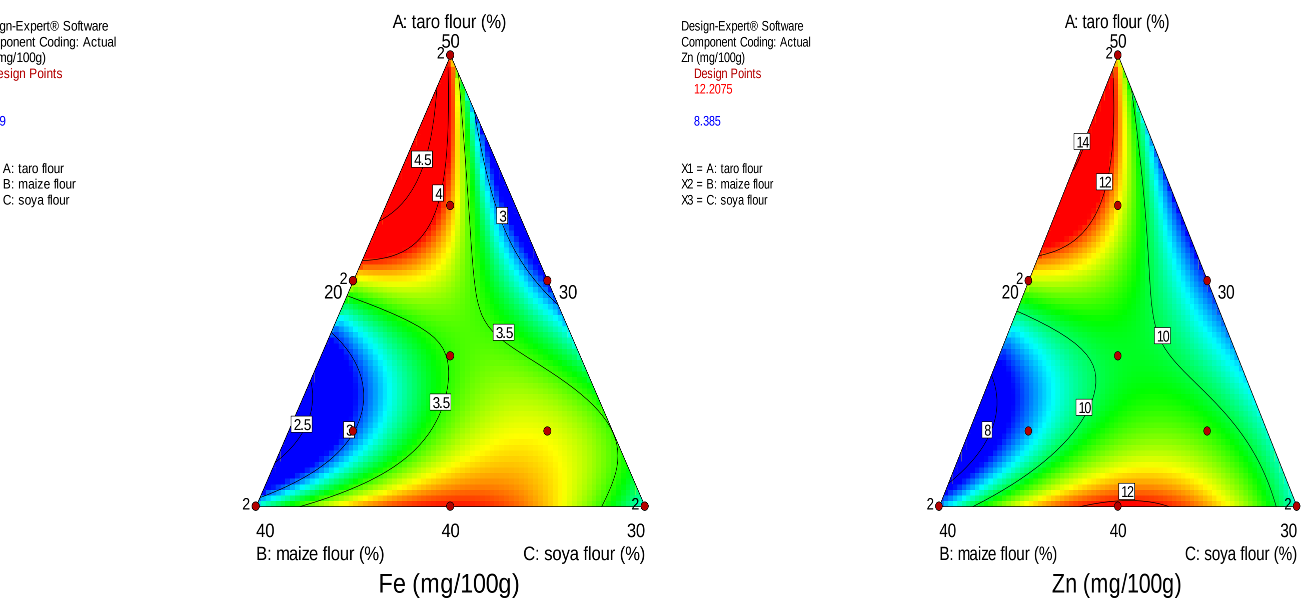

Figure 4.2. 5: Contour plots for total Fe and Zn (mg/100g) contents

The model equation for Fe was: Y=3.84*A + 3.19 *B +3.19*c + 0.77*AB – 2.59*AC + 3.11*BC. The model equation for Zn was: Y= 18.61*A + 3.91 *B + 3.41*C + 0.186*AB + 0.041*AC +0.039*BC. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

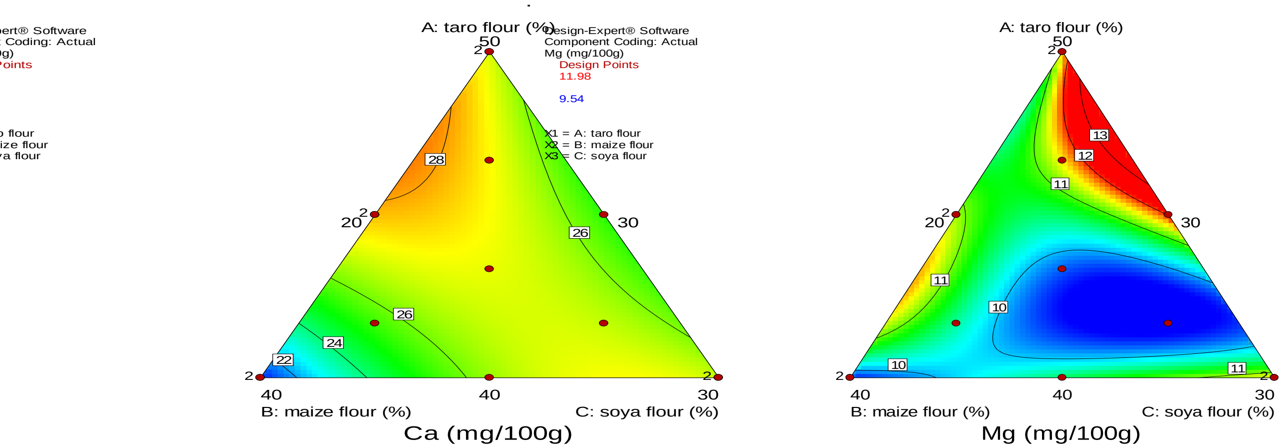

Figure 4.2. 6: Contour plots for total Ca and Mg (mg/100g) contents

The model equation for Ca was: Y= 26.78*A + 20.77 *B + 26.63*C + 15.96*AB – 6.01*AC + 10.48*BC. The model equation for Mg was: Y= 11.45*A + 9.67*B + 11.29*C + 2.25*AB + 2.65*AC - 4.56*BC.

Table 4.2.6: Beta carotene and other constituents of complementary food samples

	Components			Constituents			
				ß-carotene	Phenols	Phytate	Oxalate
Formulation	A	B	C	(µg/100g)	(mg /100g)	(mg/100g)	(mg/100g)
1.	47	32	22	190.45±0.32 ^{a}	71.78±0.23 ^{ab}	0.89±0.54 ^{b}	10.43±0.45 ^{b}
2.	43	33	23	162.65±0.32 ^{c}	61.65±0.23 ^{cd}	1.45±0.32 ^{a}	10.32±0.54 ^{ab}
3.	40	40	20	162.67±0.22 ^{c}	61.65±0.32 ^{cd}	1.53±0.32 ^{a}	10.45±0.55 ^{ab}
4.	40	30	30	190.41±0.43 ^{a}	71.43±0.43 ^{ab}	0.8±0.34 ^{b}	9.76±0.44 ^{a}
5.	40	40	20	162.43±0.32 ^{c}	60.46±0.43 ^{cd}	1.08±0.34 ^{ab}	10.43±0.56 ^{ab}
6.	40	30	30	170.43±0.32 ^{ab}	64.78±0.54 ^{c}	1.43±0.45 ^{a}	10.65±0.54 ^{a}
7.	50	30	20	190.43±0.43 ^{a}	70.43±0.45 ^{b}	0.99±0.45 ^{b}	10.76±0.45 ^{a}
8.	45	30	25	193.61±0.43 ^{a}	74.65±0.54 ^{a}	0.96±0.45 ^{b}	10.98±0.45 ^{a}
9.	40	35	25	162.45±0.76 ^{c}	57.65±0.56 ^{d}	1.45±0.54 ^{a}	10.32±0.45 ^{ab}
10.	42	32	27	157.64±0.23 ^{d}	57.65±0.54 ^{d}	1.56±0.65 ^{a}	10.78±0.45 ^{a}
11.	45	35	20	190.21±0.65 ^{a}	74.54±0.54 ^{a}	0.95±0.34 ^{b}	10.43±0.45 ^{ab}
12.	42	37	22	167.79±0.23 ^{c}	60.45±0.54 ^{cd}	1.32±0.34 ^{ab}	10.32±0.45 ^{ab}
13.	45	35	20	170.86±0.65 ^{ab}	60.43±0.54 ^{cd}	1.29±0.34 ^{ab}	10.78±0.45 ^{a}
14.	50	30	20	189.34±0.43 ^{a}	74.46±0.54 ^{a}	0.89±0.45 ^{b}	10.34±0.78 ^{ab}
15.	Control			176.21±0.57	65.66±0.78	45.68±0.8	3.65±0.78

$^{a-c}$ *Means±SD within a column with different superscripts differ significantly ($p<0.05$); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.*

Beta-carotene content

The ß-carotene content (µg/100 g) in complementary food prepared for this study ranged from 157.64±0.23 to 193.61±0.43 (Table 4.2.6). The ß-carotene content was similar to the complementary food values reported in the previous study on soybean-sweet potato supplementary food (Onoja, 2014). Among the components, taro flour contained highest beta-carotene and also the binary combination between taro and maize or soybean flour was also shown synergetic effect. This might happen due to high content of beta-carotene in taro. Infant food prepared by this work could supply adequate requirement of beta-carotene content as similar to suggested by WHO (2003). Report as presented by FAO/WHO (2010) that the estimated average requirement of ß-carotene content is 286 µg/100 g which is higher than the complementary food formulated in this work. Other reports showed that the recommended daily allowance (RDA) of ß-carotene content for infants and young children has been suggested is 400 µg/100 g (Jose, 2003 ; WHO/FAO, 2009).

Total phenolic content

The total phenolic content in Gallic Acid Equivalence (mg GAE/100 g) of complementary food prepared for this study ranged from 57.65±0.56 to 74.65±0.54 Table 4.2.6. The total phenolic content of the blended formulas were significantly ($p<0.05$) different. The result shows that there is a high content of phenolic compounds in taro than maize and soya bean. This might be due to phenolic compound difference in the components. The other reason for reducing phenolic compound in maize and soybean might be associated with processing effects and the application of soaking and drying reduced the phenols. Dewanto *et al*. (2002) also reported that total phenolics were significantly ($p< 0.05$) lower after soaking and boiling. Therefore, the time and temperature for soaking and drying of ingredients should be optimized and controlled for recovering water soluble bound phenols (Akubor, 2008). With this context, the present study is consistent with the previous studies and the notable finding from this study suggests soaking and drying of the components could reduce phenolic compound (Umar *et al.,* 2005).

The beta carotene and phenolic contents of famix (176.21±0.57, 65.66±0.78) were similar to that of the blended products; however, the phytate content (45.68±0.8) of famix was higher than of all blended samples in the study. This might be due to high phytate content in soybean (Nunes, *et al.,* 2006).

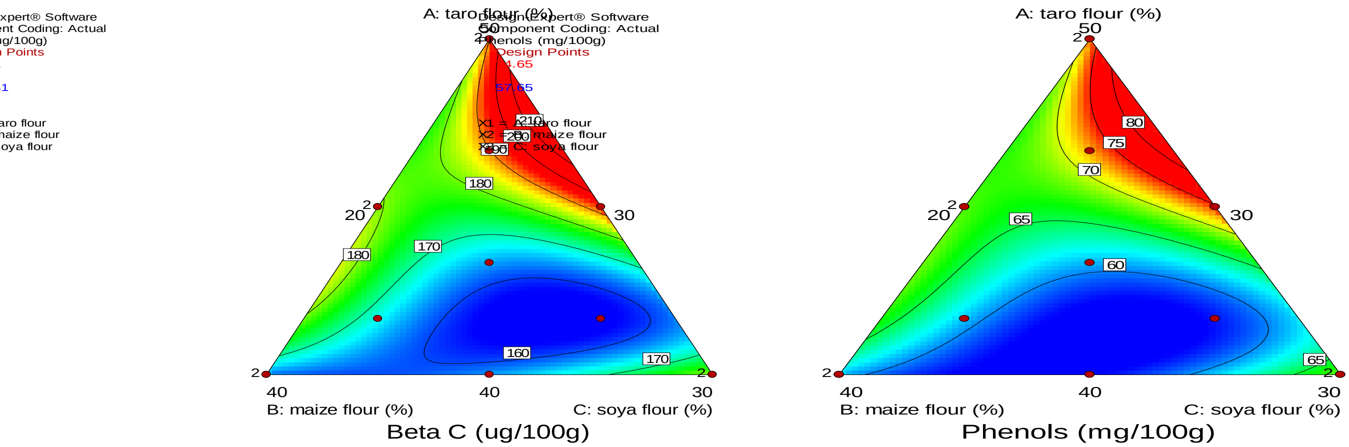

Figure 4.2.7: Contour plots for Beta C (µg/100g) and Phenols (mg/100g) contents

The model equation for ß-carotene was: Y= 11.45*A + 9.67*B + 11.29*C + 2.25*AB + 2.65*AC - 4.56*BC. The model equation for Phenols was: Y= 72.40*A + 61.01*B + 68.06*C + 2.77*AB + 17.00*AC – 28.22*BC. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

Phytate and oxalate level of the complementary food

Phytate and oxalate are the most important anti-nutritional factors accounted for complementary food development in this study. As result seen from Table 4.2.6 of the present study, the phytate level (mg/100 g) ranged from 0.8±0.34 to 1.56±0.65. The blended products prepared from maize and soybean had higher phytate values than taro. To reduce the adverse effect of phytates on mineral absorption, phytate content has to be reduced to concentrations of less than 200mg/100 g DM (Hurrell, 2004). Results from this study showed that, most of the food had less phytate (Siddhuraju and Becker, 2001).

Earlier experiments, for instance (Kirby and Nelson, 1988; Adewusi, 2001), clearly noted that anti-nutritional content of infant foods could be reduced through raw material selection and using effective domestic processing methods such as soaking, drying and other thermal treatments. Therefore, the anti-nutritional content of any complementary food should be reduced to maximize the mineral level in the diet for optimal growth, development and healthy maintenance of the body over a life span (Harland, *et al.,* 1988; Hurrell, 2004). Whole grain and soybean based foods, the content of phytate increased due to high phytate in the bran, so processing methods that reduce the phytate and foods with low phytate content were recommended for infants and all consumers. However, the phytate content of test food samples is very low compared with the maximum recommended daily intake of phytate from food which is 300–500 mg/day (Kikafunda, *et al.,* 2003; Obayanju and Ikujenlola,2002; American dietetic association, 2005; Salunke, 2006; Hotz and Gibson, 2007).

The study also revealed that there is slight variation in oxalate content (mg/100 g) was observed among the components. The oxalate level (mg/100 g) ranged from 9.76±0.44 to 10.98±0.45. The higher oxalate was occurring at the binary effect of taro and soybean flour. This might be due to the difference in oxalate contents of the individual components and their combined effect elevated the oxalate level. The results are in agreement with the finding of Cartherwood *et al.* (2007); Hang *et al.* (2009); Hang and Binh (2013). The oxalate content of test food samples is very low compared with the maximum recommended daily intake of oxalate from food which is 40–50 mg/day (American Dietetic Association, 2005). Foods with oxalate levels greater than 50 mg/100 g are categorized as high oxalate foods, and these include whole bran cereals and legumes (Chai and Liebman, 2005; Boontaganon, *et al.,* 2009). The oxalate content of these foods was within the range reported in the literature (Chai and Liebman, 2005; Siener and Hesse, 2006; Boontaganon *et al.,* 2009).

The importance of reducing anti-nutritional factors and fiber in complementary foods was emphasized by WHO complementary food requirement (WHO, 2008). Whole grains (often used in traditional complementary foods) contain high levels of dietary fiber and anti-nutrients. Therefore, use of processing methods such as soaking, drying and milling is crucial to reduce fiber and anti-nutrients in whole grains (Nnam, 2002).

For this reason, in this study taro was pre-gelatinized, both maize and soybean was soaked under water, followed by drying and milling before mixing them all to reduce the oxalate level (Omwamba and Mahungu, 2014).

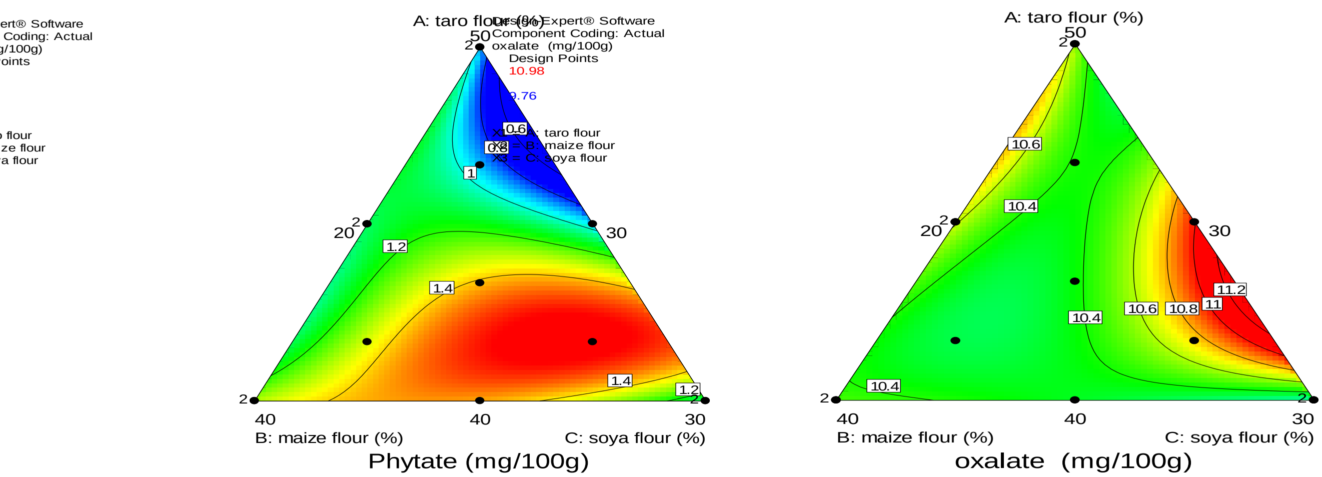

Figure 4.2. 8: Contour plots for Phytate and Oxalate (mg/100g) contents

The model equation for phytate was: Y= 0.90*A + 1.29 *B + 1.14*C + 0.07*AB + 0.11*AC + 1.48*BC. The model equation for oxalate was: Y= 10.50*A + 11.27*B + 10.79*C + 1.49*AB + 1.64*AC +4.49 *BC.

There were higher phytate and oxalate values for three combinations of taro, maize and soybean flour blended products (Fig. 4.2.8). The general trend observed since the components were the increased in taro flour content showed antagonistic effects on phyate content. The possible reason for this might be the effect of pre-treatments on taro flour preparation prior to blending with the components. Although taro is claimed for high oxalate containing crop, most of the oxalate found are water soluble and more than 65% of the entire oxalate can be eliminated through simple processing such as boiling water and cooking (Hang *et al.* (2009); Cartherwood *et al.* (2007); Hang and Binh (2013). In previous studies (Adewusi, 2001; Siddhuraju and Becker, 2001 and Bothwell and Chalton, 2002), processing methods was reported as having to lower the level of anti-nutritional factors. Therefore, the anti-nutrients in any complementary food should be minimized so as to increase other nutrient dense and bio-availability. Hence, the presence of anti-nutrients in food are bad because they inter bind mainly with mineral, proteins and carbohydrates result in reducing nutrient density, digestibility and utilization (Young, 2011).

Table 4.2.7: Water holding, solubility and hydration capacity

	Components			Functional properties (%)		
Formulation	A	B	C	holding capacity	Solubility capacity	Hydration capacity
1.	47	32	22	0.77±0.21 ^{ab}	22.7±0.32 ^{a}	1.76±0.34 ^{a}
2.	43	33	23	1.13±0.21 ^{a}	13.45±0.34 ^{c}	1.02±0.23 ^{ab}
3.	40	40	20	1.17±0.21 ^{a}	15.43±0.42 ^{b}	1.04±0.23 ^{ab}
4.	40	30	30	1.18±0.32 ^{a}	21.87±0.43 ^{a}	1.77±0.23 ^{a}
5.	40	40	20	1.09±0.12 ^{a}	12.65±0.32 ^{b}	1.04±0.34 ^{ab}
6.	40	30	30	0.67±0.21 ^{ab}	19.34±0.54 ^{ab}	1.46±0.34 ^{a}
7.	50	30	20	0.65±0.32 ^{ab}	21.98±0.34 ^{a}	1.57±0.45 ^{a}
8.	45	30	25	0.62±0.32 ^{ab}	22.76±0.45 ^{a}	1.98±0.34 ^{a}
9.	40	35	25	1.26±0.46 ^{a}	14.7±0.34 ^{b}	1.08±0.44 ^{ab}
10.	42	32	27	1.21±0.34 ^{a}	11.87±0.45 ^{c}	1.07±0.45 ^{ab}
11.	45	35	20	0.64±0.23 ^{ab}	22.43±0.34 ^{a}	1.86±0.86 ^{a}
12.	42	37	22	1.19±0.34 ^{a}	17.43±0.32 ^{ab}	1.32±0.32 ^{ab}
13.	45	35	20	1.17±0.34^{a}	19.45±0.32 ^{ab}	1.32±0.53 ^{ab}
14.	50	30	20	0.61±0.53 ^{ab}	22.45±0.43 ^{a}	1.46±0.45 ^{a}
15.	Control			1.89±0.56	19.56±0.89	1.52±0.34

$^{a-c}$ Means±SD within a column with different superscripts differ significantly ($p<0.05$); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.

Water holding, solubility and hydration capacity of complementary food samples

As shown in Table 4.2.7, the highest value of water holding capacity (WHC) was 1.19±0.34 and the lowest value was 0.61±0.53 on a DM basis. Complementary food samples prepared from maize flour showed a greater water holding capacity (WHC) following soya bean flour and taro flour. In other words, the WHC value of blended products decreased towards taro flour and this may be related to the difference in degree of starch fragmentation. Similar findings were reported by Jirarat *et al.* (2007); Obatolu *et al.* (2009). The functional properties, water holding, solubility and hydration capacity of famix was in the ranges of the blended products.

Higher water holding related with the presence of larger size, starch fragments (Seker, 2005) in grain-cereal based food and Compaore *et al*. (2011) had reported low water holding capacity in legume based complementary foods.

Water holding in food is related with water dissolving (hydrophilic) constituents of carbohydrate, lipids and proteins in the diet (Mbaeyi, 2005; Ugwuona, *et al.,* 2012). Complementary foods with high water holding are bad because the more water absorbed from the diet, reduce the uptake of solid constituents so that the required nutrient will not taken by the child (Ikpeme-Emmanuel *et al.,* 2009). Taro flour has small size, starch granules that reduce water holding potential than that of large size granules of cereal flour such as maize (Hallen *et al.,* 2004; Seker, 2005). The result from this study were in agreement with the findings of Blazek and Copeland, (2008) and taro blended products from this study were recommended for infant food.

The solubility capacity (SBC) of complementary food samples in this study was ranged from 11.87±0.45 to 22.76±0.45 (Table 4.2.7). The SBC in different samples differs significantly ($p<0.05$) in the present study. The result founded for this study predicted that blended products prepared from taro flour had high SBC than maize and soybean blended samples. This increase in the SBC for taro blended products might be due to the pre-gelatinization effect and smaller size starch in taro flour. The solubility enhanced also may be due to taro starch contains amylose/amylopectin ratio is 1:7 and an amylopectin content which is higher compared to cereals. As the amylopectin content increase, also swelling power and solubility increase. Therefore, gelatinization increases the mobilization of starch granules and high solubility function (Jirarat *et al*., 2006).

The pre-gelatinized starches have a higher tendency to soluble by water than non-gelatinized starch in food (Jones *et al.,* 2000). These findings were similar to the work of Colonna *et al*. (1989); Ollet *et al*. (1990); Sharma and Gujiral (2010) who reported increased SBC of haricot bean after pre-gelatinizing. Starch has the tendency to become soluble after different thermal treatment and such phenomenon contribute towards an increased SBI (Srichuwong, 2005b; Singh *et al.,* 2003; Jones *et al.,* 2000). When starch heated with water, the granules absorb more water and ruptured many times that leads to granules fragility and solubility (Ojinnaka, *et al.,* 2013).

The water hydration capacity (WHC) in this study ranged from 1.02±0.23 to 1.98±0.34 and did not differ significantly (p>0.05). The general trend observed from this study was that the blended products prepared from taro had slightly higher water hydration capacity than the others. This variation in WHC in the blended products might be due to starch size difference and the level of water soluble constituents in the ingredients. The smaller size, starch fragments and more water soluble substances favor hydration properties in food (Foh *et al.,* 2010). Previous studies also provided evidence that WHC was related to the degree of starch gelatinization (Owusu-Ansah *et al.,* 1993; Adebowale, *et al.,* 2005; Kaur & Singh, 2006). Gelatinization which leads to the transformation of raw starches to a cooked soluble form is one of the important effects on starch component of foods. Interactions of water with soluble contents in complementary food are very important because of their effects on the flavor and texture of foods (Barbut, 1999; Singh, 2001; Kaushal, *et al.,* 2012).

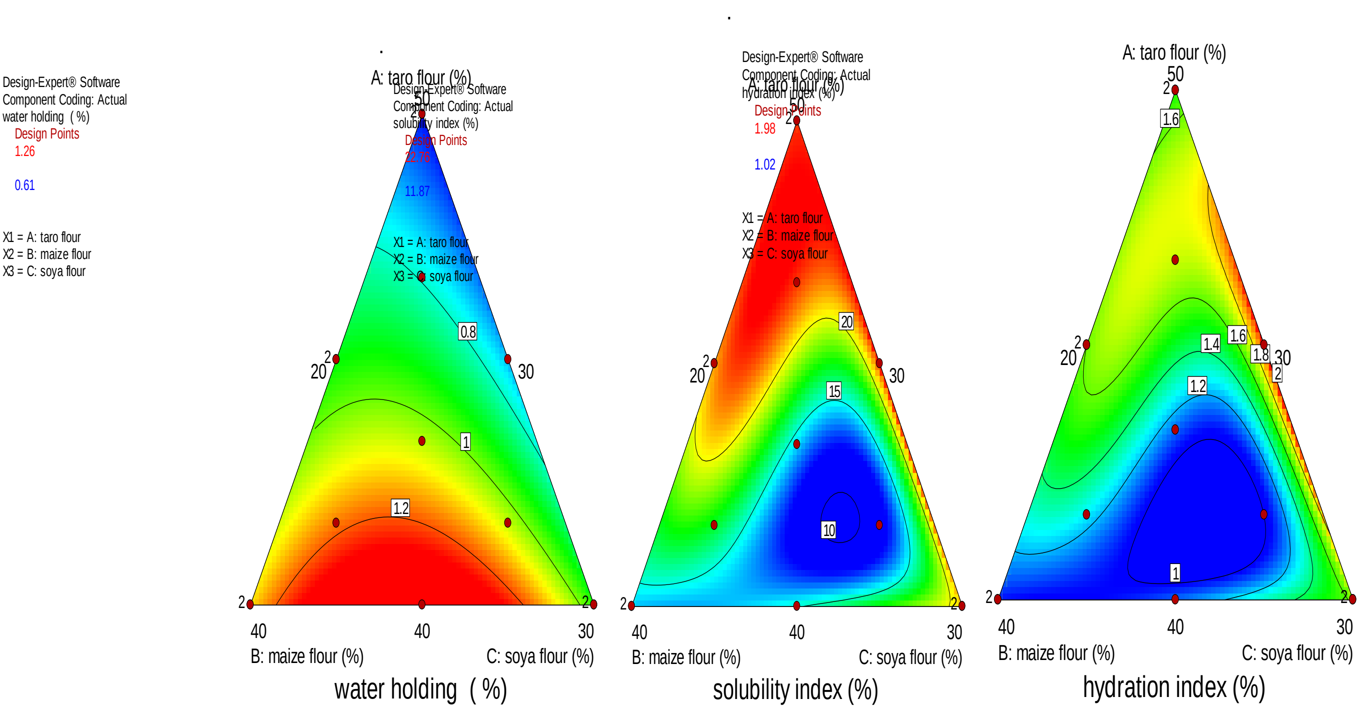

Figure 4.2. 9: Contour plots for water absorption, solubility and hydration capacity

The model equation for water absorption capacity was: $Y= 0.62*A + 1.23 *B + 1.02*C+ 0.26*AB - 0.22*AC + 1.37*BC$. The model equation for water solubility capacity was: $Y= 22.26*A+ 14.08*B + 20.64*C + 11.42*AB + 5.92*AC - 9.97*BC$. The model equation for water hydration capacity was: $Y= 1.52*A + 1.05*B + 1.62*C + 1.27*AB + 1.73*AC - 0.93*BC$. The positive (+) sign in the equations means that the response value increased with the increase of the variable combination while the negative (-) sign means that the response value decreased with the increase of the variables.

The analyzed model shows that, the blending ratio had an effect on absorption, solubility and hydration index (%) of the mixed ingredients. There were lower values of water absorption and higher values of solubility and hydration index for taro flour blended products (Fig. 4.2.9). The general trend observed from the components were the increase in maize and soya bean contents showed antagonistic effects on solubility property. There are evidences that complementary food with high solubility and low water absorption property are more desirable for infant nutrition (Ikpeme-Emmanuel *et al.,* 2009). Therefore, formulating complementary food with the addition of taro showed good functional properties.

Sensory qualities

For sensory analysis, 2 medium size cups (200g) of each mixed formula were mixed with 4 cups of water (460 ml). Then, placed in cooking pan on moderate heat and stirred constantly (to avoid lump formation) until boiling. One cup of water boiled in another pot was added gradually until a moderately light consistency was achieved. The porridge was then allowed to boil slowly under low heat for 20 minutes and served in labeled cups with plastic spoons. Mothers are used for the sensory test, because their ability to objectively evaluate the sensory characteristics of the formulations.

Table 4.2.8: Sensory qualities of taro based complementary food

Components				Attributes				
Formulations	A	B	C	Mouth feel	Color	Taste	Given as baby food	Overall acceptability
1	47	32	22	6.11±0.32^{ab}	5.76±0.12^{ab}	6.43±0.21^{ab}	6.43±0.12^{a}	6.35±0.23^{ab}
2	43	33	23	6.12±0.13^{ab}	6.21±0.12^{a}	6.56±0.21^{a}	6.12±0.32^{ab}	6.56±0.23^{a}
3	40	40	20	6.16±0.32^{ab}	6.32±0.21^{a}	6.56±0.32^{a}	6.07±0.21^{ab}	6.54±0.32^{a}
4	40	30	30	6.16±0.34^{ab}	6.23±0.21^{a}	6.54±0.21^{a}	6.08±0.23^{ab}	6.56±0.21^{a}
5	40	40	20	6.49±0.45^{a}	6.22±0.32^{a}	6.53±0.34^{a}	6.12±0.21^{ab}	6.46±0.12^{ab}
6	40	30	30	6.49±0.34^{a}	5.98±0.23^{ab}	6.52±0.21^{a}	6.12±0.21^{ab}	6.47±0.23^{ab}
7	50	30	20	6.25±0.53^{ab}	5.53±0.23^{ab}	6.45±0.13^{ab}	6.45±0.21^{a}	6.35±0.21^{ab}
8	45	30	25	6.16±0.34^{ab}	5.78±0.23^{ab}	6.45±0.23^{ab}	6.45±0.32^{a}	6.45±0.23^{ab}
9	40	35	25	6.36±0.45^{a}	5.98±0.12^{ab}	6.55±0.13^{a}	6.21±0.23^{ab}	6.45±0.23^{ab}
10	42	32	27	6.27±0.34^{ab}	5.76±0.12^{ab}	6.56±0.13^{a}	6.12±0.32^{ab}	6.43±0.23^{ab}
11	45	35	20	6.38±0.32^{a}	5.54±0.21^{ab}	6.43±0.43^{ab}	6.32±0.21^{a}	6.43±0.43^{ab}
12	42	37	22	6.36±0.43^{a}	5.65±0.32^{ab}	6.45±0.43^{ab}	6.23±0.23^{ab}	6.45±0.32^{ab}
13	45	35	20	6.11±0.34^{ab}	5.45±0.23^{ab}	6.32±0.32^{ab}	6.34±0.12^{a}	6.43±0.23^{ab}
14	50	30	20	6.12±0.54^{ab}	5.53±0.21^{ab}	6.24±0.23^{ab}	6.32±0.21^{a}	6.45±0.43^{ab}
15	Control			6.51±0.65	5.8±0.46	6.57±0.65	6.34±0.56	6.85±0.65

$^{a-c}$ *Means±SD within a column with different superscripts differ significantly (p<0.05); SD: standard deviation; A: taro flour; B: maize flour; C: soybean flour.*

The sensory values of infant porridge prepared from taro, maize and soya were presented in Table 4.2.8. There is no noticeable difference on mouth feel, taste, color and acceptability between the famix and the blended complementary food samples. The average score given for mouth feel in porridge samples ranged from 6.11±0.32 to 6.49±0.45. The mouth feel is the initial perception of food in the palate and related to the softness, thickness and other physical and chemical interactions inside the mouth (Sivam *et al.,* 2010; Ivanovski, *et al.,* 2012). The mouth feel of all porridge samples was rated greater than 6 indicating that liked very much by the panelists and the addition of taro flour did not vary mouth feel acceptability. The average score given for color in different samples ranged from 5.53±0.21 to 6.32±0.21. The increase of taro flour slightly decreased the color compared to maize and soybean. However, the color acceptability did not differ significantly (p>0.05). In any new food product development color is one of the mo~~i~~st important factors to be considering and affect the acceptability of the product (Kikafunda *et al*., 2006).

The mean score given for taste ranged from 6.24±0.23 to 6.56±0.32. All products were rated greater than 6 indicating that the taste of the porridge were accepted or liked very much by panelists. However, the taste of the porridge less intensified due to wards taro flour. In spite of maize and soybean, the taste improved and this might happened due to the presence of important sugar in maize such as sucrose, fructose, maltose, glucose and raffinose and high fat content in soya (Lawless and Heyman, 1998; Kikafunda, 2006; Adenuga, 2010).

The acceptability feeling if the products given as baby food all the porridge samples in the present study were equally accepted by the panelists. The score given for as baby food had ranged from 6.12±0.32 to 6.45±0.32 (Table 4.2. 8). From this study, all samples were rated greater than 6 indicating that the formulated porridge samples were acceptable or liked very much and agreed that the formulas could be provided for baby food. All products were rated greater than 5 indicating that the color of the porridge were accepted or liked moderately by panelists.

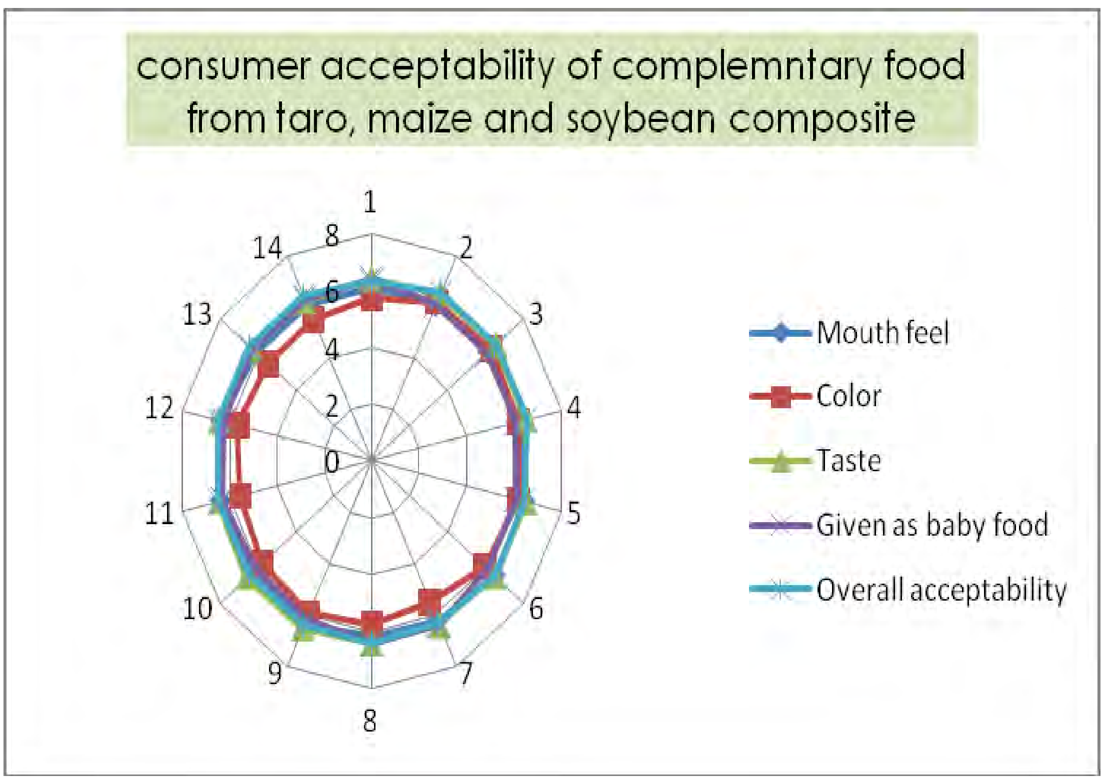

Figure 4.2. 10: Cconsumer acceptability of net score for complemntary food samples

Food ingredient optimization

Optimization is a scientific way of maximizing the desirable and minimizing the undesirable constituents of food components (Pawlak, *et al*., 2003). The protein, fat, mineral and anti-nutritional values were considered in the optimization of complementary food. The nutrient density and anti-nutrients have usually been considered as determinants in the selection of complementary food. In the case of numerical optimization, an attempt was made to maximize the responses for CP, fat , carbohydrate and minerals (Fe, Zn, Ca, Mg and P) and minimize dietary fiber, oxalate and phytate. Graphical optimization was determined by superimposing the contour plots protein, fat, carbohydrate and minerals (Fe, Zn, Ca, Mg and P) and minimize dietary fiber, oxalate and phytate were selected to develop a predicted optimum formulation for complementary food samples. The overall optimization suggested that complementary food made with 50% taro, 30% maize and 20% soybeans achieved the best formulation for this combination of variables with a desirability of 0.695 (Fig. 4.2.11).

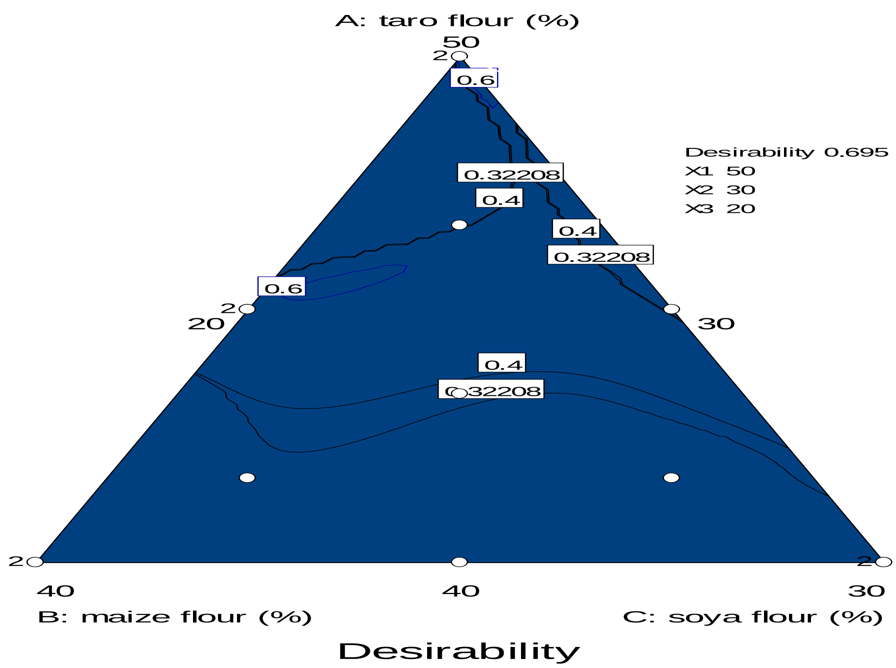

Figure 4.2. 11: Contour plot for over all optimization of complementary food

The optimum region (contour line surrounded dark area) of complementary food samples that has high nutrient and lower anti-nutrient values. Graphical optimization suggested that the level of ingredients to produce complementary food that would be in the mixture of taro 50 % taro, 30 % maize and 20 % soybean. In case of proximate optimization, the most importance was assigned to maximize responses for protein, fat, total carbohydrate while minimizing maize and maximizing taro and soya to get maximum desirability of ingredients. Based on the optimal result, the nutrient compositions for the optimized formula was crude protein (22.14), crude fat (6.33), ash (4.6), carbohydrate (56.8), and energy kcal (373) from maximizing responses and crude fiber (3.8), phytate (0.89) and oxalate (10.34) for minimizing responses.

4.2.3. CONCLUSION

The results of this study are good indicators of the possibility for better utilization of taro through developing a variety of new food products. The study showed that blending of taro with maize and soybean improved the nutritional density of complementary foods. The analyzed model shows that, the blending ratio of taro, maize and soybean had an effect on crude protein, fat, fiber, carbohydrate content and energy value of the mixed components. Complementary foods prepared from the high taro flour obtained greater mineral values while the smaller value contained in blended products prepared from high maize flour due to higher mineral content in taro than maize. Among the components, taro flour contained highest beta-carotene and also the binary combination between taro and maize or soybean flour was also shown synergetic effect. The blended products prepared from maize and soybean had higher phytate values than taro. However, the phytate content of all complementary food samples is very low compared with the maximum recommended daily intake of phytate from food which is 300–500 mg/day. The overall optimization suggested that the level of ingredients to produce complementary food that would be in the mixture of taro 50 % taro, 30 % maize and 20 % soybean. For the sensory results, the mouth feel, taste and overall acceptability of all porridge samples were rated greater than 6 indicating that liked very much by the panelists. Thus, it is believed that this study could give insights for use of taro in industry level for making complementary foods for the future to mitigate household food insecurity and malnutrition in Ethiopia.

4.3: NUTRITIONAL AND ANTI-NUTRITIONAL COMPOSITION OF TARO (*Colocasia esculenta*) LEAF

4.3.1. ABSTRACT

The analysis undertaken in this study was leaf proximate composition, mineral concentration and anti-nutritional factors of samples processed as leaf powder and curd concentrates between local taro and boloso-1 taro varieties. An UHPLC and GC-FID method was used for the determination of amino acid and fatty acid composition, respectively. The moisture content ranged from 3.44±0.19 *to* 3.73±0.69*, crude protein from* 25.06±1.29 to 26.25 ±1.53*, crude fat from* 5.84±0.16 *to* 6.44±0.88*, fiber from* 3.19±1.98 *to* 4.60±1.39[a]*, total ash from* 2.71±0.07 *to* 2.88±0.23*, total carbohydrate from* 56.22±2.74 *to* 59.38±2.94*, amylose contents from* 5.32±1.15 *to* 6.28±0.98*, beta-carotene (µg/100 g)* 2.48±0.20 *to* 2.70±0.22 *and phenolic compound (mg/100 g) from* 0.96±0.08 *to* 1.11±0.18*. Similarly, the five macro minerals, mg/100 g of Na, K, Mg, P and Ca content ranged from* 6.61±1.05*,* 14.33±0.40*,* 8.29±1.89*,* 1.40±1.19 *and* 15.26±6.21, *to* 7.83±1.82*,* 16.48±0.07*,* 9.74±1.90*,* 1.90±0.99 *and* 19.54±6.61 *value, respectively. Concerning micro minerals like Zn, Fe, and Cu contents, mg/100 g were varied from* 9.65±4.55*,* 40.37±9.83 *and* 0.42±0.1*) to* 12.75±1.81*,* 51.87±1.02 *and* 0.58±0.22 *values, respectively. The amino acid profiles, suggesting that high values were obtained from taro leaf than that of the corm sample. From the general trend observed in this study, boloso-1 taro variety subjected to curd processing provided better proximate, mineral, functional and lower anti-nutritional contents than that of the local taro variety.*

Key words: boloso-1 taro, curd extract, leaf powder and composition

4.3.2. INTRODUCTION

Taro leaf is edible part of the taro crop which also has nutritional significance and economic importance (FAO, 1999; Carmichael, *et al.,* 2008). The leaves are used as green vegetables after thorough removal of the acrid elements through processing (de Oliviera, 2012). The leaves contain significant levels of protein and are also an excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fiber (Ahmed, 2014). The high level of dietary fiber found in the taro leaf is also advantageous for their active role in the regulation intestinal transit, increasing dietary bulk and feces consistency due to their ability to absorb water (Saldanha, 1995).

Most rural people suffer from malnutrition, not because of the economic status, but because of inability to utilize the available nutritional raw materials to meet their daily requirements (Annan and Plahar, 1995). While taro has many nutritional, economic and health attributes, it has some anti-nutritional compounds such as oxalic acid, cyanide, alpha-amylase inhibitors, lectins, and tannins that are harmful to health and which can be eliminated through appropriate processing (Savage and Catherwood, 2007; Ramanatha *et al.,* 2010).

Taro (*Colocasia esculenta*) is well adapted food crop across many agro-ecological zones of sub-Saharan Africa and In Ethiopia, taro is one of the most important food crops as well as an income source for the farmers (CSA, 2005). It has a great potential to supply high quality food and one of the cheapest sources of energy (Patrick *et al.,* 1999) and the most underutilized root crop (Umeta, 2000; Adane *et al.,* 2013). People are eating taro corm as boiled, but there is no awareness about the quality the leaf has. Njintang *et al.* (2006) stated that the taro leaf has not gained sufficient research attention with regards to its potential having nutritional, industrial and health importance. Previous researches done in Ethiopia are mainly on taro corm products and no research was conducted on taro leaf as human food. So that there is no awareness among people about the quality the leaf has. In this context, taro leaf was evaluated for its nutritional and anti-nutritional components.

The leaves are a nutritious like spinach vegetable, which provides a lot of minerals, vitamins and thiamine (Mwangi, *et al.,* 2007; Azeez and Madukwe, 2010). It is particularly a major vegetable and source of income to farming household in Ghana. Unfortunately, most growers and consumers of other root crops are not aware of the nutritional advantages of cocoyam (FAO, 2012). Before eating the leaves, it must be cooked or blanched to remove the calcium oxalate, an irritant substance, which limit the maximum utilization by peoples across the world (Savage and Dubois, 2006; Oscarsson and Savage, 2007; Pang, *et al.,* 2010; Kaushal, *et al.,* 2013).

This study was undertaken, to explore the nutritional importance of taro leaf and to recommend taro leaf as a sustainable approach for the alleviation of malnutrition through transforming to edible products. Generally, this research brought extensive outputs on how to use taro leaves accompanied with improved processing methods for human consumption at a high level of nutrient sources which have direct impact on the improvement of human nutrition. Therefore the crude protein (CP), crude fat, ash, crude fiber (CF), carbohydrate, energy, minerals, phytate, oxalate, tannin and total phenols constituents of taro leaf were determined and the results obtained were discussed in discussion part and some works not carried out in the study are recommended for further study.

4.3.3. MATERIALS AND METHODS

4.3.3.1.Sample source

Taro leaf: Local and boloso-1 were harvested at 7 months of age from the Areka Agricultural Research Center (AARC) grow in same condition and brought to Addis Ababa, Ethiopia. The detailed procedure of preparation and analysis is presented in chapter 3.

4.3.3.2.Experimental samplespreparation

The leaf samples were prepared according to (Cornelissen *et al.,* 2003).

4.3.3.3. Experimental analysis

The leaf samples were analyzed for moisture, crude fiber, total ash, crude fat and crude protein using the (AOAC, 2000, and AACC, 2000) reference methods. The total carbohydrate content was determined by nutrient difference (100 – % moisture + % protein + %fat +% fiber

+ % ash] according to (Egan *et al.,* 1981). The energy density (ED) of taro leaf samples was calculated using standard food energy conversion factors: 4 kcal/g for carbohydrates; 4 kcal/g for proteins; 9 kcal/g for crude fat; according to Livesey (1995). The amyloses (%) content was determined by a calorimetric method (Zhu, *et al.,* 2008).

The five macro minerals, of Na, K, Mg, P and Ca and three micro minerals Zn, Fe and Cu were analyzed by using atomic absorption spectrophotometer (AACC, 2000). Beta-carotene and total phenolic compounds were determined according to Laurie, *et al.,* (2012) and (Sharma and Gupta, 2010). The anti-nutritional content, i.e., mucilage was determined according to methods noted by (Yamazaki *et. al*., 2008). Phytic acid was determined through phytate phosphorus (Ph-P) analysis according to (Plaami and Kumpulainen, 1991). The AOAC (1990) method was used to determine the oxalate content of taro sample. Tannins were determined using the method of (Burns, 1972). Lectins, Alpha-amylase inhibitors and protease (trypsin) inhibitors were determined according to (Leo *et al.* 2001; Noman, *et al.,* 2006; Bhattacharyya and Babu, 2009) respectively. For amino acid analysis, leaf samples were hydrolyzed in acid and alkaline medium to the complete hydrolysis of the protein fraction according to (Luyen, *et al.,* 2012). UHPLC instrumentation and analytical procedure were conducted according to (Kim, *et al.,* 2013). For the fatty acid of the leaf samples, lipid extraction with hexane: isopropanol (3:2 v/v) with, methylation and thin layer chromatography (TLC) checking was conducted using the reference method according to (Ostermann, *et al.,* 2014). Finally, Fatty acids were analyzed with a Gas chromatography-Flame ionization detector (GC-FID) system.

4.3.3.4. Statistical analysis

All data were analyzed using the general linear model procedures of Statistical Analysis Systems software (version 9.4 SAS Institute Inc., Cary, USA).

4.3.4. RESULTS AND DISCUSSION

Moisture Content

The moisture content (%) of different samples ranged from 3.44±0.19 to 3.73±0.69 (Table 4.3.1). In the case of fresh taro leaf the moisture content composed 85%, indicating that a leaf could not be stored favorably for a long period of time due to deterioration and microbial attack. Regarding to the dried sample, the moisture content is very low and add quality and shelf life stability for a longer time (Kabganian, *et al.,* 2002; Park, *et al.,* 2002; Emmanuel-Ikpeme *et al.,* 2007). Drying at adequate temperature and time to insure the lowest moisture that preserve for longer time is necessary for drying leaf samples (Ghosho and Hasan, 1999; Veselinov, *et al.,* 2004).

Crude Protein Content

As presented in Table 4.3.1, the crude protein content in the study varied from 25.06±1.29 to 26.25±1.53. In the present study, the crude protein contents in different samples differ significantly ($p<0.05$). From the result, the crude protein contents of boloso-1 leaf curd contained the highest (26.25±1.53) followed by leaf powder of the same variety. However, the lowest (25.06±1.29) protein contents were obtained from local leaf powder samples.

This implies that, curd samples exhibited better crude protein content than powder samples. This might be due to the curd processing method that would have increased the solubility of proteins and increase protein content (Hang and Preston, 2009; Ezeocha *et al.,* 2012). The observed result from the study is consistent with the report of (Nip *et al.,* 1998; Agbor-Egbe and Rickard 1990; Shanthakumari *et al.,* 2008). Taro leaf contains about 23% CP on a DM basis (Ezeocha *et al.,* 2012). The notable findings from this study were the crude protein values were higher than the reported value might be due to variety difference, age at which the leaf harvested and may be because of all the major anti-nutrients identified in fresh taro leaves were significantly reduced during the curding period and agree with (Chittavong, *et al.,* 2008; Rodríguez and Preston, 2009) reported a significant reduction of about 86% in the oxalate content of the raw leaves of taro.

Fat Content

As summarized in Table 4.3.1, the crude fat content in different samples ranging from 5.84±0.16 to 6.44±0.88%. The crude fat content from the study in percentage (%) differ significantly ($p<0.05$). The crude fat content (%) of boloso-1 leaf curd composed the highest and the least value was obtained in the local taro leaf powder. The curd samples in taro varieties provided higher fat content than that of powder samples. Little reduction of fat content was observed in powder samples might be because drying leaf into powder form would decrease due to drying treatment induced dehydration and oxidation of the samples the decrease fat content (Adeyeye and Fagbohon, 2005). Therefore, the results from the study suggest that drying methods affect the fat content. The result obtained in this work agreed with the works of (Adeboye and Babajide, 2007; Abiodun *et al.,* 2010; Aletor and Abiodun, 2013) in which drying method was found to affect nutritional component of dried leafy vegetables. However, curd processing in this study is found to be better way to decrease fat degradation (Ndabikunze*, et al.,* 2011). Also, the result of the study is similar to the reports of (Albihn and Savage, 2001; Ndabikunze*, et al.,* 2011) reported about, the effects of the drying methods fat contents.

Total Ash

As results presented in Table 4.3.1, the total ash content in percentage (%) ranged from 2.71±0.07 to 2.88±0.23 values and differ significantly ($p<0.05$). The result from this study revealed that higher ash content was existed in boloso-1 leaf curd while the lowest ash content was detected in local leaf curd. The ash contents of the study were within the range of 2.354 to 3.128 % reported by (Njoku and Ohia, 2007; Ifon, *et al.,* 2009; Kolawole, *et al.,* 2011).

Crude Fiber Content

As it can be seen from Table 4.3.1, crude fiber content of in leaf curd and powder samples from two taro varieties differ significantly ($p<0.05$). The crude fiber contents decreased in leaf curd samples than powder samples and local taro had lower values than that of taro boloso-1 variety. As the result the present study indicated that reduction of fiber content was observed in curd samples than powder samples (Table 4.3.1). This might be due to the effects of the thermal processing break down the fiber components into smaller and soluble forms and results chemical changes (Achanta and Okos, 2000; Morris, *et al.,* 2004; Adane *et al.,* 2013).

Total carbohydrate

The total carbohydrate content (%) in different samples ranged from the 56.22±2.74 to 59.38±2.94 (Table 4.3.1). The carbohydrate content from the study differ significantly ($p<0.05$). The result from this study revealed that the carbohydrate content was higher in local taro leaf than a boloso-1 taro leaf in both curd and powder samples. The high carbohydrate value was comprised in local taro leaf curd and the lowest carbohydrate values were observed in boloso-1 taro leaf curd. According to the results reported by (Jane *et al.,* 1992; USDA, 2002; Benesi *et al.,* 2004) taro is a good source of starch and could supply high carbohydrates. Taro leaf has been reported to have 40-50% starch on dry weight basis (Jane *et al.,* 1992; Deo, *et al.,* 2009).

Energy value

As indicated in Table 4.3.1, the total energy value (%) in the study was ranged from 386.69±11.59 to 394.69±9.86. The total energy value in different samples differs significantly ($p<0.05$). The result from the study examined leaf curds of both taro varieties obtained higher energy content than that of leaf powder samples. The high energy content was existed in local taro leaf curd followed by boloso-1 taro leaf curd. The energy content is associated with carbohydrate content and leaf samples with high carbohydrate maintained highest energy content (Bradbury and Holloway, 2002).

Leaf amylose content

As shown in Table 4.3.1, the highest value of taro leaf amylose percentage is 5.32±1.15 to 6.28±0.98 in dry weight basis. The amylose content in different samples differs significantly at ($p<0.05$). Taro leaf powder samples in both varieties showed greater amylose values than that of curd sample. In the other words, taro starch amylose decreased in curd processing and this may be due to leaf become de-starched during heat processing (Tattiyakul et al., 2006; Gordon, 2000). Such small reduction of amylose may be associated with hydrolysis of leaf starch by heat (Matazu and Haroun, 2004; Hasan, *et al.,* 2007; Ekwe *et al.,* 2009). Therefore, leaf powder samples kept bettre amylose than that of curd samples and boloso-1 taro was good sources of leaf amylose according to the result provided for this study.

The result showed in Table 4.3.1 also summarizes the β-carotene and phenolic compounds (mg/100g) of the analyzed taro leaf samples. The first higher β-carotene (μg/100 g) 2.70±0.22 was detected in taro boloso-1 powder and followed by least β-carotene 2.48±0.20 mg/100 g in taro local curd. The β-carotene content from this study differ significantly at ($p<0.05$). Taro leaf powder samples in both varieties showed greater β-carotene content than that of curd sample. In other words, β-carotene content decreased slightly during curd processing and this might be due to leaf de-pigmentation during heat processing in sweet potato leaves (Dewanto *et al.,* 2002; Horvathova *et al.,* 2007; Faber and Laurie, 2011b; Laurie, *et al.,* 2012a).

Taro leaves contain a moderate amount of β-carotene and is a good source of Vitamin C which keeps the body tissues strong, helps the body use iron and chemical actions in the body (Dumnnt and Vernier, 1997; Negi and Roy, 2000; Yamini, *et al.,* 2001). Therefore, this study indicated that leaf powder samples held good β-carotene content than that of curd sample and boloso-1 taro was good sources of leaf β-carotene content.

When it comes to phenolic compound composition (mg/100 g), in this study was ranged from 0.96±0.08 to 1.11±0.18. The total phenolic content in different samples differs significantly at ($p<0.05$). Taro leaf curd samples in both varieties provided greater phenolic compound values than that of powder samples. The highest phenolic compound concentration was prevailed from local taro leaf curd followed by boloso-1 leaf curd while the lowest phenolic compound was recorded in curd processing increased the level of phenolic compound in the final analyzed samples (Ejoh, *et al.,* 1996; Jayasinghe *et al.,* 2003). Nowadays, the consumers" demand for a higher content of bioactive compounds in the diet has increased. Bioactive metabolites, like polyphenols, are not strictly required in the diet, but when present at sufficient levels promote good health and prevent diseases of different origins (Prajapati, *et al.,* 2011; Ferreres, *et al.,* 2012b).

Table 4.3.1: Proximate (%), β-carotene (μg/100 g) and total phenols (mg/100 g) content

	Taro varieties (A)				
	Boloso-1 lea		Local leaf		
	Processing method (B)				
Parameters	Powder	Curd	Powder	Curd	CV (%)
Moisture	3.73±0.69^{a}	3.73±0.69^{a}	3.73±0.69^{a}	3.44±0.19^{a}	2.0
protein	26.07±1.82^{b}	26.25 ±1.53^{a}	25.06±1.29^{cd}	25.43±1.92^{c}	5.58
Crude fat	6.37±0.95^{ab}	6.44±0.88^{a}	5.84±0.16^{cd}	6.32±0.98^{c}	4.0
Crude fiber	4.60±1.39^{a}	4.60±1.39^{a}	4.034±2.36^{b}	3.19±1.98^{cd}	9.85
Total ash	2.83±0.27^{ab}	2.88±0.23^{a}	2.85±0.26^{cd}	2.71±0.07^{d}	5.71
Carbohydrate	56.22±2.74^{d}	57.61±4.77^{c}	58.12±4.75^{b}	59.38±2.94^{a}	4.02
Energy value	387.21±12.46^{c}	392.63±13.43^{b}	386.69±11.59^{d}	394.69±9.86^{a}	1.5
Amylose	6.28±0.98^{a}	5.51±1.46^{c}	5.96±1.52^{ab}	5.32±1.15^{cd}	7.3
β-carotene	2.70±0.22^{a}	2.56±0.34^{ac}	2.63±0.337^{bd}	2.48±0.20^{d}	3.22
Phenols	0.96±0.08^{bd}	1.07±0.23^{b}	1.06±0.24^{ac}	1.11±0.18^{a}	0.81

$^{a-d}$ *Means±SD within a raw with different superscripts differ significantly (p<0.05); SD: standard deviation; CV: coefficient of variation.*

Macro and micro minerals concentration (mg/100 g)

The mg/100 g values in DM basis of five major minerals were presented in Table 4.3.2. The mg/100 g of Na in different samples ranged from 6.61±1.05 to 7.83±1.82 and differ significantly ($p<0.05$). The present result from this study states that the Na concentration differ significantly due to taro varieties at ($p<0.05$) and boloso-1 has a higher concentration in comparison with the local taro variety. The present result was in agreement with the work of (Umar *et al.,* 2005). Thus, this study revealed that curd processing is a good way of reducing the Na level from food samples and recommended for human consumption (Baruah, 2002).

Table 4.3. 2: Concentration of Na, K, Mg, P, Ca Zn, Fe and Cu (mg/100 g) of taro leaf

	Taro varieties (A)				
	Boloso-1 leaf		Local leaf		
	Processing method (B)				
Parameters	Powder	Curd	Powder	Curd	CV (%)
Na	7.83±1.82[a]	7.22±2.11[c]	7.83±1.82[ab]	6.61±1.05[d]	9.83
K	16.03±.20[bc]	16.03±4.20[bc]	14.33±0.40[d]	16.48±0.07[b]	10.4
Mg	8.29±1.89[cd]	9.74±1.90[a]	8.55±2.33[c]	9.49±2.32[ab]	7.68
P	1.18±0.81[d]	1.90±0.99[a]	1.40±1.19[cd]	1.83±1.11[ab]	5.73
Ca	15.26±6.21[d]	19.54±6.61[a]	15.89±7.24[c]	19.40±6.90[ab]	5.68
Zn	10.75±5.26[b]	12.75±1.81[a]	9.76±4.71[c]	9.65±4.55[cd]	5.86
Fe	51.72±17.24[ab]	51.87±1.02[a]	40.37±9.83[d]	46.12±19.78[c]	9.04
Cu	0.56±0.25[ab]	0.42±0.19[cd]	0.58±0.22[a]	0.46±0.27[c]	0.74

[a-d] Means±SD within a rows with different superscripts differ significantly ($p<0.05$); SD: standard deviation; CV: coefficient of variation.

Table 4.3.2 also presents the mg/100 g of K, Mg, P and Ca 14.33±0.40 to 16.48±0.07, 8.29±1.89 to 9.74±1.90, 1.18±0.81to 1.90±0.99 and 15.26±6.21 to 19.54±6.61 value, respectively, and micro minerals like Zn, Fe and Cu contents mg/100 g were significantly ($p<0.05$), varied from 9.65±4.55, 40.37±9.83 and 0.42±0.19 to 12.75±1.81, 51.87±1.02 and 0.58±0.22 values, for boloso-1 taro variety. The result obtained from this study predicted that curd concentrate samples had high K, Mg, P, Ca, Zn and Fe mg/100 g concentration than powder samples. This indicates the mineral concentration was elevated during curd processing and increased the available mineral contents in the final curd concentrate samples (Siddhuraju and Becker, 2001; Adejumo and Bamidele*,* 2012*)*. In addition, during curd processing, there was a simultaneous reduction in anti-nutritional contents that bind with mineral and reduce their bioavailability (Kordylas, 1990; Lima, *et al.,* 2009).

Previous studies also provided evidence that thermal processing would reduce anti-nutritional factors in different food samples (Kordylas, 1990; Bothwell and Chalton, 2002). Therefore, the present study is consistent with reviewing literatures and notable finding from the study suggests processing with heat can be an appropriate method for reducing toxic compound from leaf samples (Pradee and Susanta, 2001; Schippers, 2005; Smith and Eyzaguirre, 2007; Nahar, 2009).

Anti-nutritional contents of taro leaf

The high tannin content 504.24±92.06 is (mg/100 g) exhibited in taro local leaf powder while the smaller content 301.31±38.40 (mg/100 g) was found from a boloso-1 variety subjected with curd method. The mg/100 g of phytate in different samples varied from 8.53±2.31 to 9.88±2.45 and differ significantly ($p<0.05$). The mg/100 gm of mucilage in the study was ranged from 0.13±0.05 to 0.16±0.06 and differ significantly ($p<0.05$). The mg/100 g of oxalate in the study ranged from 152.30±9.64 to 257.92±13.34 and differ significantly ($p<0.05$). From this study boloso-1 taro leaf curd had the low tannin, phytate, mucilage and oxalate (mg/100 g) content and the local leaf powder showed the highest. This indicates that curd concentrate processing has an effect on reduction of tannin, phytate, mucilage and oxalate (Ndimantang *et al.,* 2006). More reduction of tannin, phytate, mucilage and oxalate in curd samples were exhibited and this might be due to curd processing, reducing the concentration of anti-nutritionals, which is consistent with the previous work (Onayemi and Nwigwe, 2007).

Condensed tannin and other anti-nutritional factors in food samples can be reduced during thermal processing and boiled with water in leaf (Prajapati *et al.,* 2011). Not all taros are edible and should not be eaten raw because wild varieties are usually contain high anti-nutritionals and increase the loss of essential nutrients from the body, interfering with the metabolism of absorbed essential nutrients, decreasing the digestion of food, or decreasing food intake (Bothwell and Chalton, 2002). Phytate is the main inhibitors of many nutrients and mainly inhibits the absorption of iron and, to some extent, zinc (Kordylas, 1990). Therefore, it is important to apply different methods of preparation and cooking that could reduce the risk of absorbing excess anti-nutritional compound when consumed. Therefore pretreatment and drying had a positive effect on the nutritional composition of taro leaf as it boosts the nutrient content and reduces the anti-nutritional factors to a more tolerable level (Shahnaz, *et al.,* 2003; Antia, *et al.,* 2006;Savage and Dubois, 2006; Oscarsson and Savage, 2007).

Table 4.3. 3: Anti-nutritional contents of taro leaf

	Taro varieties (A)				
	Boloso-1 leaf		Local leaf		
	Processing method (B)				
Parameters	Powder	Curd	Powder	Curd	CV (%)
Tannin(mg/100g)	419.80±43.45[c]	301.31±38.40[d]	504.24±92.06[a]	494.36±35.81[b]	6.32
Phytate(mg/100g)	8.59±2.44[b]	8.53±2.31[b]	9.88±2.29[a]	9.88±2.45[a]	7.45
Mucilage(mg/100g)	0.13±0.05[b]	0.13±0.06[b]	0.16±0.06[a]	0.17±0.061[a]	0.94
Oxalate(mg/100g)	198.61±7.61[c]	152.30±9.64[d]	257.92±13.34[a]	243.19±58.80[b]	2.37
Lectin (%)	0.03±0.08[a]	0.03±0.06[a]	0.02±0.03[a]	0.03±0.08[a]	0.52
A-amylase (%)	0.01±0.002[a]	0.010.001[a]	0.010.002[a]	0.010.002[a]	0.001
Protase (%)	0.02±0.04[a]	0.02±0.04[a]	0.02±0.09[a]	0.03±.01[a]	0.01

[a-d] Means±SD within a raw with different superscripts differ significantly (p<0.05); SD: standard deviation; CV: coefficient of variation.

As demonstrated in Table 4.3.3, lectin, α-amylase and protease inhibitors in leaf curd and powder samples from two taro varieties with and without peel did not differ significantly (p>0.05). The protease inhibitor in taro local leaf curd showed high values (0.03±0.01 mg/100 g) while the smaller (0.02±0.01 mg/100 g) protease inhibitor level was found in a taro boloso-1 variety. However; protease inhibitor in different samples did not significantly differ. The dominant protease inhibitor found in taro leaf is trypsin inhibitor and is a type of serine protease inhibitor that reduces the biological activity of trypsin. As a result, protease inhibitors that interfere with trypsin activity can have an anti-nutritional effect (Kiran and Padmaja, 2003). It is noted that the lectin and protease inhibitor effect can be reduced using different processing methods which are processed by heat, for increasing the safety of taro leaves (Mosolov and Valueva, 2005; Ravindarn *et al.,* 2006; Sumathi and Pattabiraman, 2007).

Table 4.3. 4: Phytate mineral molar ratio in taro leaf samples

	Taro varieties (A)			
	Boloso-1 leaf		Local leaf	
	Processing method (B)			
Parameters	Powder	Curd	Powder	Curd
[Phy]:[Na]	0.17±0.11[a]	0.14±0.06[ab]	0.14±0.12[ab]	0.14±0.10[ab]
[Phy]:[K]	0.38±0.11[ab]	0.37±0.13[ab]	0.44±0.11[a]	0.44±0.13[a]
[Phy]:[Fe]	0.05±0.02[ab]	0.42±0.05[ab]	0.61±0.05[a]	0.52±0.04[ab]
[Phy]:[Ca]	0.21±0.06[a]	0.21±0.05[a]	0.24±0.06[a]	0.24±0.06[a]
[Phy]:[Mg]	0.35±0.11[ab]	0.34±0.09[ab]	0.41±0.10[a]	0.40±0.1[a]
[Phy]:[Zn]	0.04±0.02[a]	0.04±0.04[a]	0.05±0.04[a]	0.05±0.04[a]
[Phy]:[Cu]	0.03±0.04[a]	0.03±0.02[a]	0.31±0.03[a]	0.34±0.04[a]
[Phy]:[P]	0.12±0.10[a]	0.104±0.07[a]	0.17±0.09[a]	0.16±0.09[a]

[a-ab] Means±SD within a raw with simlar superscripts did not differ significantly (p>0.05); SD: standard deviation.

Table 4.3.4 shows the phytate: mineral ratio of the analyzed samples of taro leaf powder and curd samples. The result obtained from this study was, powder samples in both varieties prevailed slightly greater phytate mineral molar ratio than that of curd sample. However, the phytate mineral molar ratio of a1l samples did not differ significantly at ($p<0.05$). Therefore mineral levels in all samples are more bio-available due to the low phytate mineral ratio (Subramony, 2002; Bothwell and Chalton, 2002). It was also reported that different domestic processing, including peeling and heating maintained high mineral bioavailability and low phytate constitutes (Gibson *et al.,* 2006).

To reduce the adverse effect of phytates on mineral absorption, phytate contents have to be reduced to concentrations of less than 200 mg/100 g DM (Hurrell, 2004). Foods with a molar ratio of phytate: mineral less than 10 showed adequate availability of mineral and problems were encountered when the value was greater than 15 (Siddhuraju and Becker, 2001). These results are in accordance with Mbofung *et al.,* 2006; Njintang, *et al.,* 2011).

Amino acid composition in (g/100g)

As summarized in Table 4.3.5, the amino acid composition (g/100g) of the analyzed taro leaf powder and curd samples were quantified with their relative area comparing with amino acid standard and the concentration of each amino acid (g/100g) was also calculated by multiplying the percentage of each amino acid with their average crude protein content (Table 4.3.1). After HPLC analysis, fully separated peaks were manual integrated and compared the area with standard amino acids.

The essential amino acids are ranging for His (2.4±0.30 to 3.54±0.31), Thr (4.9±0.31to 6.19±0.32), Val (5.65±0.52 to 6.67±0.51), Metto Cys (4.08±0.25 to 6.98±0.26), Try (2.65±0.28 to 3.36±0.27), Phe (3.67±0.13 to 5.78±0.12), Iso (2.53±0.13 to 4.54±0.12), Leu (6.54±0.32 to 9.78±0.31), and Lys (4.54±0.35 to 7.64±0.34). Similarly, the non essential amino acids are ranging for Asp (8.45±0.06 to 11.97±0.05), Glu (8.65±0.44 to 11.98±0.43), Asp (5.67±0.45 to 7.73±0.44), Ser (3.87±0.16 to 6.75±0.15), Glu (2.86±0.17 to 7.36±0.15), Gly (4.65±0.04 to 7.65±0.08), Ala (2.35±0.13 to 2.87±0.16), Arg (4.55±0.69 to 6.61±0.68), Tyr (5.76±0.52 to 7.65±0.51), Nor (1.54±0.20 to 3.8±0.18), Hyd (2.34±0.13 to 2.98±0.16) and Pro (2.55±0.52 to 2.76±0.53).

The glutamic acid, aspartic acid and asparagine are the three dominant non-essential amino acids, respectively. The amino acid profile of the leaf samples showed a favorable balance of both essential and non-essential amino acids to support the nutrient requirement of human and animal if mutually mixed with other foods. Similarly, other studies have shown that taro leaf has high scores of amino acid. These results are in range of aminio acid reported by (Agbor-egbe, *et al.,* 1990a; FAO, 1991; Agbor-Egbe and Treche, 1995; Afoakwa and Sefa-Dedeh, 2001; Bhandari, *et al.,* 2003; Mbofung. *et al.,* 2006).

From the result, boloso-1 taro leaf curd is relatively rich in leucine, valine, phenylalanine, and lysine and also adequate in threonine, tryptophan, methionine and iso-leucine amino acids of all. The second high essential amino acids compositions were found in local taro leaf curd followed by boloso-1 taro leaf powder.

Table 4.3.5: Amino acids composition (g/100g) of taro leaf samples

Types of AAs	Treatments				
	Abbreviations	BLP	BLC	LLP	LLC
Essential amino acids (EAA)					
L-Histidine	His	2.4 ± 0.30^{ab}	3.54 ± 0.31^{a}	2.76 ± 0.32^{ab}	2.98 ± 0.33^{ab}
L-Threonine	Thr	4.9 ± 0.31^{c}	6.19 ± 0.32^{a}	5.78 ± 0.33^{ab}	5.87 ± 0.34^{ab}
L-Valine	Val	6.32 ± 0.50^{a}	6.67 ± 0.51^{a}	5.65 ± 0.52^{ab}	5.98 ± 0.52^{ab}
L-Methionine + Cys	M-Cy	4.08 ± 0.25^{c}	6.98 ± 0.26^{a}	5.65 ± 0.27^{ab}	5.87 ± 0.27^{ab}
L-Tryptophan *	Try	3.36 ± 0.26^{a}	3.36 ± 0.27^{a}	2.65 ± 0.28^{ab}	2.98 ± 0.29^{ab}
L-Phenylalanine	Phe	4.34 ± 0.11^{ab}	5.78 ± 0.12^{a}	3.67 ± 0.13^{b}	3.78 ± 0.14^{b}
L-Isoleucine	Iso	3.99 ± 0.11^{ab}	4.54 ± 0.12^{a}	2.53 ± 0.13^{b}	3.51 ± 0.14^{ab}
L-Leucine	Leu	9.51 ± 0.30^{a}	9.78 ± 0.31^{a}	6.54 ± 0.32^{b}	7.56 ± 0.33^{ab}
L-Lysine	Lys	6.09 ± 0.33^{ab}	7.64 ± 0.34^{a}	4.54 ± 0.35^{b}	6.54 ± 0.36^{ab}
Non-essential amino acids (NEAA)					
L-Aspartic acid	Asp	9.97 ± 0.04^{ab}	11.97 ± 0.05^{a}	8.45 ± 0.06^{ab}	10.87 ± 0.07^{a}
L-Glutamic acid,	Glu	11.66 ± 0.42^{a}	11.98 ± 0.43^{a}	8.65 ± 0.44^{ab}	9.98 ± 0.45^{ab}
L-Asparagines	Asp	7.66 ± 0.43^{a}	7.73 ± 0.44^{a}	5.67 ± 0.45^{ab}	5.86 ± 0.46^{ab}
L-Serine	Ser	4.90 ± 0.14^{ab}	6.75 ± 0.15^{a}	3.87 ± 0.16^{b}	3.87 ± 0.17^{b}
L-Glutamine	Glu	7.36 ± 0.15^{a}	6.92 ± 0.16^{a}	2.86 ± 0.17^{b}	2.9 ± 0.18^{b}
L-Glycine	Gly	5.01 ± 0.07^{ab}	7.65 ± 0.08^{a}	4.65 ± 0.09^{b}	5.87 ± 0.10^{ab}
L-Alanine	Ala	2.35 ± 0.13^{ab}	2.75 ± 0.14^{a}	2.65 ± 0.15^{ab}	2.87 ± 0.16^{a}
L-Arginine	Arg	5.91 ± 0.67^{ab}	6.61 ± 0.68^{a}	4.55 ± 0.69^{b}	5.79 ± 0.70^{ab}
L-Tyrosine	Tyro	6.32 ± 0.50^{ab}	7.65 ± 0.51^{a}	5.76 ± 0.52^{ab}	5.87 ± 0.53^{ab}
L-Norleucine	Nor	3.8 ± 0.18^{a}	3.78 ± 0.19^{a}	1.54 ± 0.20^{b}	1.98 ± 0.21^{b}
L-Hydroxproline	Hyd	2.34 ± 0.13^{b}	2.65 ± 0.14^{ab}	2.45 ± 0.15^{ab}	2.98 ± 0.16^{a}
L-Proline	Pro	2.61 ± 0.50^{ab}	2.76 ± 0.51^{a}	2.55 ± 0.52^{ab}	2.76 ± 0.53^{a}

$^{a\text{-}c}$ Means ± SD within a raw with similar superscripts did not differ significantly (p>0.05); SD: standard deviation; AAs: Amino acids; BLP: boloso-1 taro leaf powder; BLC: boloso-1 taro leaf curd; LLP: Local taro leaf powder and LLC: Local taro leaf curd.

Through this study amino acid composition was affected by both taro leaf varieties and processing methods, there is limited research results on processed taro leaf amino acid composition and the findings of this study was reviewed with other amino acid composition of yam, cassava and Moringa leaves. As noted by different researchers (Sheela *et al.,* 2004; Ayodele and Olajide, 2011; Busani *et al.,* 2011), the amino acid profile of the present study was potentially enough than yam and cassava leaf. For the present study, the amino acid profiles, suggesting that high values were obtained from taro leaf curd concentrate and had adequate sulphur containing amino acid. The findings of this study were agreement with the work of Aletor and Fasuyi (1997) who reported an increased amino acid composition of cassava leaf concentrate due processing.

Table 4.3. 6: Fatty acid profile (g/100 g) of taro leaf samples

Class of FAs	Treatments			
	BLP	BLC	LLP	LLC
$C_{14:0}$	2.51±0.34^{a}	2.35±0.22^{a}	2.07±0.43^{ab}	1.28±0.34^{ab}
$C_{16:0}$	210.68±0.34^{a}	212.81±0.45^{a}	192.92±0.33^{b}	96.47±0.45^{c}
$C_{16:1(n-7)}$	15.30±0.23^{a}	15.28±0.23^{a}	13.80±0.54^{b}	1.27±0.34^{c}
$C_{17:0}$	0.32±0.43^{a}	0.13±0.23^{ab}	0.06±0.67^{b}	0.39±0.34^{a}
$C_{17:1}$	3.47±0.21^{a}	3.32±0.34^{a}	2.95±0.34^{ab}	0.49±0.34^{c}
$C_{18:0}$	105.92±0.34^{a}	106.89±0.22^{a}	96.87±0.34^{b}	35.72±0.34^{c}
$C_{18:1(n-9)}$	214.61±0.32^{a}	216.78±0.43^{a}	196.52±0.45^{b}	140.70±0.34^{c}
$C_{18:2(n-6)}$	55.18±0.21^{b}	55.59±0.21^{b}	50.36±0.34^{c}	334.07±0.32^{a}
$C_{18:3(n-3)}$	0.32±0.22^{b}	0.13±0.32^{b}	0.06±0.65^{c}	16.08±0.32^{a}
$C_{20:0}$	0.32±0.12^{b}	0.13±0.32^{b}	0.06±0.43^{c}	3.25±0.54^{a}
$C_{20:1(n-9)}$	0.32±0.43^{b}	0.13±0.23^{b}	0.06±0.65^{c}	1.37±0.34^{a}
$C_{20:2(n-6)}$	0.32±0.43^{a}	0.13±0.21^{ab}	0.06±0.45^{b}	0.13±0.43^{ab}
$C_{20:3(n-6)}$	0.32±0.56^{a}	0.13±0.32^{ab}	0.06±0.56^{c}	0.13±0.43^{ab}
$C_{20:4(n-6)}$	26.34±0.21^{a}	26.43±0.54^{a}	23.91±0.45^{b}	0.13±0.45^{c}
$C_{22:6(n-3)}$	5.85±0.33^{a}	5.72±0.33^{a}	5.13±0.45^{ab}	0.13±0.23^{c}
Total FAs	641.78	645.93	584.87	631.57
	Proportion (g/100 g), SFA, MUFA, PUFA and UFA:SFA			
SFAs	319.75	322.30	291.98	137.10
MUFAs	233.70	235.50	213.32	143.82
PUFAs	88.32	88.13	79.57	350.65
UFAS	322.03	323.63	292.90	494.47
UFA:SFA	1.01	1.00	1.00	3.61

$^{a-d}$ Means ± SD within a raw with similar superscripts did not differ significantly (p>0.05); SD: standard deviation; FAs: Fatty acids; BLP: boloso-1 taro leaf powder; BLC: boloso-1 taro leaf curd; LLP: Local taro leaf powder and LLC: Local taro leaf curd.

The fatty acid composition in mg/100 g (Table 4.3.6) of the analyzed taro leaf powder and curd samples was identified with their retention time and quantified with relative area compared with the fatty acid standard. The occurrence of trans fatty an acid in taro has not been detected and it is not expected (Kalac, 2009). The overall FA differences in boloso-1 taro leaf powder and curd extract were not significant (p>0.05). However, there were significant (p<0.05) differences in local taro leaf powder and curd extract. The dominant fatty acid was oleic acid ($C_{18:1,\ n-9}$) ranging from (140.70±0.34 to 216.78±0.43) g/100 g.

The next three dominant fatty acids were palmitic acid ($C_{16:0}$), oleic acid ($C_{18:0}$) and linoleic acid ($C_{18:2,\ n\text{-}6}$) ranging from (96.47±0.45 to 212.81±0.45, 35.72±0.34 to 106.89±0.22 and 50.36±0.34 to 334.07±0.32) g/100 g values, respectively. The essential fatty acids $C_{18:2}$ and $C_{18:3}$ are found in low concentration as compared to oleic acid. From taro leaf samples boloso-1 taro leaf curd had relatively high levels of fatty acid fractions and low levels of fatty acid fractions were obtained in local taro leaf powder (Table 4.3.6). This might be due to variety differences and effect of pre-treatments prior to analysis. Valasco *et al.* (2008) concluded that differences in FA concentrations have been found between taro varietys even harvested at the same stage of development. Again, similar findings have been made that the predominant fatty acids in the leaf of root crops were oleic, linoleic, palmitic and linolenic acids orderly (Katiyar *et al.,* 2005).

From the proportion (g/100 g) of saturated, monounsaturated and polyunsaturated fatty acids, unsaturated fatty acids were little higher than saturated fatty acids (Table 4.3.6). This is further verified by calculating the ratio of unsaturated: saturated fatty acids, which all are greater than one. This is consistent with the observation that, in taro and other root crops, unsaturated fatty acids greater than saturated fatty acids, in the total fatty acid content (Alozie *et al.,* 2010; Bhandari *et al.,* 2003; Brown *et al.,* 2008).

Table 4.3.6 summarizes the fatty acid composition in g/100 g of the analyzed pre-gelatinized taro corm flour samples. Taro corms pre-gelatinized without peel were analyzed for fatty acid. Fatty acids were identified with their peak retention time and quantified with relative area computing. The occurrence of trans fatty acids in taro have not been reported and it is not expected (Kalac, 2009).

4.3.5. CONCLUSION

The study highlighted the proximate composition, the major and minor mineral contents and anti-nutritional contents of taro leaf processed as leaf powder and leaf curd. Chemical compositions of cured and powder taro leaf samples were affected by processing methods used in the study (twigging, washing, curding, drying and powdering) and variety difference. From the result, boloso-1 leaf curd contained higher crude protein, crude fat, ash and mineral contents and lower crude fiber and anti-nutrients than that of local taro variety. The study showed that curd taro leaf samples had a better nutrient composition than powder samples. In addition, during curd processing, there was a simultaneous reduction in anti-nutritional contents and consequently, enhanced the mineral bioavailability. It is noted that the inhibitory effect can be reduced using curd and drying methods which are processed by heat, for increasing the safety of taro leaves. Therefore, the nutritional and anti-nutritional composition of taro leaf samples was affected by both variety difference and preparation method. For the leaf, boloso-1 taro leaf had relatively high levels of amino acid and fatty acid fractions while low levels of fractions were obtained in local taro leaf.

The study has shown that taro leaf has high scores of amino acid than that of corm samples. This was due to high crude protein content in taro leaf than corm used for multiplying the percentage of each amino acid with crude protein. The leucine, lysine and tryptophan are the three highest essential amino acid found in the present study, respectively. The dominant fatty acid was oleic acid ($C_{18:1,\ n\text{-}9}$) followed by palmitic acid ($C_{16:0}$), oleic acid ($C_{18:0}$) and linoleic acid ($C_{18:2,\ n\text{-}6}$). The essential fatty acids $C_{18:2}$ and $C_{18:3}$ are found in low concentration as compared to oleic acid. From taro leaf samples boloso-1 taro leaf curd had relatively high levels of fatty acid fractions and low levels of fatty acid fractions were obtained in local taro leaf powder. This means that taro leaf would be considered a good source of essential amino and fatty acid, than the corm, but still it is not clear why taro leaf is not considered for human food in Ethiopia.

4.4: TARO LEAF AS ALTERNATIVE FEED INGREDIENTS: EFFECTS ON DIGESTIBILITY AND NUTRIENT INTAKE OF BROILERS

4.4.1. ABSTRACT

The study was carried out using four treatments in which the treatments were inclusion of TLM at 0% (T_1), 3% (T_2), 5% (T_3) and 7% (T_4). The analysis undertaken in this study was feed DM, Crude Fiber (CF), Total Ash (TA), Ether Extract (EE), Crude Protein (CP) and functional properties (WHC, WA, WSI and WAI) of feed offered. The result obtained from the study shows that variation was found as taro leaf level increase. The crude protein in feed ranged from 21.27±0.05 to 21.62±0.05*, ether extract from 4.15±0.04 to 4.51±0.01, total ash from 10.96±0.04 to 12.89±0.05, utilizable carbohydrate from 54.44±0.04 to 55.36±0.03 and metabolizable energy from 2962.41±0.02 to 3117.57±0.07. The functional properties of the feed, water holding capacity (%) ranged from 1.41±0.03to 1.53±0.04, solubility capacity (%) from 19.63±0.043 to 20.98±0.06 and hydration capacity (%) from 0.83±0.02 to 0.98±0.04. The (mg/100g) Na, K, Fe, Ca, Zn, Cu and P of feed samples ranged from* 4.25±0.03 *to* 6.21±0.03*,* 10.15±0.01 *to* 19.63±0.03*,* 0.16±0.04 *to* 0.28±0.03*,* 5.11±0.02 *to* 7.98±0.04*,* 4.46±0.06 *to* 8.99±0.05*,* 3.42±0.03 *to* 3.44±0.04 *and 58.07±0.02 to 59.09±0.06 of values, respectively. The DM intake increased from T_2 to T_4 in all feed groups. Body weight gain and change (g/bird) was in the order of $T_4 > T_3 > T_1 > T_2$. The protein intake (g) was in the order of $T_4 > T_3 > T_2 > T_1$. In breast muscle, the nutrient compositions were in the order of $T_4 > T_3 > T_2 > T_1$ except the crude fiber content and also the amino acid composition of chicken meat was affected by dietary supplementation with taro leaf.*

Keywords: chicken, meat quality, nutrient composition, taro leaf

4.4.2. INTRODUCTION

In developing countries, like Ethiopia, in highly populated areas where it may be complex to continue agronomic activities, subsistence poultry can be the best alternative form of income resources, plays a significant role in family nutrition and above all, smallholder poultry provides a good opportunity to address poverty alleviation (Balakrishnan, 2004; Meseret, *et al.,* 2012; Tolera, *et al.,* 2012; Etalem, *et al.,* 2013). However, scarcity of conventional feedstuffs and food insecurity problems by rapidly increasing population growth are the most aggravating factors at present (FAO, 2014; Davis, 2014). Most of the population in Ethiopia depends on cereals as staple foods which are also required for poultry feeding (Alemu, 1995; CSA, 2013). This means, it is difficult to use cereals for poultry with having the human food insecurity burden and replacing expensive cereals and less available agro-industrial by-products by unconventional feedstuffs, which are less exploited by man, is one of the solutions to reduce cost of production and contribute to increased supply of animal protein to reach of poor people‟s (Silva, 2004; Amire, 2010).

Diets formulated on a least cost basis are vital and essential. It is generally assumed that improvements in alternative feed resources utilization will be associated with increased rate of productivity and product quality (Tion and Adeka, 2000; Ravindran, 2012). However, in Ethiopia this strategy is not practiced because of the less characterization of non-conventional feed sources from rural areas rather dependent on the high costs of conventional feed ingredients (maize, soybean and others), many of are utilized primarily in the human food and industrial input application (FAO, 2011). This elevates the prices of the products like egg and chicken meat. Thus, the high cost of cereals and protein supplements and uncertainty about their sustainable supply pressed the need to search for other potential non-conventional feed sources which are relatively less used for human consumption (Kamalzadeh *et al.,* 2008).

To bridge the gap and tackling the problems, inclusion of taro leaf meals (TLM) in the diets of chicken may bring products rich in essential nutrients for the consumers due to its good essential amino acid and fatty acid profiles. (McDonald *et al.,* 2002; Haftu, 2016).

Taro leaves can be harvested from plants grown from tubers any time during the season and used as animal feed (An *et al.,* 2003). Harvesting taro leaves did not affect tuber yield rather frequent harvesting increases the leaf yield. Taro leaf is rich in proteins and can be of supplementary protein, carotene and trace minerals in chicken diets (Delgado *et al.,* 1999; FAO, 2003). Moreover, most of poultry diets are made of cereals and lack the most important amino acids for humans, such as lysine, threonine, the sulphur-bearing amino acids (methionine and cysteine) and occasionally tryptophan unless supplemented in the ration. However, the above mentioned amino acids are very expensive and beyond the rich of poultry farmers. Since this is a great problem in nutrition and that is why important to identify alternative high quality protein feed ingredients that contain most of essential amino acids.

To achieve maximum efficiency in meat quality and production performance, properly balanced ration is a pre-requisite and taro could be possible alternative feed ingredients in this regard of the poultry industry (Kitalyi, *et al.,* 2012).

4.4.3. . MATERIALS AND METHODS

4.4.3.1. Study site

The broiler experiment was conducted at Debre Zeit Agricultural Research Center (DZARC) where located at an altitude of 1900 meters above sea level, at $8^0$44' N latitude and 38^0 38‟E longitudes and 47 km east of Addis Ababa. The day old chick used for the study was obtained from Alema farms, chicken breeder, Debre Zeit, Ethiopia.

4.4.3.2. Treatment formulation

The major feed ingredients were mixed based on a feed formulation recipe according to (Zhu *et al*., 2007) and carried out for various chemical composition analyses. The detailed procedure of for the treatment ration formulation is presented in chapter 3.

4.4.3.3. Management of experimental birds

The experimental broilers were managed and feeding trials were carried out according to (Picard *et al.,* 2002).

4.4.3.4. Measurement of experimental birds

Birds were measured weekly and the initial and final weights were used to estimate weight gains, according to (Tahir and Pestig, 2012).

4.4.3.5. Laboratory analysis

Samples of feed offered and pooled refusal samples from the respective treatments were analyzed for crude fiber (CF), total ash (TA), ether extract (EE) and CP uses the AOAC reference methods (AACC, 2000). The functional properties of feeds such as water holding capacity (WHC) were determined according to Anderson *et al.,* (1992). The water solubility index (WSI) in percentage was calculated as a ratio of dry residue to the original mass used to estimate WAI (Anderson *et al.,* 1992) and the water holding capacity (WHC) was estimated (AACC, 2000 method 56-20). Metabolizable energy (ME) content of the experimental diets was determined according to Wiseman (1987) as: ME (kcal/kg DM) = 3951+54.4EE-88.7CF-40.80Ash. Minerals of Na, K, Fe, Zn, Cu and P were analyzed by using atomic absorption spectrophotometer (AACC, 2000).

The total phenolic compound was analyzed using Biswas *et al*. (2011). The AOAC (1990) method was used to determine the oxalate, tannin and phytate contents of feed samples. After slaughter, carcass cuts of breast and thigh muscles without skin were taken, freeze dried and homogenized and has been tested for proximate content (AOAC Official Method 925.10). For the other protein quality parameter analysis, digestibility was calculated as apparent and true digestibility (FAO/WHO, 1993). Protein efficiency ratio (PER) was calculated based on the weight gain of birds divided by intake of a particular food protein during the test period (AOAC, 2000 Official Method 982.30).

Protein digestibility corrected amino acid score (PDCAAS) was determined from protein digestibility multiplied by the amino acid score (AOAC, 2000 Official Method 999.29). The amino acid score was measured as gram of essential amino acid in the sample divided by a mille gram of reference essential amino acid. The detailed amino acid and fatty acid analysis protocol were presented in chapter 3.

4.4.3.6. Statistical analysis

The statistical analysis was conducted to examine the effects of taro leaf meal on feed composition, functional properties, body weight gain, digestibility, protein efficiency ratio and finally chicken meat composition analysis Data were analyzed using the general linear model procedures of Statistical Analysis Systems software (SAS, 2000) with the model containing treatments. Probability values < 0.05 were considered significant.

4.4.4. RESULTS AND DISCUSSIONS

The dry matter (DM) content (%) of rations from different levels of taro leaf containing diets ranged from 93.33±0.02 to 93.39±51.05 and showed no difference among the treatments (Table 4.4.1). The values resulted from the present study was in a similar range with the values of DM% reported by Etalem *et al*. (2013) for *Moringa oliefera* leaf meal containing diets. The result is also in accordance with (Bediye, *et al.,* 2007; Leta and Bekana 2010; Worku *et al.,* 2012).

Crude Protein Content

As presented in Table 4.4.1, the crude protein content in the study varied from 21.27±0.05 to 21.62±0.05 and there was no difference among the treatments. From the study, the CP was a little high in T_4. Therefore, in order to consider taro leaf as a protein sources the inclusion level should be considered and as the increasing inclusion level of taro leaf, the CP was also consistently increased. The protein content of different feedstuffs was reported in harmony with this study (Whittemore, *et al.,* 2002; Thanh, *et al.,* 2007; Rodríguez and Preston, 2009; Nguyen, *et al.,* 2010). Therefore, supplementing green leaf in chicken feeding has great value to enhance the protein content (Nguyen and Ogle, 2005). When formulating broiler diets, the main emphasis is placed on the crude protein (CP), because protein is the critical constituent of poultry diets, and together with the other main nutrients such as carbohydrates, fat, water, vitamins, and minerals, is essential for life (Cheeke, 2005).Therefore, nutritionally and economically, proper protein usage is essential in all feeding systems.

Crude Fiber Content

The CF content (%) in the rations (Table 4.4.1) from the different levels of TLM differ significantly ($p<0.05$). A lower value of CF% 6.19±0.07, 6.44±0.05 was observed in T_3 and T_4, respectively, than T_1 8.87±0.04 and T_2 7.81±0.05. This might be due to the presence of soluble and digestible fibers in taro (Adane *et al.,* 2013). Also, it was noted that taro leaf is low in insoluble fiber content and the fiber in taro is easily soluble and digestible (Dari *et al.,* 2005; Jones, *et al.,* 2013; Vries; *et al.,* 2015). Therefore, feed with low fiber like TLM, is desirable for digestibility and improve the bulkiness of the feed. In contrary, the high insoluble dietary fiber intake is often associated with slower rate of passage and may inhibit optimal digestion (Perttilä *et al.,* 2002). Therefore, the present study encourage using TLM as a good source of digestible fiber for broilers like other tropical leaves (Pham, *et al.,* 2010; Abuye *et al.,* 2003) at the inclusion level of 5-7%.

Table 4.4.1: Nutrient composition (%) and functional properties of taro leaf meal

	Treatments			
Parameters	T_1(control)	T_2	T_3	T_4
DM	93.33±0.02^{a}	93.35±0.02^{a}	93.34±5.90^{a}	93.39±5.05^{a}
CP	21.30±0.02^{a}	21.27±0.05^{a}	21.62±0.05^{a}	21.77±0.06^{a}
CF	8.87±0.04^{a}	7.81±0.05^{a}	6.19±0.07^{b}	6.44±0.05^{b}
EE	4.51±0.01^{ab}	4.15±0.04^{a}	4.23±0.06^{a}	4.34±0.06^{a}
ash	10.96±0.04^{ab}	12.33±0.05^{a}	12.61±0.07^{a}	12.89±0.05^{a}
Carbohydrate	55.36±0.03^{ab}	54.44±0.04^{a}	55.35±0.08^{a}	54.86±0.05^{a}
ME	2962.41±0.02^{d}	2980.95±0.03^{c}	3117.57±0.07^{a}	3116.57±0.06^{b}
WHC	1.53±0.04^{a}	1.45±0.03^{a}	1.41±0.03^{ab}	1.42±0.07^{ab}
SBC	19.63±0.04^{ab}	20.68±0.07^{a}	20.30±0.02^{a}	20.98±0.06^{a}
HDC	0.98±0.04^{a}	0.83±0.02^{ab}	0.92±0.02^{a}	0.90±0.05^{a}

$^{a-d}$ Means in the same row with a common superscript did not differ significantly ($p>0.05$). DM: dry matter; CP: crude protein; CF: crude fiber; EE: ether extract; ME: metabolizable energy; T_1: ration containing 0% TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM; TLM: taro leaf meal; WHC: water holding capacity; SBC: water solubility cappacity and HDC: hydration capacity; TLM: taro leaf meal.

The ether extract content (%) of the treatment rations was ranged from 4.15±0.04 to 4.51±0.01 (Table 4.4.1). From the present study, the results showed a non-significant difference ($p>0.05$) among the treatments. However, the ether extracts the content of T_1 composed little highest and the lowest value obtained in T_2.

Table 4.4. 2: Mineral and anti-nutritional (mg/100 g) composition of taro leaf meal

	Treatment			
Parameter	T_1(control)	T_2	T_3	T_4
Na(mg/100 g)	6.21±0.03 ^{a}	4.74±0.04 ^{b}	4.67±0.02 ^{b}	4.25±0.03 ^{b}
K(mg/100 g)	10.15±0.01 ^{c}	15.33±0.04 ^{b}	19.41±0.02 ^{a}	19.63±0.03 ^{a}
Fe(mg/100 g)	0.16±0.04 ^{b}	0.26±0.03 ^{a}	0.27±0.02 ^{a}	0.28±0.03 ^{a}
Ca(mg/100 g)	7.98±0.043 ^{a}	5.6±0.03 ^{b}	5.11±0.02 ^{b}	5.112±0.05 ^{b}
Zn(mg/100 g)	4.46±0.06 ^{bc}	5.46±0.03 ^{b}	7.66±0.02 ^{ab}	8.99±0.05 ^{a}
Cu(mg/100 g)	3.44±0.04 ^{a}	3.43±0.03 ^{a}	3.42±0.03 ^{a}	3.42±0.03 ^{a}
P(mg/100 g)	59.09±0.06 ^{a}	58.07±0.02 ^{ab}	58.90±0.05 ^{ab}	58.99±0.03 ^{ab}
Phenols(% g)	1.68±0.04 ^{ab}	1.88±0.032 ^{a}	1.88±0.04 ^{a}	1.89±0.03 ^{a}
Oxalate(mg/100g)	0.18±0.05^{a}	0.18±0.02 ^{a}	0.16±0.03 ^{ab}	0.19±0.03^{a}
Tannin (mg/100g)	0.13±0.04 ^{ab}	0.11±0.02 ^{b}	0.11±0.04 ^{b}	0.10±0.03 ^{a}
Phytate (mg/100g)	0.17±0.05^{ab}	0.19±0.03 ^{a}	0.19±0.04 ^{a}	0.18±0.03 ^{ab}

$^{a\text{-}c}$ *Means in the same row with a common superscript did not differ significantly (p>0.05).* T_1: ration containing 0% TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM and T4: ration containing 7% TLM; TLM: taro leaf meal.

The mg/100 g values in DM basis of Na, K, Fe, Ca, Zn, Cu and P were presented in Table 4.4.2. The (mg/100g) Na, K, Fe, Ca, Zn, Cu and P of feed samples ranged from 4.25±0.03 to 6.21±0.03, 10.15±0.01 to 19.63±0.03, 0.16±0.04 to 0.28±0.03, 5.11±0.02 to 7.98±0.04, 4.46±0.06 to 8.99±0.05, 3.42±0.03 to 3.44±0.04 and 58.07±0.02 to 59.09±0.06 of values, respectively. The mg/100 g of K, Fe and Zn values among the treatments were in the order of $T_4 > T_3 > T_2 > T_1$ and in this study it revealed that TLM in the ration was a good way in enriching the mineral supply of feeds mainly with K, Fe and Zn levels but with low levels in Ca and P contents. Therefore, the obtained results encourage the use of taro leaf, which meant that a ration with more minerals containing formula is good to meet requirements for minerals (Okitoi, et al., 2009; Malavanh, et al., 2008a; Anjorin et al., 2010).

The content of phenols, oxalate, tannin, and phytate in the treatment rations differ significantly ($p<0.05$) among the treatments and ranged from 1.68±0.04 to 1.89±0.03, 0.16±0.03 to 0.19±0.03, 0.11±0.03 to 0.13±0.04 and 0.17±0.05 to 0.19±0.03 g/100 g, respectively (Table 4.4.2). For the present study, the ration containing TLM had a better total phenols and lower anti-nutritional contents of oxalate, tannin and phytate.

This could imply that the inclusion of the TLM in the ration did not hamper the feed intake and feed utilization of birds for appreciating growth rate because of the lower anti-nutritional factors and better nutrient promoting factors such as phenolic substance (Nworgu, 2009).

In animal feeds the presence of high anti-nutritional factors can decrease feed intake and acceptability of feed nutrients which resulted in poor growth (Udedibie and lgwe, 1989; Esonu, 2003; Nworgu, 2009). With this context for proper nutrient utilization of feed and appreciated growth rate in chicks, it is vital to reduce the nutrient limiting factors to the threshold level. Condensed tannin and other anti-nutritional factors can be reduced during thermal processing and boiling (Pham, *et al.,* 2005; Joh *et al.,* 2007; Prajapati *et al.,* 2011). Therefore, it is important to apply different methods of preparation and cooking that could reduce the risk of absorbing excess anti-nutritional compound when consumed as part of the diet (Chittavong, *et al.,* 2008).

With this regard, the present study provided an overview of improved processing methods as good ways of reducing the most dominant anti-nutritional components in taro leaf samples and most of the samples contained low anti-nutritional substances when subjected with curd processing. These results were in line with the findings of Abeke *et al*. (2007) who found that the processing (cooking) method had a significant improvement in the mineral content that could increase the performance of chicks. The increase in the performance of chicks might be happening due to intake of low anti-nutritional factors in the ingredients (Wu and Inglett, 2004). Du Thanh Hang and Preston (2010) noted that oxalate content ranged from 1326-3567mg/100g DM for taro stem and 770-2531 mg/100g DM taro leaf. Processing methods such as air-drying, soaking, cooking or ensiling could reduce oxalate content, by as much as 50% by cooking and ensiling.

Table 4.4.3: Dry matter intake, body weight change, daily growth of broilers

	Treatment			
Parameters	T_1(control)	T_2	T_3	T_4
DMI	76.33±0.32 ^{ab}	74.25±0.45 ^{ab}	82.18±0.56 ^{a}	79.19±0.54 ^{a}
IBW(g/bird)	43.5±0.45 ^{a}	42.95±0.56 ^{a}	42.25±0.54 ^{a}	40.56±0.21 ^{b}
FBW(g/bird)	1106±0.65 ^{c}	1010.632±0.23 ^{d}	1279.658±0.32 ^{b}	1324.503±0.34 ^{a}
BWC(g/bird)	1062.5±0.67 ^{c}	967.682±0.65 ^{d}	1237.408±0.45 ^{b}	1283.943±0.34 ^{a}
ADG	18.899±0.43 ^{c}	18.662±0.23 ^{c}	25.283±0.34 ^{b}	29.534±0.34 ^{a}
DMCE	0.072±0.43 ^{a}	0.076±0.45 ^{a}	0.066±0.56 ^{ab}	0.062±0.45 ^{ab}

$^{a-d}$ Means in the same row with a common superscript did not differ significantly (p>0.05). T_1: ration containing 0% TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM; DMI: dry matter intake; IBW: initial body weight; FBW: final body weight; BWC: body weight change; ADG: average daily growth and DMCE: dry matter conversion efficiency

As demonstrated in Table 4.4.3, DM intake, body weight change, average daily growth and dry matter conversion efficiency of broilers fed ration containing different levels of TLM differ significantly ($p<0.05$). The DM intake (g/bird) for chicks in T_3 had the greatest value 82.18±0.56 while the smaller value 74.25±0.45 in DM intake was found in T_2. The DM intake increased from T_2 to T_4 in all feed groups. Body weight gain and change (g/bird) was in the order of $T_4 > T_3 > T_1 > T_2$. The dietary inclusion of the TLM in broiler diets depressed feed intake at 3% (T_2) inclusion level compared to the control. However, at T_3 and T_4 total feed intake was higher and significantly ($p<0.05$) different than the control. Therefore, BW change was affected by the TLM level in the study. This showed that the average daily growth was in the order of $T_4 > T_3 > T_1$. However, the average daily growth for T_2 was less than T_1. The differences may be related to composition of diet. The nature of the diet is considered the factor that most influences weight gain and feed conversion on broilers (Péron, *et al.,* 2005; Zang, *et al.,* 2009). Consistent with this study improvements were achieved in *Moringa oleifera* leaf meal up to 20% level of inclusion replacement for soybean meal (Etalem, *et al.,* 2013).

The maximum inclusion level of leaf in poultry feed is 8-10 % and if added more than the inclusion level, the color of feed may be changed and this would cause feed intake depression and mortality in chickens (Khajarem, *et al*., 1997; Omoregie and Osagie, 2002; Onu, 2012). Therefore, the recommended inclusion level of leaf in feed formulation must be taken in account.

It was observed that as the level of TLM inclusion increased the percentage of weight gain and daily growth up to 7% level of inclusion. Therefore, this study showed that the nutrient status of the experimental diets was adequate for the broiler growth and performance. Concerning the DMCE, had an increasing trend and it was in the order of $T_4 < T_3 < T_2 < T_1$. It is assumed that the presence of anti-nutritionals have attributable effect on depressed feed intake and reduced feed utilization (Agwunobi 2002, Ndimantang *et al* 2006; Okereke 2012).

Table 4.4.4: Total protein intake and protein efficiency ratio of broilers

	Treatment			
Parameters	T_1(control)	T_2	T_3	T_4
PI (%)	469.77±0.05 ^{d}	503.02±0.05 ^{c}	603.12±0.04 ^{b}	1109.92±0.06 ^{a}
AD (%)	99.06±0.01 ^{ab}	99.04±0.02 ^{b}	99.05±0.02 ^{b}	99.22±0.04 ^{ab}
TD (%)	99.6±0.01 ^{ab}	99.63±0.01 ^{ab}	99.65±0.01 ^{ab}	99.68±0.01 ^{ab}
PER	2.26±0.04 ^{a}	1.92±0.05 ^{ab}	2.12±0.03 ^{a}	1.16±0.07 ^{ab}

$^{a\text{-}d}$ *Means in the same row with a common superscript did not differ significantly (p>0.05).* T_1: ration containing 0% TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM; PI: protein intake; AD: apparent digestibility; TD: true digestibility and PER: protein efficiency ratio

As presented in Table 4.4.4 protein intake in rations containing different levels of TLM differ significantly ($p<0.05$), but digestibility and protein efficiency ratio did not differ significantly. The protein intake (g) was in the order of $T_4 > T_3 > T_2 > T_1$. The higher protein intake (g) was exhibited in T_4 compared to a T_1. This might be due to T_4 diet with a high protein level that contained 7% TLM and that attributed for higher protein intake. Similarly, as reported by Patterson and Adrizal (2005) protein intake and digestion increased with protein levels in the diet.

Concerning digestibility, there was no significant difference ($p>0.05$) in the apparent digestibility, but the true digestibility had greater values in the order of $T_4 > T_3 > T_2 > T_1$. In general, digestibility improved from in the order of T_1 to T_4. Therefore, TLM is a good digestible alternative feed source than the control feed. The quality of a feed protein depends not only on nitrogen content, but also on their digestibility (Amata, 2010; Emmans and Kyriazakis, 2001; Vuong and Van Kinh, 2010).

Digestibility depends on amount of true protein intake and fecal protein out; therefore, the present study revealed that digestibility improved as protein level increased in TLM. In general, the result of this study showed that TLM contained appreciable amounts of digestible nutrients (protein, fiber and minerals) to promote weight gain and performance. Therefore, the present study answers the questions regarding digestibility of diet and feed intake of broilers affected by the level and nature of protein in feed diets. From previous works, little has been reported concerning the use of taro leaf as feed. From the present study digestibility results would contribute to scale up the use of taro leaf for feed purpose in developing countries (Fasuyi *et al.,* 2008). The protein efficiency ratio was calculated from body weight gain over their protein intake from the total dry matter intake and not varied much among the treatments this might be due to protein intake and weight gain variation among the feed.

The finding was in harmony with results of dietary supplementation of *Moringa oleifera* on protein efficiency and growth of boilers (Du *et al.,* 2007). Similarly, several studies are in line with the digestibility of feed (Hetland *et al.,* 2002; Goodband *et al.,* 2002; Maiorka *et al.,* 2005; Vuong *et al.,* 2010; Ayssiwede *et al.,* 2011; Svihus, 2011).

Table 4.4. 5: Nutrient composition of chicken meat after feeding taro leaf meal

Treatments		Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	vitaC (mg/100gm)
Breast Meat	T_1	75.29±0.56	80.87±0.34	2.58±0.34	2.56±0.54	4.27±0.34	3.61±0.67
	T2	74.00±0.43	89.36±0.55	3.31±0.54	0.67±0.34	4.39±0.54	4.66±0.56
	T_3	76.21±0.54	92.68±0.34	3.3±0.56	0.07±0.34	4.48±0.44	8.39±0.45
	T_4	74.99±0.45	88.62±0.67	3.37±0.45	0.027±0.34	4.89±0.34	10.56±0.32
Thigh meat	T_1	75.01±0.45	70.67±0.65	2.67±0.45	3.24±0.32	4.06±0.54	3.12±0.43
	T2	69.87±0.65	63.14±0.65	3.35±0.75	1.21±0.34	4.13±0.43	3.88±0.43
	T_3	76.27±0.64	73.08±0.54	3.45±0.23	0.11±0.43	4.32±0.34	7.86±0.53
	T_4	75.25±0.34	76.80±0.54	3.46±0.34	0.07±0.43	4.53±0.34	9.46±0.45

T_1: ration containing 0% TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM; TLM: taro leaf meal.

The nutrient content of chicken meat was determined and differs significantly as shown in Table 4.5.5. The breast muscles have a better nutrient composition than that of the tight muscles. In both breast and tight muscle the nutrient compositions were in the order of $T_4 > T_3 > T_2 > T_1$ except the crude fiber content. Rations containing increased levels of TLM had greater values than the controlled feed. Rukchon *et al.* (2011); Abdou *et al*. (2006) and Sahraei et al. (2012), suggested that the chemical composition of chicken meat affected feed protein quality.

The quality of a feed protein highly depends on the protein composition of final products such as meat and egg. The present study was consistent with the findings of Melesse *et al*. (2011) who proved that the quality of protein and amino acid content of feed are known to enhance muscle protein. Therefore, diet formulation based on enriched nutrients, mainly protein is clearly superior to improve the nutritional quality of chicken meat (Dari *et al.,* 2005; Musa, *et al*., 2006; Abou Dozier, *et al.,* 2008; Dyubele, *et al.,* 2010; Elezz, *et al.,* 2011). Also the observation from the present study was in harmony with Perttilä *et al*. (2002); Lemme *et al*. (2004) noted that diet formulation based on high quality protein would allow the use of alternative protein sources would improve the precision of least-cost diets and reduce nitrogen excretion from chicken operations. Therefore, protein sources are especially limiting factors in chicken feed production and the quality of their end products (Gous and Cherry, 2004; Wood, *et al.,* 2004; Atawodi *et al.,* 2008; Madruga, *et al.,* 2008).

As summarized in Table 4.4.6, the amino acid composition in (g/100g) of the analyzed chicken breast meat samples were quantified with their relative area comparing with amino acid standard and the concentration of each amino acid (g/100g) was also calculated by multiplying the percentage of each amino acid with their average crude protein content (Table 4.4.5). The dominant essential amino acid in breast muscle was leucine, lysine and valine ranging from 62.99±0.04 to 66.43±0.32, 71.92±0.04 to 75.36±0.3 and 39.99±0.32 to 43.43±0.04, respectively. Similarly, glutamic acid, aspartic acid and arginine are also the three dominant non-essential amino acid ranging from 104.53±0.04 to 107.97±0.04, 74.18±0.04 to 77.62±0.04 and 58.4±0.3 to 61.84±0.12 in T_4 and T_1 respectively.

Table 4.4. 6: Amino acids composition (g/100g) of breast meat after feeding taro leaf meal

Types of Amino Acids		Treatments			
Essential Amino Acids (EAA)		T_1(control)	T_2	T_3	T_4
L-Histidine	His	38.62 ± 0.32^{ab}	39.29 ± 0.43^{ab}	40.74 ± 0.23^{a}	42.06 ± 0.43^{a}
L-Isoleucine	Iso	36.48 ± 0.04^{b}	37.15 ± 0.34^{b}	38.6 ± 0.43^{ab}	39.92 ± 0.34^{a}
L-Leucine	Leu	62.99 ± 0.04^{ab}	63.66 ± 0.32^{ab}	65.11 ± 0.04^{a}	66.43 ± 0.32^{a}
L-Lysine	Lys	71.92 ± 0.04^{b}	72.59 ± 0.04^{a}	74.04 ± 0.04^{ab}	75.36 ± 0.3^{a}
L-Methionine	Met	14.97 ± 0.32^{b}	15.64 ± 0.45^{b}	17.09 ± 0.44^{ab}	18.41 ± 0.45^{a}
L-Phenylalanine	Phe	19.1 ± 0.04^{b}	19.77 ± 0.21^{b}	21.22 ± 0.34^{ab}	22.54 ± 0.45^{a}
L-Threonine	Thr	30.75 ± 0.32^{d}	31.42 ± 0.43^{c}	32.87 ± 0.04^{b}	34.19 ± 0.04^{a}
L-Tryptophan	Try	29.4 ± 0.04^{b}	30.07 ± 0.13^{b}	31.52 ± 0.43^{ab}	32.84 ± 0.04^{a}
L-Valine	Val	39.99 ± 0.32^{b}	40.66 ± 0.04^{b}	42.11 ± 0.24^{ab}	43.43 ± 0.04^{a}
Non-essential Amino Acids (NEAA)					
L-Alanine	Ala	43.33 ± 0.54^{b}	44 ± 0.65^{b}	45.45 ± 0.56^{ab}	46.77 ± 0.12^{a}
L-Arginine	Arg	58.4 ± 0.3^{b}	59.07 ± 0.54^{b}	60.52 ± 0.56^{ab}	61.84 ± 0.12^{a}
L-Asparagines	Asp	34.13 ± 0.04^{b}	34.8 ± 0.56^{b}	36.25 ± 0.56^{ab}	37.57 ± 0.04^{a}
L-Aspartic acid	Asp	74.18 ± 0.04^{b}	74.85 ± 0.53^{b}	76.3 ± 0.04^{ab}	77.62 ± 0.04^{a}
L-Glutamic acid,	Glu	104.53 ± 0.04^{ab}	105.2 ± 0.54^{ab}	106.65 ± 0.56^{a}	107.97 ± 0.04
L-Glutamine	Glu	34.78 ± 0.04^{b}	35.45 ± 0.65^{b}	36.9 ± 0.04^{ab}	38.22 ± 0.23^{a}
L-Glycine	Gly	31.65 ± 0.04^{ab}	32.32 ± 0.56^{ab}	33.77 ± 0.54^{a}	35.09 ± 0.23^{a}
L-Serine	Ser	24.78 ± 0.04^{b}	25.45 ± 0.43^{b}	26.9 ± 0.04^{ab}	28.22 ± 0.32^{a}
L-yrosine	Tyro	29.4 ± 0.32^{b}	30.07 ± 0.04^{ab}	31.52 ± 0.04^{ab}	32.84 ± 0.22^{a}
L-Hydroxy	Hyd	13.22 ± 0.32^{b}	13.89 ± 0.04^{b}	15.34 ± 0.04^{ab}	16.66 ± 0.54^{a}
L-Norleucine	Nor	14.19 ± 0.34^{b}	14.86 ± 0.04^{b}	16.31 ± 0.04^{ab}	17.63 ± 0.54^{a}
L-Proline	Pro	13.97 ± 0.34^{b}	14.64 ± 0.04^{b}	16.09 ± 0.45^{ab}	17.41 ± 0.54^{a}

$^{a\text{-}b}$ *Means ± SD within a raw with similar superscripts did not differ significantly (p>0.05); SD: standard deviation; AAs: Amino acids; T_1: ration containing 0%TLM; T_2: ration containing 3% TLM; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM;* TLM: taro leaf meal.

In the present study, amino acid composition of chicken meat was affected by dietary supplementation with taro leaf and similarly Fujimura and Kadowaki (2006) reported the amino acid composition of meat could be improved and varied due to dietary components. Dietary amino acid concentration should closely meet the quantitative requirement of chicken dependent on genotype, gender, age, performance and housing conditions (Dari, *et al.,* 2005; Taherkhani, *et al.,* 2008; Pastor, *et al.,* 2013).

In general, the study shows that the amino acid composition of chicken meat varied by feed ingredients and therefore, the findings of this study are found to be in agreement with the previous research works (Qiong, *et al.,* 2001; Strakova, *et al*. 2002; Peganova, *et al.,* 2003; Olugbemi, *et al.,* 2010). From the findings of this study, as increasing taro leaf in the feed rations from 3-7%, the amino acid composition also increased in chicken meat and therefore the study was found to be very significant for future use of taro leaf in chicken diet formulation so as to improve mainly the limiting amino acids of the feed and final meat products as reported (Creswelland Swick, 2001; Straková, *et al.,* 2006; Wecke and Liebert, 2010). The presence of adequate amount of essential amino acid in chicken diet is very critical because amino acids in the feed also serve vital metabolic roles as blood plasma proteins, enzymes, hormones, and antibodies, each of which has a specific role in the body (Pond *et al*., 1995).

Table 4.4. 7: Amino acids score of chicken breast meat after feeding taro leaf (TL)

	Chicken meat Amino acid score , ASS			
Amino acids	T_1	T_2	T_3	T_4
Histidine	15.45	15.72	16.30	16.82
Isoleucine	14.59	14.86	15.44	15.97
Leucine	28.77	29.04	29.62	30.14
Lysine	25.20	25.46	26.04	26.57
Methionine + cys	5.99	6.26	6.84	7.37
Phenylalanine	7.66	7.91	8.49	9.02
Threonine	12.30	12.57	13.15	13.68
Tryptophan	11.76	12.03	12.61	13.14
Valine	16.00	16.26	16.84	17.37

As shown in Table 4.4.7, the amino acid score of chicken breast meat samples is quantified. The highest score in breast muscle is found in leucine, lysine and valine ranging from (30.14 to 28.77), (26.57 to 25.20) and (17.37 to 16.00) respectively. The essential amino acid score is decreases in the order of $T_4 > T_3 > T_2 > T_1$. Protein quality is dependent on the availability of essential amino acids in a given protein source. In the present study, an amino acid score of chicken meat is affected by dietary supplementation with TL. Similarly Fujimura and Kadowaki (2006) reported the amino acid score could be affected and varied due to dietary components.

In general, the study concluded that the amino acid score of chicken meat varied by feed ingredients and therefore, the findings of this study are found to be in agreement with the previous research works (Hamm, 2001; Moughan, *et al.,* 2005; Limin *et al*. 2007). As an increasing taro leaf in the fed rations from 3 to 7%, the amino acid score also increasing in chicken meat and therefore the study is found to be very significant for future use of taro leaf in chicken diet formulation so as to improve mainly the essential amino acid score of the feed and final meat products as reported (Gruber, *et al*.,. 2000; Lemme, *et al.,* 2004; Darragh*, et al.,* 2008; Tillman and Sriperm, 2011b; Boye, *et al.,* 2012).

Table 4.4. 8: Protein digestibility corrected amino acid score of breast meat

Chicken breast meat PDCAAS				
Amino acids	T_1	T_2	T_3	T_4
Histidine	0.15	0.16	0.16	0.17
Isoleucine	0.15	0.15	0.15	0.16
Lysine	0.25	0.25	0.26	0.27
Leucine	0.29	0.29	0.30	0.30
Methionine + cys	0.06	0.06	0.07	0.07
Phenylalanine	0.08	0.08	0.08	0.09
Threonine	0.12	0.13	0.13	0.14
Tryptophan	0.12	0.12	0.13	0.13
Valine	0.16	0.16	0.17	0.17

Table 4.4.8 summarizes the protein digestibility corrected amino acid score (PDCAAS) of chicken breast meat samples. The highest PDCAAS are found in lysine, leucine, and valine ranging from 0.29 to 0.30, 0.25 to 0.27 and 0.16 to 0.17 respectively. This means, digestibility of lysine is from 30 % to 29 %, leucine from 25% to 27% and valine from 16% to 17%. The PDCAAS value is decreases in the order of T_4 >T_3 >T_2 >T_1. In T_4 the digestible amino acids are greater than T_1. On the other hand, as the increase of the taro leaf inclusion level, the percent of digestible amino acids also increased.

In the present study, PDCAAS of chicken meat are affected by dietary supplementation with taro leaf and similarly Mcnab (2004) reported the PDCAAS value could be affected and varied due to dietary components. The higher PDCAAS values represent the higher intake of digestible proteins or amino acids from the test diet (WHO, 2007; Wolfe, 2012).

Similar observations have been made by (Fuller, 2012; Rutherfurd and Moughan 2012; and Schaafsma, 2000) that the PDCAAS in broilers were affected by a variety of diets. Therefore, the findings of this study are found to be in agreement with the previous works (Zarkadas, 2002; Fenwick*, et al.,* 2005; Schaafsma, 2005; Rutherfurd, and Moughan, 2012; FAO, 2015).

Table 4.4. 9: Fatty acid composition (%) of breast meat after feeding taro leaf meal

Class of FAs	T_1	T_2	T_3	T_4
$C_{14:0}$	0.60±0.31 [a]	0.02±0.34 [a]	0.64±0.23 [a]	0.72±0.34[a]
$C_{16:0}$	13.58±0.32 [c]	46.79±0.45 [a]	31.85±0.23 [b]	31.93±0.23[b]
$C_{16:1(n-7)}$	1.76±0.12 [b]	3.92±0.34 [a]	2.85±0.23 [ab]	2.93±0.43 [ab]
$C_{17:0}$	0.02±0.02 [a]	0.02±0.34 [a]	0.02±0.03 [a]	0.10±0.43 [a]
$C_{17:1}$	0.38±0.32 [ab]	1.53±0.24 [a]	0.15±0.32 [ab]	0.23±0.34 [ab]
$C_{18:0}$	7.11±0.23 [b]	4.82±0.33 [c]	15.30±0.33 [a]	15.38±0.43 [a]
$C_{18:1(n-9)}$	25.47±0.12 [a]	21.74±0.43 [ab]	25.08±0.23 [a]	25.16±0.43 [a]
$C_{18:2(n-6)}$	35.35±0.35 [a]	17.15±0.03 [ab]	17.22±0.43 [ab]	17.30±0.34 [ab]
$C_{18:3(n-3)}$	0.79±0.33 [a]	0.02±0.04 [b]	0.75±0.44 [a]	0.83±0.23 [a]
$C_{20:0}$	0.02±0.43 [ab]	0.02±0.04 [ab]	0.20±0.34 [a]	0.28±0.32 [a]
$C_{20:1(n-9)}$	0.42±0.12 [a]	0.02±0.03 [ab]	0.31±0.34 [ab]	0.39±0.33 [a]
$C_{20:2(n-6)}$	0.77±0.34 [a]	0.02±0.04 [b]	0.24±0.34 [ab]	0.32±0.44 [ab]
$C_{20:3(n-6)}$	1.03±0.34 [a]	0.02±0.02 [b]	0.61±0.45 [ab]	0.69±0.43 [ab]
$C_{20:4(n-6)}$	0.02±0.02 [a]	0.02±0.03 [a]	0.02±0.34 [a]	0.10±0.23 [a]
$C_{24:1(n-9)}$	0.02±0.02 [a]	0.02±0.03 [a]	0.02±0.32 [a]	0.10±0.32 [a]
$C22:6(n-3)$	1.21±0.21 [a]	0.02±0.01 [b]	0.49±0.23 [ab]	0.57±0.23 [ab]
Total FA	88.55	96.15	95.75	96.97
Proportion (%), SFA, MUFA, PUFA and UFA:SFA				
SFAs	21.33	51.67	48.01	48.39
MUFA	28.05	27.23	28.41	28.79
PUFA	39.17	17.25	19.33	19.79
UFA	67.22	44.48	47.74	48.58
UFA:SFA	3.15	0.86	0.99	1.00

[a-c] Means within a raw with same superscripts did not differ significantly (p>0.05; FAs: Fatty acids; SFAs: Saturated Fatty acids; MUFAs: Mono-unsaturated Fatty acids; PUFAs: Poly-unsaturated Fatty acids; UFAs: Unsaturated Fatty acids; T_1: ration containing 0%TLM; T_2: ration containing 3% TML; T_3: ration containing 5% TLM; T_4: ration containing 7% TLM; TLM: taro leaf meal.

Table 4.4.9 summarizes the total fatty acid composition (%) for chicken breast meat samples. Fatty acids were identified with their retention time and quantified with relative area computing. The overall breast meat fatty acid of TL was significantly different (p<0.05).

The dominant fatty acid in percentage was palmitic acid ($C_{16:0}$) ranging from 13.58±0.32 to 46.79±0.45 for T_1 and T_2. The next three dominant fatty acids were oleic acid ($C_{18:1,\ n\text{-}9}$), linoleic acid ($C_{18:2,\ n\text{-}6}$) and stearic acid ($C_{18:0}$) and ranging from 21.74±0.43 to 25.47±0.12, 17.15±0.03 to 35.35±0.35 and 4.82±0.33 to 15.38±0.43 (%) values, respectively. In the present study, quantified fatty acids of breasts meat are affected by dietary supplementation with TL (Table 4.4.9).

Similar observations have been made by Cortinas *et al*. (2004) and Osek *et al*. (2004) that the muscle fatty acid compositions in broilers were affected by a variety of diets. The fatty acid composition of breast muscles obtained in the present study was in agreement with values reported for broiler meat (Cherian *et al.,* 2002; Cortinas *et al.,* 2004). From the proportion (% of total fatty acids) in the present study, the UFAs were of very higher concentration than those SFAs in control feed. However, there were no significant difference between saturated and unsaturated fatty acids in T_2, T_3 and T_4. The possible reason might be included of TL did not changed the fatty acid content in the breast meat. These results were in agreement with other studies showing that improvement of UFAs in feed also increased the UFAs contents of breast the muscle (Crespo and Esteve-Garci, 2002). In poultry diet, inclusion of ingredients with high unsaturated fatty acid content is recommendable, because one of the criteria for the determination of fat quality is the content of essential UFA like linoleic, linolenic, and arachnodic acids (Koreleski *et al.,* 2007; Meseret *et al.,* 2011). In human nutrition, polyunsaturated fatty acids (PUFAs) have great role, especially the in the management of coronary heart disease (Sanz *et al.,* 2000; Koreleski *et al.,* 2007). Since, the presence of high levels of unsaturated fatty acids, of the total lipids is nutritionally desirable.

Table 4.4. 10: Cost benefit analysis of feeding taro leaf in broiler experiment

Cost benefit parameters	Treatments(feed rations)			
	T_1(control)	T_2	T_3	T_4
Total benefit parameters				
feed cost total (Birr)	1624.61	823.55	810.85	793.05
feed intake total (kg)	42.85	40.11	38.45	40.34
weight gain (g)	640.00	580.00	775.00	840.00
sale of live chicken total (Birr)	3600.00	3400.00	4000.00	4000.00
ratio(total cost/wt gain)	2.54	1.42	1.05	0.94
net profit(Birr)	1975.39	2576.45	3189.15	3206.95
profitability index	10.97	15.16	15.95	16.03
Individual benefit parameters				
cost per bird (Birr)	81.23	41.18	40.54	39.65
intake per bird	2.23	2.32	2.26	2.29
ratio (cost/wt gain)	0.00	17.75	17.94	17.32
Sale of live chicken(Birr)	180.00	170.00	200.00	200.00
profits individual(Birr)	180.00	128.82	159.46	160.35

From cost and benefits analyses of the study, taro leaf meal is cost effective meal when used as replacement of soybean meal in chicken (Table 4.4.10). As reported by several scholars the use of locally available resources for feed have better cost benefit in poultry diets (Dessie and Ogle, 2001; Mwalusanya, *et al.,* 2002; Awuni, 2002; Batal and Parsons, 2002; Swain *et al.,* 2007a; Swain *et al*., 2007b; Swain *et al.,* 2009; Mekonnen *et al.,* 2009; Momoh *et al.,* 2010; Ironkweand Bamgbose, 2012).

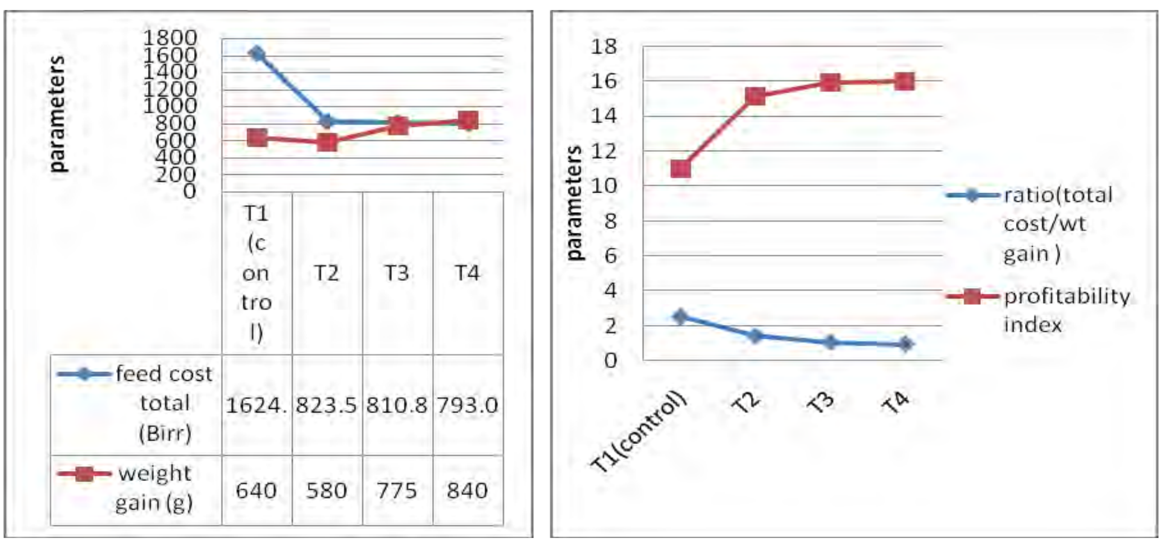

Figure 4.4. 1: Cost benefiting parameters feeding taro leaf for chicken

4.5.5. CONCLUSION

According to the findings, the variation was found as taro leaf level increasing in the fed rations from 3-7%, the nutrient composition, mineral and the functional properties of the feed, also changed. Also a lower value of crude fiber was observed as increase level of taro leaf the treatments and the lower fiber content improved the bulkiness, digestibility and functional properties the of the feed and consequent effect on body weight gain and growth. Thus, taro leaves from this study were recommended for chicken diets. It was observed that as the level of TLM inclusion increased the percentage of weight gain and daily growth up to 7% level of inclusion. Similarly, the dry matter intake and daily growth of the chickens were increased as taro inclusion level increased. Rations containing increased levels of taro leaf had greater breast muscle nutrient compositions. The amino acid, fatty acid and PDCAAS of breast muscle compositions were affected by levels of taro leaf in the rations. From cost and benefits analyses of the study, taro leaf meal founded cost effective and alternative resource when used as replacement of soybean in chicken feed. Therefore, in order to consider taro leaf as a protein sources the inclusion level should be considered.

5. CHAPTER FIVE: GENERAL DISCUSSION

5. CHAPTER FIVE. DISCUSSION

The purpose of this work was to investigate the Ethiopian taro with respect to corm and leaf potential use for human food and chicken feed development. The corm and leaf samples were subjected to proximate composition, mineral content, anti-nutritional content, functional properties analysis. The amino and fatty acid was investigated using HPLC and GC-FID instruments. Complementary food samples were development using mixture design software package (Design- Expert ®, version 10.0, Stat-Ease, SaMeep104 Inc.). The poultry experiment was conducted using four treatments, inclusion of taro leaf at 0% (T_1), 3% (T_2), 5% (T_3), and 7% (T_4). Digestibility and protein efficiency ratio analysis were done at the end of the feeding trial

5.1. Nutritive and Non-nutritive Constituents of Taro (Colocasia esculenta) Corm,

In Ethiopia, farmers produce taro for their own consumption and considers difficult to store and sometimes wasteful because of spoilage and the demand for taro in urban areas has not gained the attention and neglected yet. However, it has a great potential to supply high quality food and one of the cheapest sources of energy (Patrick, *et al*., 1999). Still now the crop is underutilized and has not gained sufficient research attention with regards to its potential. The characterization of such less domesticated and unexploited food sources from the rural sources is essential to increase food choices and habits that support rural food security. Therefore, this study was aimed to investigate the nutritional and anti-nutritional content of taro and whether different improved methods of preparation and processing can reduce dominant anti-nutritional compound when consumed as part of human diet.

Two taro corm varieties (boloso-1, local) were collected from Areka Research Centre and prepared in the form of gelatinized flour and were analyzed for proximate, mineral anti-nutritional contents. For the proximate composition results, The moisture content ranged from 4.23±2.16 to 6.22±1.83, crude protein from 10.49±2.38 to 12.13±2.32, crude fat from 2.61±1.1to 3.42±0.65, crude fiber from 2.11±0.26 to 2.80±0.93, total ash content ranged from 3.29±0.49 to 3.88±0.79, carbohydrate content from 72.53±2.11 to 75.49±4.43, amylose content ranged from 12.89±0.67 to 13.56±0.49, beta (β)-carotene content μg/100 g from 2.11±0.50 to 2.31±0.16.

The result from this study revealed that the higher nutrient and lower anti-nutrient content was found in boloso-variety than local taro variety. Additionally, pre-gelatinization without peel had a positive effect on reducing the anti-nutrients and increases the bioavailability of the minerals. With this regard, all of the analyzed samples had lower phytate: mineral molar ratio less than 10 and below the critical value. Therefore, this study investigated the effect of peeling, washing and pre-gelatinization in order to reduce inhibitory effects and enhance the nutritional composition.

5.2. Development of Complementary Food from Taro-Maize-Soybean formulation

In Ethiopia, the concept of improved feeding of infant and young children is not well understood by most families and there is no ingredient optimization method during infant food formulation and this could be also other gap to solve (WHO/UNICEF, 2005 and Ali *et al*., 2011). The cheapest and fastest way of meeting the growing demand for nutrient dense infant food is through increasing the development of low-cost and locally available infant ingredients and availing the products at affordable prices by lowering the cost of production to bring processed infant foods within the reach of poor people (Bhandari, et al., 2004; Silva *et al.,* 2006 and Fahmida, *et al*., 2014). Such an approach would increase the nutrient density of complementary foods. Therefore, this study is initiated to produce optimized complementary food from pre-gelatinized taro flour, mixing with maize and soybean in order to develop low-cost and easily available complementary food as noted by (WHO, 2003; Gibson, *et al*., 2015).

Taro flour was used together with soybean and maize to develop the optimized complementary food. Both maize and soya bean were obtained from the Oromia commodity exchange in Addis Ababa. A statistical software package (Design- Expert ®, version 10.0, Stat-Ease, SaMeep104 Inc.) mixture design was used for the generation of test formulations and analysis of the results. An attempt was made to maximize the responses for CP, fat, carbohydrate and minerals (Fe, Zn, Ca, Mg and P) and minimize dietary fiber, oxalate and phytate in the complementary food. For optimized complementary food had a proximate content of crude protein, crude fat, total ash, carbohydrate and energy value were 22.23±0.6, 6.32±0.78, 4.8±0.32 and 56.5±0.46, respectively. All the crude protein results are greater than minimum requirement (14%) of WHO/UN specification.

The development of complementary food showed that blending of taro (50 %) with maize (30%) and soybean (20%) improve the nutritional density of complementary foods. For the sensory results, the mouth feel, taste and overall acceptability of all porridge samples were rated greater than 6 indicating that liked very much by the panelists. The analyzed model shows that, in the blending ratio of taro, maize and soybean had an effect on nutrient and anti-nutrient composition of the formulated samples. Accordingly, as soybean flour increased the protein content and the binary combination of maize and soya bean produced a positive effect on protein content.

The binary effects of soybean and maize crude fat did differ with the binary effects of soybean and taro flour. As soybean flour increased the crude fiber content and the binary combination of maize and soybean produced positive effects on CF content. There were higher total ash values for taro and soybean blended products and this might be high ash content of taro and soybean than maize. The general trend observed from the components were both taro and soya flour, blending ratio contained the highest ash. The analyzed model shows that, the blending ratio had an effect on Fe, Zn, Ca, Mg and P content (mg/100 g) of the mixed components. There were higher mineral values for taro flour blended products and this might be higher mineral content in taro than maize and soya bean. The blended products prepared from maize and soybean had higher phytate values than taro. However, the phytate content of all complementary food samples is very low compared with the maximum recommended daily intake of phytate from food which is 300–500mg/day.

5.3. Nutritional and Anti-nutritional Composition of Taro (Colocasia esculenta) Leaf

The young taro leaves are a nutritious spinach-like vegetable, which provides a lot of minerals, vitamins and thiamine (Mwangi, *et al.,* 2007; Azeez and Madukwe, 2010). In Asia and western Africa, the leaves are used as green vegetables after thorough removal of the acrid elements through special processing (de Oliviera, 2012). However, that there is no awareness among people about the quality the leaf has in Ethiopia. In this context, taro leaf was evaluated for its nutritional and anti-nutritional components.

Two taro leaf samples (boloso-1 and local), were harvested at 7 months of age from Areka Research centre. The leaf samples were prepared in to curd and powder forms according to Cornelissen et al., (2003) and subjected to different chemical analysis. The moisture content ranged from 3.44±0.19 to 3.73±0.69, crude protein from 25.06±1.29 to 26.25 ±1.53, crude fat from 5.84±0.16 to 6.44±0.88, fiber from 3.19±1.98 to 4.60±1.39[a], total ash from 2.71±0.07 to 2.88±0.23, total carbohydrate from 56.22±2.74 to 59.38±2.94, amylose contents from 5.32±1.15 to 6.28±0.98, beta-carotene (μg/100 g) 2.48±0.20 to 2.70±0.22 and phenolic compound (mg/100 g) from 0.96±0.08 to 1.11±0.18.

From the result, boloso-1 variety contained the highest nutrient contents than that of local variety and in terms of processing method; the curd samples exhibited better value. The mg/100 g of K, Mg, P and Ca ranged (14.33±1.40), (8.29±1.89, (1.18±0.81) and (15.26±6.21) and micro minerals like Zn, Fe and Cu contents mg/100 g were significantly ($p<0.05$), varied from (9.65±4.55), (40.37±9.83) and (0.42±0.19) to (12.75±1.81, (51.87±1.02) and 0.58±0.22) values, for boloso-1 taro variety. The result obtained from this study predicted that curd concentrate samples had high K, Mg, P, Ca, Zn and Fe mg/100 g concentration than powder samples. Chemical compositions of cured and powder taro leaf samples were affected by processing methods used in the study (twigging, washing, curding, drying and powdering) and variety difference.

5.4. Taro Leaf as Alternative chicken Feed Ingredients:

Most of the population in Ethiopia depends on cereals as staple foods which are also required for poultry feeding (Alemu, 1995; CSA, 2013). This means, it is difficult to use cereals for poultry with having the human food insecurity burden and replacing expensive cereals and less available agro-industrial by-products by unconventional feedstuffs, which are less exploited by man, is one of the solutions to reduce cost of production and contribute to increased supply of animal protein to rich of poor people‟s (Silva, 2004; Amire, 2010). To bridge the gap and tackling the problems, inclusion of taro leaf meals (TLM) in the diets of chicken may bring products rich in essential nutrients for the consumers due to its good essential amino acid and fatty acid profiles.

The major feed ingredients were mixed based on a feed formulation recipe according to (Zhu et al., 2007) and carried out for various chemical composition analyses. The experimental broilers were managed and feeding trials were carried out for 56 days. The treatments were inclusion of taro leaf meal at 0% (T_1), 3% (T_2), 5% (T_3) and 7% (T_4). The result obtained from the study shows that variation was found as taro leaf level increase. The crude protein in feed ranged from 21.27±0.05 to 21.62±0.05, ether extract from 4.15±0.04 to 4.51±0.01, total ash from 10.96±0.04 to 12.89±0.05, utilizable carbohydrate from 54.44±0.04 to 55.36±0.03 and metabolizable energy from 2962.41±0.02 to 3117.57±0.07.

The functional properties of the feed, water holding capacity (%) ranged from 1.41±0.03to 1.53±0.04, solubility capacity (%) from 19.63±0.043 to 20.98±0.06 and hydration capacity (%) from 0.83±0.02 to 0.98±0.04. The (mg/100g) Na, K, Fe, Ca, Zn, Cu and P of feed samples ranged from 4.25±0.03 to 6.21±0.03, 10.15±0.01 to 19.63±0.03, 0.16±0.04 to 0.28±0.03, 5.11±0.02 to 7.98±0.04, 4.46±0.06 to 8.99±0.05, 3.42±0.03 to 3.44±0.04 and 58.07±0.02 to 59.09±0.06 of values, respectively.

The inclusion of the taro leaf meal in the ration did not hamper the feed intake and feed utilization of birds for appreciating growth rate because of the lower anti-nutritional factors. The dry matter intake increased from T_2 to T_4 in all feed groups. Body weight gain and change (g/bird) was in the order of $T_4 > T_3 > T_1 > T_2$. It was observed that as the level of taro leaf meal inclusion increased the percentage of weight gain and daily growth up to 7% level of inclusion. The protein intake (g) was in the order of $T_4 > T_3 > T_2 > T_1$. Finally, rations containing increased levels of taro leaf had greater breast muscle nutrient compositions. The amino acid, fatty acid and PDCAAS of breast muscle compositions were affected by levels of taro leaf in the rations.

5.5. Limitation of the study

Promotion of underutilized crop like taro can bring about better nutrition and fights hidden hunger and thereby helps in combating malnutrition, improving the health status of the population, and sustainably contributing to poverty reduction. The results of this study would assist the promotional activities by providing valuable information about the potential values of taro. However, there were some limitations:

The samples were collected at one season of cultivation and similar maturity levels of the two varieties. The maturities have major effects on the nutritional composition and phytochemical contents of edible parts of the crop. These factors, major sources of variability were not controlled.

- ✓ The leaf samples were not taken at different interval of the growing season to see the difference between young and matured leaf composition.
- ✓ There are also other two taro varieties and many accessions in the country and therefore, including varieties in different regions was not done.
- ✓ Characterizations of taro leaf constituents were done. However, promoting and utilizing as human food was not possible due to the eating habit and lack of previous information about taro leaf. Therefore, to make taro leaf eadible as a human food effort needed.
- ✓ The product development from the taro flour is only for infant and other products like cheeps, slices and breads were not tested from taro.
- ✓ In chicken feed digestibility, both the apparent and true digestibility of feed was determined. But, the true digestability was only based on the fecal drop out nitrogen and the intestinal or illial digestibility was not considered. For true digestibility determination, endogenious protein loss should be considered.

6. CHAPTER SIX. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The nutritional profiles and product development of two popular and socially significant varieties of taro in Souther region of the country were investigated. The investigation took the form of evaluation of macro and micronutrients, phytochemical contents, anti- nutrient contents, functional properties and finally new products were formulated from the corm and leaf. From the results of the present study it was understood that taro corm and leaf contains appreciable quantity of nutrients. The data presented in Table 4.1.1 suggest that taro corm is good sources of macro and micro (minral, beta carotene content) nutrients. Nutrient compositions of peeled and unpeeled taro flours were affected by taro varieties and processing methods applied in the study (peeling, washing, drying and milling) and variety difference. The essential fatty acids $C_{18:2}$ and $C_{18:3}$ are found in low concentration as compared to oleic acid.

In complementary food development, the results are good indicators of the possibility for better utilization of taro through developing variety of new food products. The blending ratio of taro, maize and soybean had an effect on crude protein, fat, fiber, carbohydrate content and energy value of the mixed components. The overall optimization suggested that the level of ingredients to produce complementary food that would be in the mixture of taro 50 % taro, 30 % maize and 20 % soybean. All complementary food samples were also found to be safe regarding the anti-nutrient contents. The phytate, tannin, oxalate and other inhibitory content in the different samples analyzed were less than mimimum daily intake recommended by WHO. It is noted that the inhibitory effect can be reduced using physical and thermal methods, for increasing the safety of taro leaves.

In chicken feed formulation, as increase level of taro leaf in the treatments, improved the bulkiness, digestibility and functional properties of the feed and consequent effect on body weight gain and growth of the chicks. Similarly, the dry matter intake and daily growth of the chickens were increased as taro inclusion level increased.

6.2. RECOMMENDATIONS

Due to the low attention given for utilization of taro in the country, it is underutilized and only populat in the southern parts of the country. Hence, taro has potential for improving food and nutrition security, attention and promotion should be given by consumers and researchers. Even if, very few researchs were done previouselly, still taro is less characterized compared to the other root crops such as cassva, sweet potato and potato. The following recommendations have been drawn from the results of this study:

1) There is need efforts to investigate and domesticate different varieties of taro leaf for food sources in different rural areas of the country as alternative food diets.(the different varietys)

2) Different processing methods need to be promoted so as to reduce the anti-nutritional substances that limits the potential utilization by the peoples (fermentation, ensiling, chemical)

3) Different product development from both taro corm and leaf need to be continued to utilize the potential of taro there by diversify dietery food and industrial raw materials(cheeps, beverage, breads, injera)

4) The findings obtained from this study need to be assisted with other relevant works to maximize the utilization of underutilized food crops as a key solution for food insecurity problems in the country. (sociao economic studies, productivity and)

5) This study did not address the nutrient and anti-nutrient composition of taro samples at different age of the growing season to assess composition difference due to the age of maturity especially for taro leaf. Therefore, investigations at different age of samples need to be studied. (the young and the matured, the raw and the processed, storage condition)

6) The anti-oxidant and other health promoting phytochemicals found in taro need to be studied. (the ANF are good for the crop at field but bad for human/animal, some are still important fro health and protective effcet)

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APPENDIXES

Appendix A: Published papers

Paper I

Journal of Nutrition & Food Sciences
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Research Article | Open Access

Effect of Pre-Gelatinization on Nutritive and Non-Nutritive Constituents of Taro (*Colocasia Esculenta L*) Corm

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Abstract

The study was conducted using factorial combination of two independent variables (taro leaf varieties and processing methods) with three replications in a completely randomized design (CRD) at the same significant level (α<0.05). The analysis undertaken in this study was leaf proximate composition, mineral concentration and anti-nutritional factors of samples processed as pre-gelatinized taro starch with and without peel. The moisture content (%) between taro varieties in this study was ranged from 6.218 ± 1.835 to 4.218 ± 2.162, crude protein (CP%) from 12.127 ± 2.326 to 10.498 ± 2.384, crude fat (EE%) from 3.421 ± 0.659 to 2.608 ± 1.116, crude fiber (CF%) from 2.801 ± 0.938 to 2.110 ± 0.262, total ash (TA%) content ranged from 3.886 ± 0.794 to 3.296 ± 0.494, carbohydrate content (%) from 75.489 ± 4.436 to 72.534 ± 2.107, amylose content (%) ranged from (12.894 ± 0.679) to (13.557 ± 0.490), beta (β)-carotene content mg/100 g from 2.307 ± 0.162 to 2.107 ± 0.502. The five macro minerals, mg/100 g of Na, K, Mg, P and Ca in this study were significantly (p<0.05) different and ranged from minimum 9.716 ± 4.52, 17.743 ± 1.364, 7.683 ± 0.185, 0.736 ± 0.307 and 11.280 ± 4.78 to maximum13.443 ± 5.704, 18.923 ± 1.543, 8.233 ± 0.830, 1.063 ± 0.307, 16.280 ± 4.68 values, respectively and micro minerals like Zn, Fe, and Cu (mg/100 g) were significantly (p<0.05) varied from minimum 8.526 ± 2.418, 88.773 ± 17.205 and 0.296 ± .125 to maximum 11.310 ± 2.674, 104.303 ± 16.109 and 0.403 ± 0.096 values, respectively. The anti-nutritional content like condensed tannin, phytate, mucilage, oxalate, lectin, protease inhibitor and alpha (α)-amylase inhibitor were determined and their inhibitory effects were reduced in flour samples pre-gelatinized without peel. From the general trend observed in this study most of the analyzed samples that boloso-1 taro variety subjected to pre-gelatinization without peel provided better proximate (except CF%), mineral, functional and lower anti-nutritional content than that of the local taro variety.

Keywords: Anti-nutritional factors; Boloso-1; Taro starch; Pre-gelatinization and proximate composition

Introduction

Taro (Colocasia esculenta L.) a family of Aracea is cultivated for its edible corms used as a subsistence food by millions of people in developing countries [1]. Root and tuber crops are second only in importance to cereals as a global source of carbohydrates. The corm of taro contains more than twice the carbohydrate content of potatoes and yield 135 kcals per 100 g and 11% crude protein (CP) on a dry matter (DM) basis. These reported values are even higher than other root crops like yam, cassava or sweet potato that have a carbohydrate(85%) and CP (11%) content respectively [2]. It contains 85-87% starch on DM basis with small granules size of 3-18 μm and other nutrients such as zinc, vitamin C, thiamin, riboflavin and niacin better than other cereals [3]. Thought, protein and fat content of taro are low but it is high in carbohydrates, fibre and minerals.

Due to lack of appropriate storage and post-harvest handling technologies preserving crops for longer period is very difficult and most rural peoples suffer from malnutrition. For instance, zinc deficiency is currently widespread and affects the health and well-being of populations of the developing countries worldwide and since taro is one of the few non-animal sources of zinc, its utilization should therefore be pursued to help in the alleviation of zinc deficiency which is associated to stunting of children. Characterization of such less domesticated and unexploited food sources from the rural sources is essential to increase food choices and habits that support rural food security. In Ethiopia, root crops are widely grown in the southern part of the country.

In Ethiopia, root crops are widely grown in the southern part of the country. Among the root crops, taro is one of the most important food crop as well as income source to the farmers. Farmers produce taro for their own consumption and considers difficult to store and sometimes wasteful because of spoilage and the demand for taro in urban areas has not gained attention and neglected yet. However, it has a great potential to supply high quality food and one of the cheapest source of energy. Even though taro has many nutritional, economic and health attributes, it has also some anti-nutritional compounds that are harmful to health. Because of these inherent factors of anti-nutritional compounds, taro demand is often unsatisfied which impose serious neglecting in both rural and urban consumers.

Generally, taro is the underutilized root crop in Ethiopia [4]. Underutilized plants when properly brought under use can be goodness to malnutrition. People are eating taro corm as boiled, but there is no awareness about various processing methods that increase the quality and safety of the corm. Njintang et al. [5] stated that taro has not

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Paper II

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PROXIMATE, MINERAL AND ANTI-NUTRITIONAL COMPOSITION OF TARO (*COLOCASIA ESCULENTA* L.) LEAF GROWN IN ETHIOPIA

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ABSTRACT: The objective of this study was designed to investigate the nutritive and non- nutritive constituents of taro leaf powder and leaf curd extracts through improved processing methods. The study was conducted using factorial combination of two independent variables (taro leaf varieties and processing methods) with three replications in a completely randomized design (CRD). The analysis undertaken in this study was leaf proximate composition, mineral concentration and anti-nutritional factors of samples processed as leaf powder and curd concentrates between local taro and boloso-1 taro varieties. The moisture content in the study ranged from 3.444 ± 0.192 to 3.733 ± 0.693, crude protein from 25.061 ± 1.297 to 26.252 ± 1.530, crude fat from 5.835 ± 0.169 to 6.436 ± 0.889, fiber from 3.192 ± 1.988 to 4.597 ± 1.396, total ash from 2.706 ± 0.074 to 2.876 ± 0.230, total carbohydrate from 56.221 ± 2.743 to 59.383 ± 2.942, amylose contents from 5.319 ± 1.159 to 6.276 ± 0.987, beta-carotene (mg/100 g) 2.703 ± 0.225 to 2.480 ± 0.205 and phenolic compound(mg/100 g) from 0.957 ± 0.087 to 1.113 ± 0.188. The five macro minerals, mg/100 g of Na, K, Mg, P and Ca in the study differ significantly ($p<0.05$) and ranged from lowest (6.607 ± 1.055, 14.333 ± 1.470, 8.293 ± 1.899, 1.179 ± 0.81 and 15.265 ± 6.211 respectively) to highest (7.826 ± 1.827, 17.017 ± 3.4, 9.744 ± 1.908, 1.904 ± 0.995 and 19.547 ± 6.69) value, respectively. Concerning micro minerals like Zn, Fe, and Cu contents, mg/100 g were significantly ($p<0.05$); varied from minimum (9.647 ± 4.550, 40.374 ± 9.831 and 0.415 ± 0.194) to maximum (12.750 ± 1.811, 51.868 ± 17.025 and 0.580 ± 0.221) values, for boloso-1 taro variety, respectively. From the general trend observed in this study, most of the analyzed samples that boloso-1 taro variety subjected to curd processing provided better proximate, mineral, functional and lower anti-nutritional contents than that of the local taro leaf.

Key words: Boloso-1 taro, curd extract, leaf powder and composition

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Paper III

American Journal of
Food Sciences and Nutrition
(AJFSN)

AMINO ACID AND FATTY ACID COMPOSITION OF ETHIOPIAN TARO

Melese Temesgen, Negussie Retta and Etalem Tesfaye

AJP

Appendix B: Sensory Evaluation Sheet

Acceptability Test Score Sheet

Name ---------------------------- Age----- Sex ---- Date ---------------- Product Code-----------

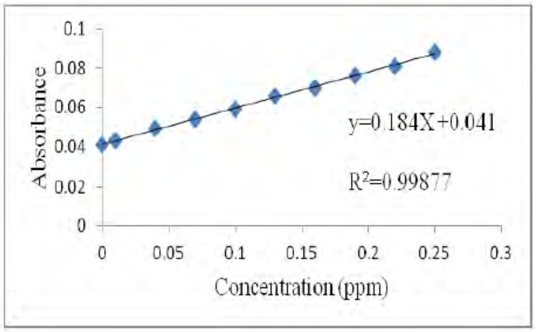
You are given servings of porridge to test and express your degree of liking of each sensory attributes provided. Test each sample and indicate your response by marking (x) in the corresponding acceptances. Please rinse your mouth between each taste to remove all traces of the previous sample.

Degree of acceptance	Scale	Sensory Attribute			
		Color	Flavor	Taste	Overall Acceptability
Like extremely	7				
Like very much	6				
Like moderately	5				
Neither like nor dislike	4				
Dislike Moderately	3				
Dis like very much	2				
Dislike Extremely	1				

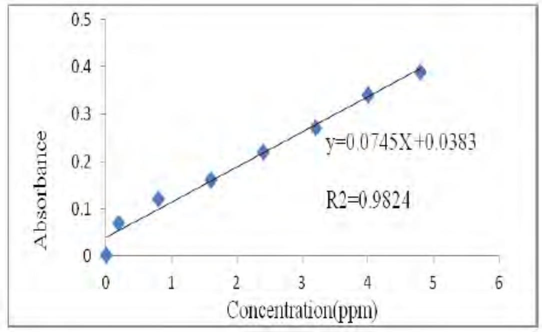
How do you get the product, if it provide for infants?

7. I agree very much	7
6. I agree medium	6
5 . I agree slight	5
4 . Neither agree nor disagree	4
3 . I disagree slight	3
2 . I disagree medium	2
1 . I disagree very much	1

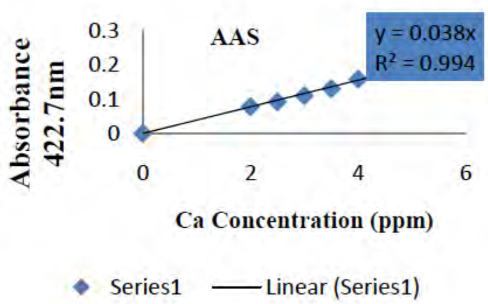
Appendix C: Figures



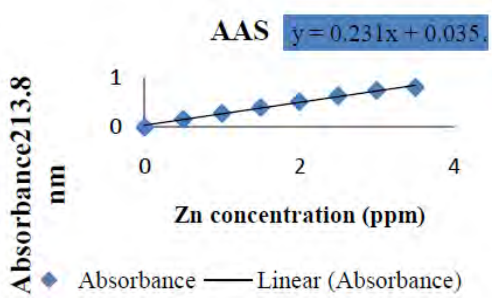
Calibration curve for amylase



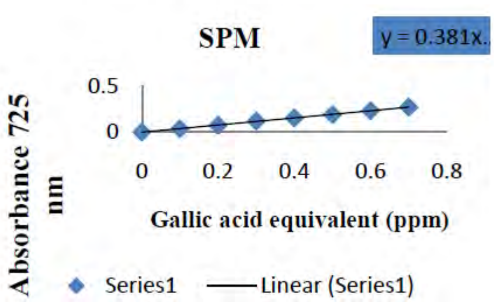
Calibration curve for P



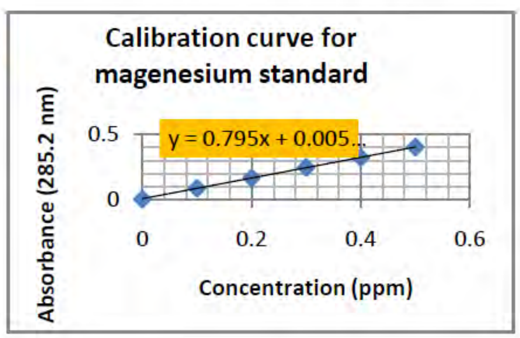
Calibration curve for Ca



Calibration curve for Zn

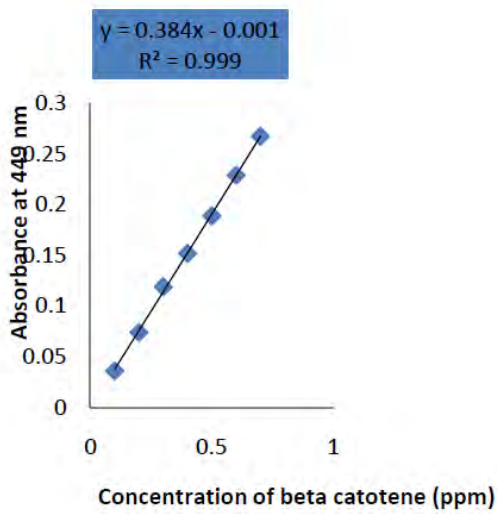


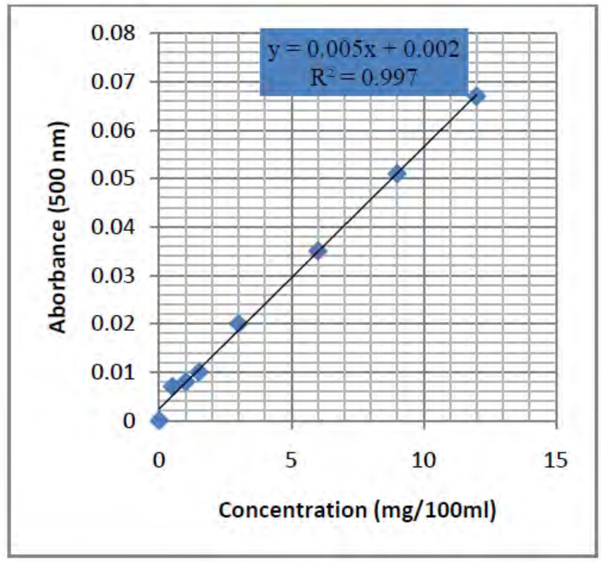
Calibration curve for Fe



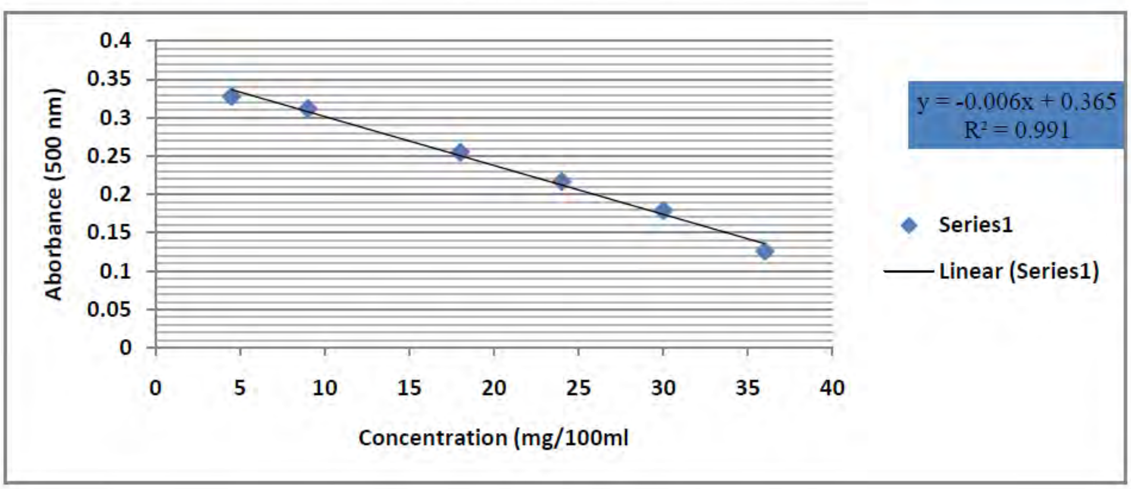
Calibration curve for Mg

Figure 1: Calibration curves for amylase, P, Ca, Zn, Fe and Mg



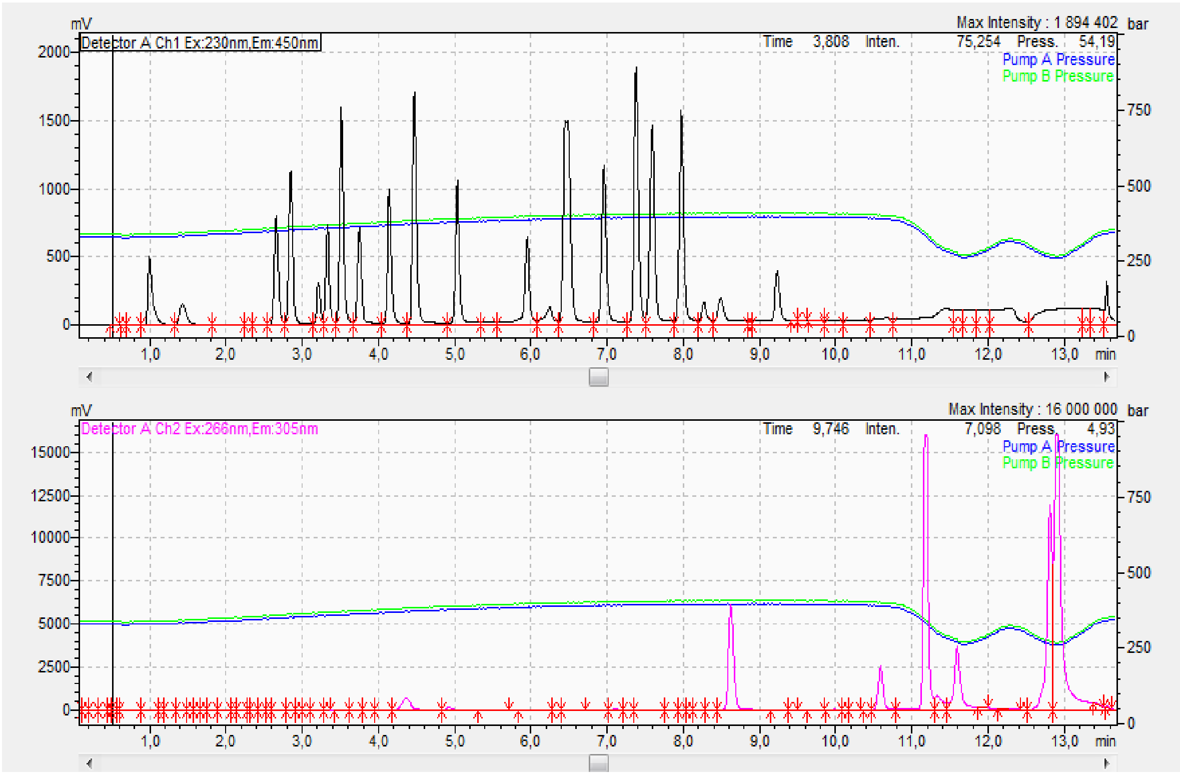


Calibration curve for beta-carotene   Calibration curve for Tannin

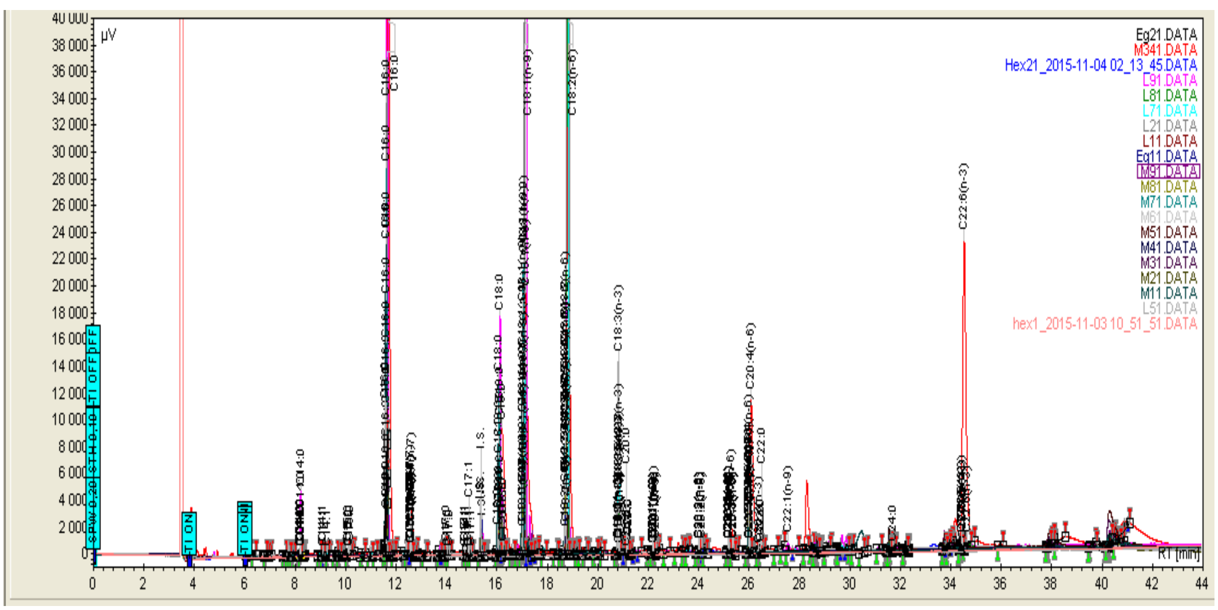


Calibration curve for phytic acid

Figure 2: Calibration curves for Fe, Mg, beta-carotene, tannin and phytic acid



(a)



(b)

Figure 3: Chromatograms for amino acid (a) and fatty acid profiling (b)


(a)Taro leaf samples

(b) Transporting polyethylene bag


(c)Leaf cutting into pieces

(d) Taro corm samples in pics bag


(e) Peeled corm soaking in hot water

(f) Corm cooking


(g) Mucilage inhibitor analysis

(h) Taro flour processing community group

Figure 4: Taro leaf and corm sample, preparation, laboratory works and taro flour processing group