Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

Effects of Boiling and Fermentation on Biochemical Composition, Physicochemical and Functional Properties of Wild Edible Pagana (*Amorphophallus gomboczianus*) in Konso Area

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

By:

Sisay Tolla

Advisors: Kelbesa Urga (Ass.prof)

Getachew Addis (PhD)

June/2017

Addis Ababa, Ethiopia

DECLARATION

I, the undersigned, declare that this is original work and has never been presented in this or any other university and that all the source materials used for this thesis have been duly acknowledged ;

Name; Sisay Tolla

Signature:_____

Place, Addis Ababa University, Addis Ababa, Ethiopia

Date of submission: June, 2017

The research proposal has been submitted for examination with any approval as university Advisor.

Name: Kelbessa Urga (Assoc. prof.), Name: Getachew Addis (PhD)

Signature:_____ Signature_____

Date: _____ Date _____

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Dedication

This work is fully dedicated to my Mother:

Shawaye Abaire

For everlasting and true love she had for me

My today’s success is her last work

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List of Abbreviations

AAS	Atomic Absorption Spectrum
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemist
ATP	Adenosine Triphosphate
$CaCl_2$	Calcium Chloride
CHO	Carbohydrate
EU	European Union
FAO	Food and Agricultural Organization for United Nation
FDA	Food and Drug Administration
FSA	Food Safety Authority
HCl	Hydrogen Chloride
HCN	Hydrogen cyanide
KGM	Konjac Glucomanan
LSD	Least Significance Difference
m.a.s.l	Meter Above Sea Level
OAC	Oil Absorption Capacity
SNNPR	Southern Nation, Nationalities and Peoples Region
TCM	Traditional China Medicine
UK	United Kingdom
US	United state
USDA	United State Department of Agriculture
WAC	Water Absorption Capacity
WHO	World Health Organization

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Abstract

Pagana is an endemic tuber plant of Ethiopia categorized under Araceae family .In this study effect of traditional processing, natural fermentation and boiling on biochemical composition, antioxidants, physicochemical and functional properties of wild edible paganna (Amorphophallus gomboczianus) collected from Konso, southern part of Ethiopia were investigated on dry basis. The moisture content, ash, crude protein ,crude fat , crude fiber total carbohydrate and energy of raw Pagana flour were 7%, 6.46%, 7.7%, 0.49%, 2.71%, 82.62% and 365.99%Kcal/100g respectively for raw sample. Minerals of raw were (2443.66, 17.56, 260.54, 7.7, 49.14, 1.02, and 3.1)mg/100g to K, Na, Ca, Fe, Mg, Mn and Zn respectively.The antinutritional factors, level of oxalate phytate and tannin were,39.15mg/100g, 2.83mg/100g, 1.64mg/100g respectively for the raw sample. Among the traditional processing methods used, fermentation had significant effect ($p<0.05$) on reducing antinutritional factors by 65.51%, 6.36%, 34.75%, for oxalate, phytate and tannin respectively. The total phenolics extract by methanol were determined using Folin-Ciocalteu methods while total flavonoid content was estimated by using aluminium trichloride($AlCl_3$), Free radical scavenging activity was determined by 2,2-diphenylpicryl-1-picrylhydrazyl activity using ascorbic acid as standards. Fermentation significantly ($p<0.05$) increases the total phenolics and total flavonoid from 67.12 to 620.42) mg Gallic acid equivalent/g and (32.29 to 42.18) mg D-Catechin equivalent/g respectively. Boiling increased the total flavonoid and reduced total phenolic amount from the raw Pagana flour. Both processing methods had also significant effect on physicochemical and functional properties of Paganna.

***Keywords**: Boiling, Fermentation antinutritional factors, physicochemical, functional property, antioxidant, Amorphophallus gomboczianus, oxalate*

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Tubers and roots are important sources of carbohydrates and serve as an energy source and are used as staple foods in tropical and sub tropical countries. These products have nutritionally beneficial components, such as a resistant starch and mucilage. Resistant starch has been attributed with a slow digestion in the lower parts of the human gastrointestinal tract which slows the liberation and absorption of glucose helping in the reduction of the risk of obesity, diabetes and other diet related diseases [1]. Root and tuber produce large quantities of dietary energy and have stable yields under conditions in which other crops may fail. Root and tubers produce remarkable quantities of energy per day, even in comparison to cereals. In addition, some roots and tubers are an important source of vitamins, minerals, and essential amino acids such, as lysine. The, Root and tuber crops play multi-purpose roles in the global food system as a starch source. These are recommended crops for food security, source of cash income, raw material for feed and processed products, and as key components in small-scale agro-enterprise development. Pagana is one of these tuber plants found in, Southern nations, and nationalities peoples region (SNNP) of Ethiopia especially around Segen zone Konso woreda. Pagana is the local name given by Konso societies in Konsogna language for *Amorphophallus gomboczianus*. It is which is a tuber crop, belongs to the genus *Amorphophallus,* family *Arecea* [2]. It is a large genus of some 200 species of tropical and subtropical tuberous herbaceous plants. Native to Asia, Africa, Australia and various oceanic Islands [3].Pagana is one of the genus *Amorphophallus that* grows wildly in southern Ethiopia at altitudes ranging from 900 to1200 m.a.s.l. It is drought tolerant and the tubers are edible particularly during times of food shortages [4]. These small to massive plants grow from a subterranean tuber. *Amorphophallus* tubers vary greatly from species to species, from the quite uniformly globose tuber of *A. konjac* to the elongated tubers of *A. longituberosus* and *A. macrorhizus* to the bizarre clustered rootstock of *A. coaetaneus*[2]. *Amorphophallus gomboczianus* tuber (aroid), which is known for its burning and irritating sensations ,the burning and irritating of Pagana

(*Amorphophallus gomboczianus*) is due to high content of oxalate in the tuber [2].The semi-managed wild food plant Pagana, producing edible tubers, was the most frequently mentioned (by more than 90% of the interviewees). It was also by far the most abundant wild food plant in Konso, mainly in agro-environments[5].The Konso communities use it as an insurance against hunger because of its good storability in the ground and it is self-proliferating. Pagana is normally consumed in association with other (staple) food items [5]. Kurkufa is one of the Konso traditional food prepared from mixing Pagana flour with another crops. Additionally Pagana is also used to prepare traditional beverage 'Chaqa'.

1.2. Statement of the problem:

One major limiting factor in the utilization of root crops is the presence of antinutritional factors, which may have adverse effect on health through inhibition of digestion, absorption, and growth. Root crops, in common with most plants, contain toxins and antinutritional factors [6]. Antinutritional factors accumulate in seeds, stems, leaves, and tubers of plant [7]. Generally. There are two mechanisms that act as toxicants in human body, the bioavailability of the nutrients in food is affected by some naturally occurring chemical compounds present in it. These compounds include plant phenolics, dietary fiber, phytates, and oxalates. The bioavailability of minerals, vitamins and proteins are limited by dietary fiber. Plant phenolics bind with proteins and make them unavailable for digestion [7]. The presence of chemicals in plant sources decreases the absorption and utilization of one or the other nutrient [6].Taro contains large amount of Phytate (855mg/100g) [6]. Cassava, cocoyam and yam contain 624 mg, 855 mg and 637 mg of phytate per 100 g respectively [8]. Abdulrashid and Agwunobi 2009, reported oxalates, phytates and tannins are the antinutritional factors found in taro. The high content of calcium oxalate crystals, about 780mg per 100 g in some species of cocoyam (*Colocasia Esculenta*), *Colocasia* and *Xanthosoma,* has been implicated in the acridity or irritation [9].Oxalic acid forms water soluble salts with Na^+, K^+, and NH_4^+ ions. It also binds with Ca^{2+}, Fe^{2+} and Mg^{2+} rendering these minerals available to animals [10].Phytate reduces protein and mineral bioavailability [11].Tannins reduce protein digestibility and adversely influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption [12].Understanding the level of antinutritional factors in Pagana and processing methods that

are effective in reducing these factors may significantly contribute in reducing health risk that were associated with consumption of Pagana.

1.3. Significance of the study

- Provide information about the effect of processing on physicochemical composition, and antinutritional factors of pagana.
- Providing use of underutilized edible plants that adapted to marginal agro climatic conditions.
- Reducing healthy disorder caused by consumption of anti nutritional factors
- Add variety to the agro-processing industry.
- This study provide baseline data for further studies that can be conducted in such type of tuber plants.

1.4. Objective:

1.4.1. General objective:

To study the effects of traditional processing methods on nutritional compositions, anti-nutritional factors, antioxidant activities and physicochemical and functional properties of Pagana.

1.4.2. Specific objective:

- Evaluation of the nutritional composition of Pagana upon traditional processing methods.
- To evaluate the effect of traditional processing methods (boiling, and natural fermentation) on antinutritional factors on Pagana
- To evaluate the effect of the traditional processing methods on minerals composition of Pagana.
- To evaluate the impacts of boiling and fermentation on antioxidant capacity of Pagana

- To evaluate the physicochemical and functional properties of Pagana up on traditional processing methods.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Origin and distribution of *Amorphophallus*

Amorphophallus belongs to the family *Araceae,* reported that there are 200 species of the genus *Amorphophallus* in the world [2, 3]. Of these, *A. gomboczianus* is the species grown for its edible tuberous roots. It is well known and eaten in Ethiopia in Southern Nations, Nationalities and Peoples Region (SNNPR),Konsodistrict.Though there is wide consumption of Pagana in SNNPR as per our literature survey ,there is no detailed study regarding its biochemical composition, physicochemical and functional properties in Ethiopia.The only study on proximate composition of Pagana well reported by [2]. Therefore further study is needed on Pagana species found in Ethiopia. *Amorphophallus konjac* and *Amorphophallus gomboczianus* are categorized under the same family and genera [3]. Due to similarities in family and genera the properties and importance of *Amorphophallus gomboczianus* is might be the same with *Amorphophallus konjac,* from the current study results there is similarities with protein content and percent of ash between the two species. *Amorphophallus konjac* in Asia, plants of the genus *Amorphophallus* has a long history of use in tropical and subtropical Asia as a food source and as a traditional Chinese medicine [13]. They are perennial plants with an underground stem in the form of a corm and a highly dissected umbrella-shaped leaf blade [3].One of the best known is *Amorphophallus konjac* which has been cultivated in China for more than 2000 years .It is highly related with *Amorphophallus gomboczianus*. Whole corm extracts of this species have been used as a TCM for the treatment of asthma, cough, hernias, breast pain, burns and skin disorders [14]. Moreover, the corm tissues are known to be a valuable source of glucomannan, a soluble, non-cellulosic polysaccharide [15].Traditionally, this polysaccharide (which has no calorific content) is extracted from corm tissues and is used to produce flour from which foodstuffs (e.g. noodles) are prepared [16].In addition to its use to prepare functional foods, a purified version of this flour may also have

potential as a nutraceutical, or pharmaceutical product for use in the treatment of obesity[17], obesity-related dyslipidemia diabetes, hyperlipidaemia and hypercholesterolemia[18], and diabetes, in countries where these are increasingly important medical problems, such as the UK. Even though there was no investigation on nutraceutical or pharmaceutical information on Pagana there is an indication of having pharmaceutical value, because traditionally Konso people used it as a relief of blood pressure [19].

Despite the fact that konjac is a widely used foodstuff and traditional medicine in the Orient, consumption in the West is relatively limited.A major challenge to the global usage of this versatile and useful foodstuff/nutraceutical is a lack of detailed information on the productivity, biosynthesis and analysis/quality assurance for both cost-effective production and safe use within the appropriate regulatory frameworks (US, UK or EU), for the various products to be brought to market. To date, the majority of literature regarding konjac glucomannan (KGM) has focused on the structural, physicochemical and pharmacological properties of this substance and to a lesser extent on the morphogenesis, cultivation, molecular biology of the crop itself and the processing chemistry of KGM. Therefore, a more detailed understanding of the science which underpins the use of konjac from cultivation to the commercialization of finished products as well as corm productivity, accumulation and metabolism of KGM is essential for further improvement of the commercial potential of this crop. This information show that further study of Pagana is very essential because the physical structure and chemical composition of Pagana is highly related with *Amorphophallus konjac*.

2.2. Botanical background

The genus *Amorphophallus* belongs to the family Araceae (Aroids), subfamily Aroideae and tribe Thomsonieae. It comprises of approximately 200 herbaceous species and is distributed in both tropical and subtropical regions of the palaeotropics, including Africa, Madagascar, India, continental South East Asia, Melanesia and North East Australia [3].They are lowland plants that grow in tropical and seasonal forest margins, in open woodlands and in humus deposits on limestone areas [20, 21]. As well as having beautifully patterned petioles and fine floral details, they also produce some of the most fascinating inflorescences in the family (Figure 2.1). The inflorescence of *Amorphophallus titanum* from Western Sumatra is one of the largest known inflorescences, reaching 3 m or more in height [22].

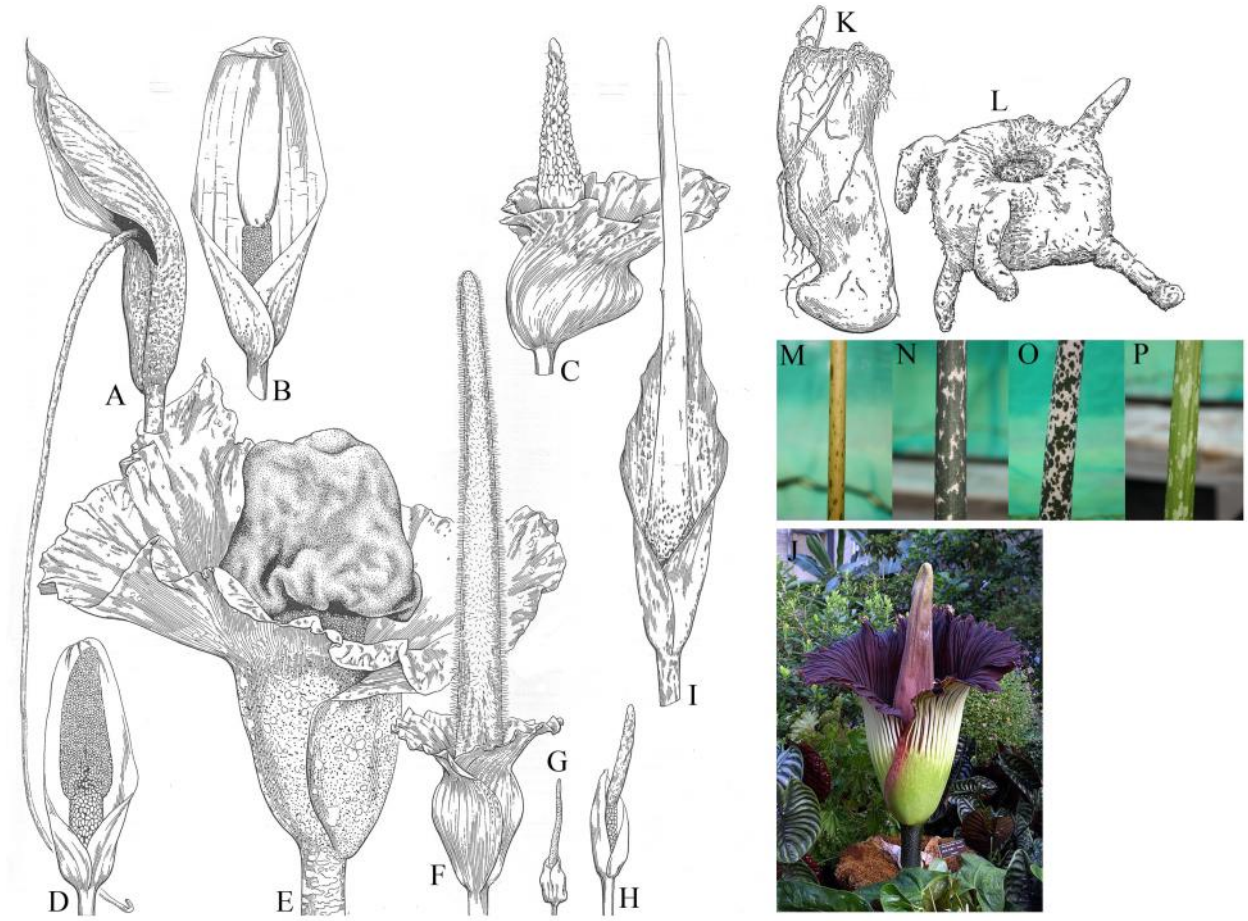


Figure 2.1 Different types of inflorescences (A - J), tubers (K, L) and petioles (M- P) of *Amorphophallus* species (A - I, K, L) , key: A= *Amorphophallus*

(A) *A. krausei*
(B) *A. albus*
(C) *A. sumawongii*
(D) *A. paeoniifolius*
(E) *A. hirtus*
(F) *A. pusillus*
(G) *A.pygmaeus*
(H) *A. gomboczianus*
(I) A. konjac
(J) A. titanum
(L) Globs corm
(M) A. albus
(*N) A. konjac*
(O) A.krausei
(P) A. paeoniifolius

2.3. Growth and development of pagana plant

Pagana grows wild in southern Ethiopia at altitudes ranging from 900 to1200 m.a.s.l. It is drought tolerant and the tubers are herbaceous, perennial monocotyledon, growing from an underground stem in the form of a corm. With similar genera *Konjac* grows well in shady environments [22] with free draining, humus-rich soils of pH 6.5 - 7.5 [3,23] .It is one of the hardiest *Amorphophallus* species and can grow in seasonal temperatures ranging between 5 and 43 °C, with an optimum temperature range of 20-25°C[3,24].With the same environmental condition and type of soil, Pagana grow in southern part of Ethiopia [4]. The shade-loving nature and tolerance to higher temperatures of this species are believed to be related to its original habitat, which is thought to have been the floor of tropical rain forests in South East Asia[24].Except for a few evergreen species (e.g. *A. arnautovii* and *A.pingbianensis*),all *Amorphophallus* species have a distinct dormancy period, which affects the planting and harvesting cycle[3,25].Most often, konjac is planted in spring (March/April) and attains maturity after 6 - 7 months (October/November). During this time, the foliage dies back and the plant over winters as a dormant corm for about 6 months, before reemerging the following spring [23] [26].The minimum temperature required to break dormancy is 14 °C. In temperate climates when this temperature is reached (during the spring), the shoot apical meristem is activated, followed by leaf bud emergence which usually takes place in May [27].

2.4. Traditional and contemporary uses of pagana corms

Figure 2.2. Pagana raw flour

Figure 2.3 Kurkufa staple food prepared from pagana

The semi-managed wild food plant Pagana, producing edible tubers, was the most frequently mentioned (by more than 90% of the interviewees). It was also by far the most abundant wild food plant in Konso, mainly in agro-environments. The Konso communities use it as an insurance against hunger because of its good storability in the ground and because it is self-proliferating, according to the interviews of local people of Konso. Pagana is prepared, first washing, chopping and drying on sun, after well dried they milled and mixed with other food to be consumed in association with other staple food items. Kurkufa is one of the staple foods prepared from Pagana and eaten in Konso.

The irritability and burning sensation of paganna is increased as it ripped. Relatively with the same genera Konjac was first listed as a Chinese medicinal herb in the "*Shen Nong Materia Medica*" as early as 206 B.C. during the Western Han Dynasty. According to the ancient Chinese pharmacopoeia, "Ben Cao Gang Mu", konjac is a toxic, pungent and cold-natured herb [28].Traditionally, corms are washed, peeled, sliced, dried and ground to produce konjac flour which is consumed in the form of cake (or gel) after boiling the flour with plant ash. In TCM, the therapeutic effects of extracts from konjac corms have partially been described to its pungent and toxic principles with the functions of detoxification, tumour-suppression, blood stasis alleviation and phlegm liquefaction [29].For more than 2000 years, konjac gel has

been used for the treatment of asthma, coughs, hernias, breast pain, burns as well as hematological and skin disorders. *Konjac* flour has also been consumed as a functional food in the form of noodles, tofu and snacks or as konjac curd, which is tasteless and is usually braised with meat in traditional Chinese cuisine [16][30]. In addition to the use of the flour derived from the corm, the leaves of the konjac plant are used as a natural insect repellent and as animal fodder by the indigenous people in Southern China [16]. In Japanese cuisine, konjac flour is pounded with lime and water into a gelatinous grey cake ("konnyaku"), a key ingredient in Japanese noodles ("shirataki") and cuisines such as "sukiyaki" and "gyudon" [26].Outside Asia, *konjac* is grown as an ornamental due to its beautiful compound foliage and marbled petioles [23].

Much of the recent interest in the use of konjac stems from its potential use as a dietary fiber. The term dietary fiber, classified as soluble or insoluble, is described as the endogenous component of plant material in the diet that is resistant to human digestive enzymes[31].The main biologically active constituent in the konjac corms is a soluble fibre which primarily consists of the non-cellulosic polysaccharide, glucomannan [15][32].As β-1,4 linkages of KGM cannot be hydrolysed by salivary and pancreatic amylase, KGM passes into the colon unchanged and is fermented by colonic bacteria [33].A highly purified form of KGM has been used in the treatment of obesity [34].Obesity-related dyslipidemia and diabetes by its action as a satiety agent [18][33]. It has been marketed as such in capsule form, as a drink mix and in food products in China and neighboring countries. The potential use of KGM as a probiotic and as an immunomodulator has also been suggested. In addition, the unique rheological and gelling properties of KGM are widely employed in emulsifier and stabilizer products for the food, drink, cosmetic and pharmaceutical industries [35]. Since 1994, KGM has been approved as a food additive by the U.S. Food and Drug Administration (FDA) [15]. In 1996, it was also passed as a binder in meat and poultry products by the U.S. Department of Agriculture (USDA).

2.5 Antinutritional factors in Pagana

Antinutritional factors have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals. Root crops, in common with most plants, contain small

amounts of potential toxins and antinutritional factors. Phytate, tannin, oxalate and cyanide are common antinutritional factors, which mostly occur in various root crops including Pagana [6].

2.5.1. Antinutritional effects of tannins

Tannins are water soluble phenolic compounds with a molecular weight greater than 500 daltons. They have the ability to precipitate proteins in aqueous solution [36][37].Tannins are secondary compounds of various chemical structures widely occurring in plant kingdom[38].They are defined as high-molecular-weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides. Tannins are generally divided into hydrolysable (glucose polyesters of gallic or hexahydroxydiphenic acids) and condensed tannins (proanthocyanidins) [39].Tannins readily form indigestible complexes with proteins and other macro-molecules under specific environmental conditions, up on Tannins can reduce protein digestibility and adversely influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption. Also carbohydrate is affected leading to reduced energy value of a diet containing tannins [12].Tannins also reduce the absorption of vitamin B_{12}. Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood stream and over a period of time cause toxicity to the organs (e.g liver and kidney) [37].Tannins may form a less digestive complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes.Tannin protein complexes involve both hydrogen bonding and hydrophobic interactions. The precipitation of the protein-tannin complex depends upon pH, ionic strength and molecular size of tannins. Both the protein precipitation and incorporation of tannin phenolics into the precipitate increase with increase in molecular size of tannins [38].However, when the molecular weight exceeds 5,000 daltons, the tannins become insoluble and lose their protein precipitating capacity and degree of polymerization becomes imperative to assess the role of tannins in ruminant. Tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Reported that condensed tannins were responsible for the testabloat trypsin inhibitor activity of faba beans [32]. Tannins also have the ability to complex with vitamin B. Other adverse nutritional effects of tannins have been reported to include intestinal damage, interference with iron absorption and

the possibility of tannins producing a carcinogenic effect [40].The total acceptable tannin daily intake for human being is 560mg/100g [41]. Reported a tannin concentration in a range of 47.69 - 59.92mg/100g and (Akpan and Umoh 2004) obtained a tannin concentration of 640mg/100g for raw samples of taro [42][43]. Cassava also found to contain a small amount of tannin vary from 3.6 to 6.9mg/100g [44].

2.5.2. Antinutritional effects of oxalate

Oxalic acid (or its salts) is widely distributed in the plant kingdom although its nutritional significance is limited to relatively few plants and forages [44]. Ingestion of foods containing oxalates has been reported to cause caustic effects, irritation to the intestinal tract and absorptive poisoning. Oxalic acid forms water soluble salts with Na^{+}, K^{+} and $NH4^{+}$ ions it also binds with $Ca^{2+,}$ Fe^{2+} and Mg^{2+} rendering these minerals unavailable to animals. However Zn^{2+} appears to be relatively unaffected. In plants with a cell sap of approximately pH 2, such as some species of *Oxalis* and *Rumex* oxalate exists as the acid oxalate (HC_2O_4), primarily as acid potassium oxalate. In plants with a cell sap of approximately pH 6, such as some plants of goosefoot family it exists as oxalate $(C_2O_4)^{2-}$ ion usually as soluble sodium oxalate and insoluble calcium and magnesium oxalates. Calcium oxalate is insoluble at a neutral or alkaline pH, but freely dissolves in acid [10].The high content of calcium oxalate crystals, about 780 mg per 100 g in some species of cocoyam, *Colocasia and Xanthosoma*, has been implicated in the acridity or irritation caused by cocoyam [9].The acridity of high oxalate cultivars of cocoyam can be reduced by peeling, grating, soaking and fermenting during processing [9]. Cooking can affect the soluble oxalate but not the insoluble oxalate content of the food. Boiling can reduce the soluble oxalate content of a food if the cooking water is discarded, while soaking, germination and fermentation will also reduce the content of soluble oxalates [45].The lethal level of oxalate in man is 3-5g as reported and relatively high oxalate content was reported by 265.88mg/100g and 243.06mg/100g for raw taro samples of two different varieties [42]. The main toxic principle which occurs in varying amounts in all parts of the tuber plants are reduced by fermentation [46].

2.5.3. Antinutritional effects of phytate

Phytate is a salt form of phytic acid. Phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability [11].Phytic acid is reported to chelate metal ions such as calcium, magnesium, zinc, copper, iron and molybdenum to form insoluble complexes that are not readily absorbed from gastrointestinal tract. Phytic acid also inhibits the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase and amylase [11]. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Especially zinc and iron deficiencies were reported as a consequence of high phytate intake [47]. Phytic acid also decreases the availability of zinc, manganese, copper, molybdenum, calcium, magnesium, iron as well as protein [48].When bound to protein, it induces a decrease of solubility and functionality of the protein. Phytate lowers the bioavailability of certain minerals through formation of insoluble complexes at intestinal pH. [41].The raw and processed cassava, reported phytate content of 543.97mg/100g to 168.24mg/100g, respectively [43]. 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 150–1400mg for mixed diets [49].The phytic acid intake of 4-9mg/100g is said to decrease iron absorption by 4-5 folds in humans [49].

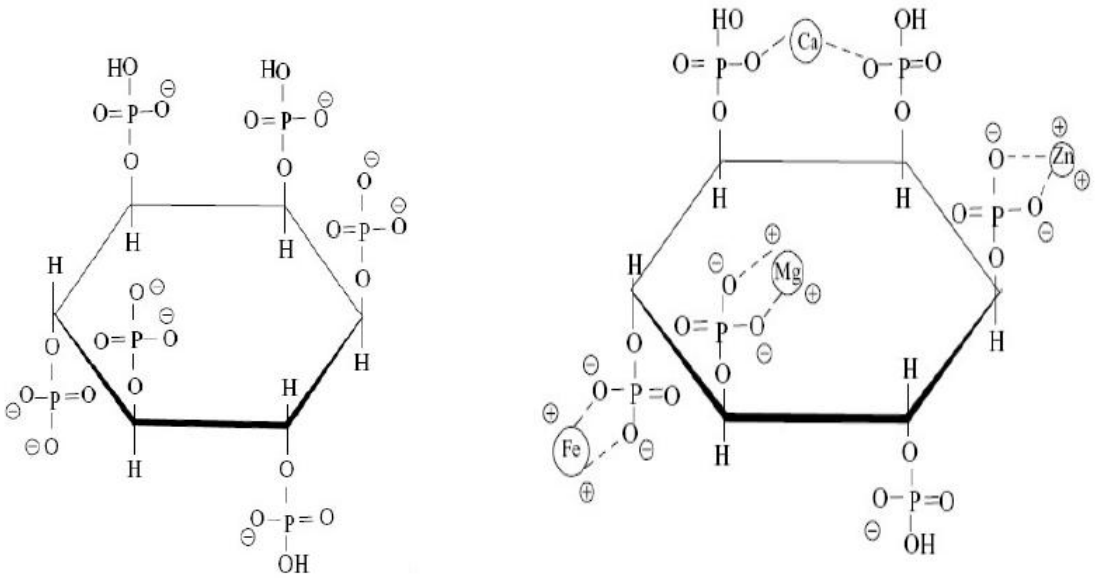


Figure 2.4: Molecular structure of phytate at neutral p^{H} and phytate chelating at neutral p^{H}

2.6. Processing methods used to reduce antinutritional factors

Processing methods are critical for the utilization of nutrients in food sources and to reduce toxicity due to antinutritional factors. Different processing methods or conditions are reported to eliminate or minimize antinutrients in root crops. However, more research is needed to evaluate the potential nutritional advantages of the new crops on the basis of agronomic and morphological characteristics as well as to determine acceptable threshold levels of each of antinutrients and to make best use of their nutritional values. Reduction or inactivation of antinutritional factors through processing requires knowledge of the type, distribution, chemical reactivity and thermal sensitivity of these factors within the seed matrix and complete knowledge of process technologies.

2.6.1 Boiling

Boiling/cooking and roasting are important food processing methods. As a thermal process, boiling/cooking could enhance the palatability and nutritional value by inactivating endogenous toxic factors. Roasting is similar to cooking/boiling but involves higher temperature and reduced time. Boiling is effective method in reducing water soluble antinutrients. For example boiling of root crops such as taro and cassava will lead to significant reduction of oxalates and cyanide [50].Boiling is also found to decrease some amount of soluble phytate. Cooking of cassava can, efficiently carried out, reduce cyanide content to non-toxic levels. Since boiling needs energy it is not economical method as other processing methods such as natural fermentation for poor rural community.

2.6.2. Fermentation

Fermentation also is a very interesting process used in plant foods to increase the nutritional quality and remove undesirable compounds. Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fibre digestibility. It also enhances micronutrient bioavailability and aids in degrading antinutritional factors [51].Over the centuries, fermentation has evolved and been refined and diversified. Today, a variety of food products are derived from this technology in

households, small-scale food industries as well as in large enterprises. Furthermore, fermentation is an affordable food preservation technology and of economic importance to developing countries. It enhances the nutritional quality of foods and contributes to food safety particularly under conditions where refrigeration or other foods processing facilities are not available [52].Organic acids produced, such as acetic, lactic, citric, formic and butryic acids, during fermentation that potentiate zinc absorption by forming ligands with zinc. Microbial fermentation enhances zinc bioavailability through hydrolysis induced by microbial phytase enzymes Reduction of phytates in the diet could also favor enhanced absorption of other minerals like calcium and iron [46].

Fermentation reduces phytate content via the action of phytases that catalyze conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phytate [46].There are differences in optimal conditions for phytate degradation between plant species. Most cereal phytases have pH optima between 4·5 and 5·6, but pH optima of some legumes are neutral or alkaline. To optimize the food process for increased mineral bioavailability by phytate degradation, it is essential to know optimal conditions for the phytases, responsible for phytate degradation in the process [46].Hydrolysis of phytate during biological food processes and preparation such as fermentation is a result of activity of phytase enzymes, naturally synthesized by plants and many microorganisms. Phytases (InsP6-phosphohydrolases) are by definition enzymes able to hydrolyse InsP6 to InsP5 and inorganic phosphate (Pi). Typically, phytases are not specific for InsP6; leading to further hydrolysis to *myo*-inositol via intermediate *myo*-inositol phosphates (penta- to monophosphates). Phytases constitute a subgroup of the family of acid phosphatases. Those that exhibit the ability to hydrolyse InsP6 can be considered to be phytase [46].It is also reported that fermentation can reduce tannin content of foods. Reduction in tannin due to processing might have been caused by the activity of polyphenol oxidase or fermented microflora on tannins [53].

All over the world, fermented foods provide an important part of human diet. Fermented foods and beverages provide about 20-40% of human food supply. Traditional food fermentation is capable of improving nutrients of the food, preserve it by generating acidic condition, detoxify and reduce cooking time of the food [53].Fermentation is found to be useful in flavoring foods, in inhibiting spoilage bacteria and pathogens, in intestinal health and other health benefits related to blood cholesterol levels, immune competence and antibiotics

production. Lactic acid fermentation is inexpensive and often little or no heat is required during the process thus making it fuel efficient [54].Fermentation generally improves extractability of minerals, probably because of the decreased content of phytic acid in the fermented product [55].

2.7. Free radical and antioxidants activity

Natural antioxidants may act as free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen [56]. The antioxidants can interfere with the oxidation process by reacting with free radicals [57]. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [58].

2.8. Physicochemical and functional properties

2.8.1Physicochemical properties

2.8.1.1. pH of the flour

The acidity or alkalinity of a food is usually expressed as pH. It gives us information on; to what extent a certain food sample is acidified. The pH of a food can dramatically alter the growth of microbes in food and is a major determinant of the type of food preservation process used for that food. Yeasts and molds usually grow best between pH 4 and 6 and bacteria usually grow best at pH near 7. In selecting a food preservation process that makes a food shelf stable, the initial pH of that food must be considered to minimize the likelihood of bacterial growth in that food[59].

2.8.1.2. Titratable acidity

Titratible acidity measures the total amount of hydrogen ions available in the food and expressed as mg lactic acid eq/g of the food sample. Titratible acidity is different than total acidity, although at times both terms are used to mean the same thing. Total acidity is the total

amount of organic acids in the food sample. The titratible acidity of any food sample in the form of solution is an approximation of the solutions total acidity usually measured by reacting the acids present in the food sample with a base such as sodium hydroxide to the chosen end point close to neutrality, as indicated by an acid sensitive colour indicator [60].

2.9. Functional properties

Functional properties are very important in determining the level of utilization in ingredient formulation and new food product development [61]. Before consideration is given to tubers as potential sources of flour and starch to produce foods, it is necessary to characterize their chemical composition, physical, physicochemical, and functional properties [62]. The chemical composition of flours and starches exhibits differences especially in amylose and phosphorous content, as a function of the botanical origin. It is significant because of the influence of amylose and phosphorous content in the functional properties of flours and starches. It is a general consensus that the influence of both amylose and phosphorous content affects the gelatinization and pasting behavior of starches and flours. These two parameters determine the functional properties of flours and starches such as: texture, consistency, binding, coating, adhesiveness, cohesiveness, thickening, viscosity, and palatability [62].

2.9.1. Water and oil absorption capacity

The ability to absorb water is a very important property of flours used in food preparation. The ability of food materials to absorb water is sometimes attributed to the protein content [59]. Water absorption capacity is an important functional property required in food formulations especially those involving dough handling [63]. WAC plays a major role in the functionality of dough. In particular, WAC has been shown to be related to dough consistency [64]. It is known that water binding by starches and flours is a function of several parameters including size, shape, conformational characteristics, steric factors, hydrophilic hydrophobic balance in the starch molecule, lipids and carbohydrates associated with the proteins, thermodynamic properties of the system (energy of bonding, interfacial tension, etc.), physicochemical environment (pH, ionic strength, vapor pressure, temperature, presence/absence of surfactant etc.), solubility of starch molecules and others [65].

2.9.2. Bulk density

Bulk density gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness which is an important factor in convalescent child feeding [63].

2.9.3. Foam capacity and stability

Foaming property is very important to improve texture, consistency, and appearance of food; such as baked and confectionary goods. Foam ability or foaming power (capacity) corresponds to the ratio of gas volume to liquid volume in foams [66]. Foaming, the capacity of proteins to build stable foams with gas by forming impervious protein films, is an important property in some food applications, including beverages, as well as angel and sponge cakes. Stable foams are known to occur when low surface tension and high viscosity occur at the interface, forming a continuous cohesive film around the air vacuoles in the foam. Soluble proteins in general play an important role in the formation of foam and this probably justify why legumes exhibit higher foaming capacity [59].

2.9.4. Swelling power and solubility

Swelling power provides evidence of non-covalent bonding between starch molecules. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and confirmation determine the degree of swelling and solubility. Solubility of flours depends on a number of factors such as sucrose, interassociative forces, swelling power, presence of other factors, etc [67]

CHAPTER THREE

3. MATERIALS and METHODS

3.1. Materials

Polyethylene bags, cooking utensils, aluminum foil, ice box, stainless steel knives, tray and different laboratory equipments and chemicals were purchased and used in this study. Sample preparation was done at Addis Ababa University College of Natural and Computational sciences Center for Food Science and Nutrition laboratory. The proximate, mineral, antinutritional composition analysis, antioxidant activities, physicochemical and functional properties were carried out at the center using(Kahjal, Muffle furnace, AAS, Spectrophotometer model). Fiber, potassium and sodium analysis were done at Ethiopian public Health and Nutrition Research Institute.

3.1.1 The Study Area

This study was conducted in South Nations Nationalities people regional state, Segen zone Konso woreda, Jarso, Dogotu, Fasha ,Darre and Nalayasegen kebele (5015' to 5030' N, and 37015' to 37030' E) about 600 km south of Addis Ababa in the southern region of Ethiopia. The study area was located in the dry land part of the southern Ethiopian Rift Valley system and the adjoining areas.

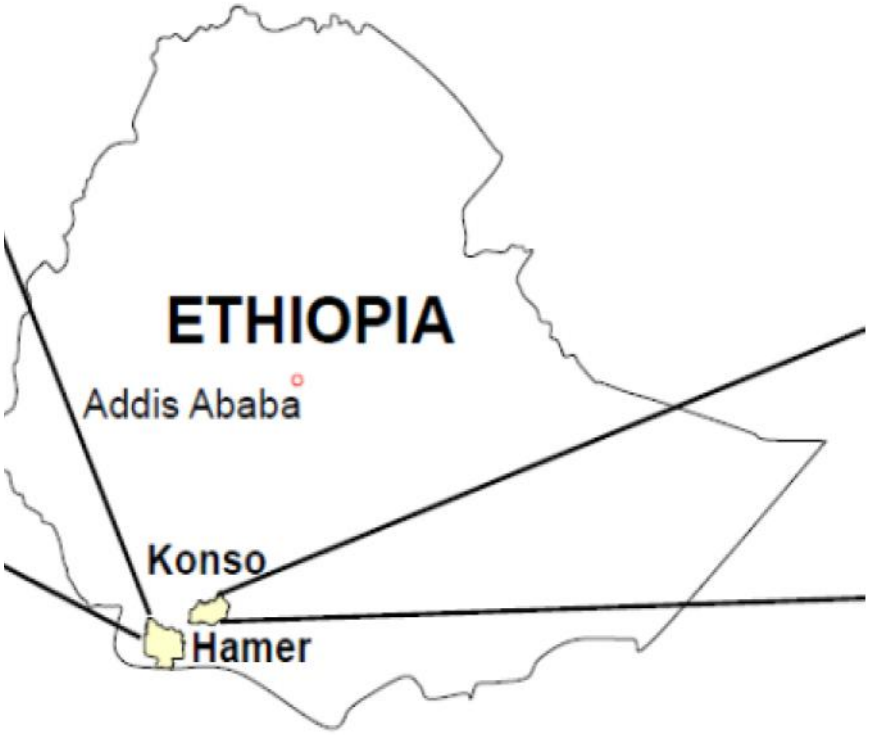


Figure 3.1 Map of sample area

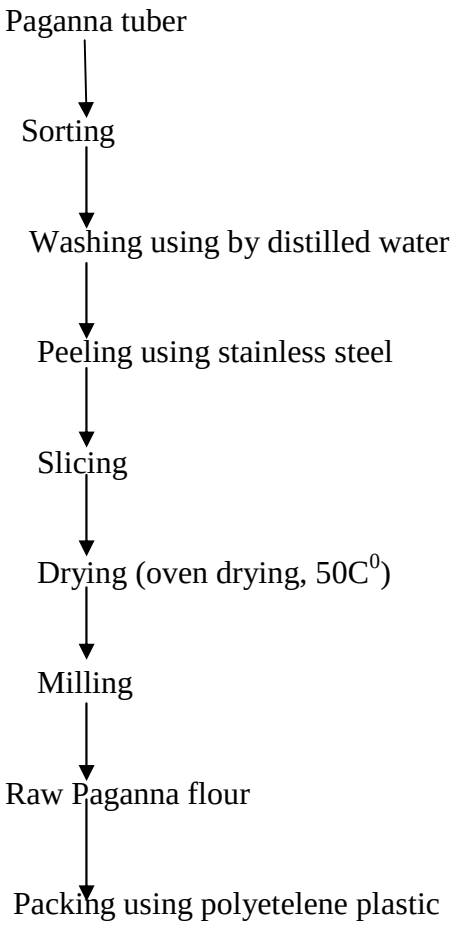
3.2. Sample collection

A total of about 15Kg samples was collected from Konso rural area of farmer association by the local participants that have a good experience and knowledge about the indigenous tuber paganna together with the investigator. All the samples were harvested within 2-6 months of planting (the maturation period of Pagana). The Pagana samples selected, contained large, middle and small corm (cormels) sizes that was not damaged during harvesting and which were not attacked by pests. The sample was kept in an ice box of about 5C ^{0} and transported to Center for Food Science and Nutrition Program laboratory of Addis Ababa University, after a day. Then the tubers were washed by distilled water all together. The washed tuber was grouped into two sections of nine kilograms for the first section and three kilograms for the second section. The first section was used for raw and boiled analysis, whereas the second section was for fermented analysis.

Figure 3.2 collection of Pagana by local people from Konso rural area

3.2.1. Flour preparation from raw Pagana tubers

The raw Pagana flour was prepared by the method described for taro flour preparation with some modifications [64]. All the samples were cleaned manually to remove foreign matters adhering to it and hand peeled carefully using stainless steel knives and the peeled Pagana was washed and sliced to 0.5cm thick slices. Then the slices were dried overnight in a hot air oven at 50c. The dried Pagana chips were milled using an electric miler and sieved to pass through 100 mesh sieve, packed, stored until analysis.



3.1. Flow diagram of preparing Paganna flour from corms

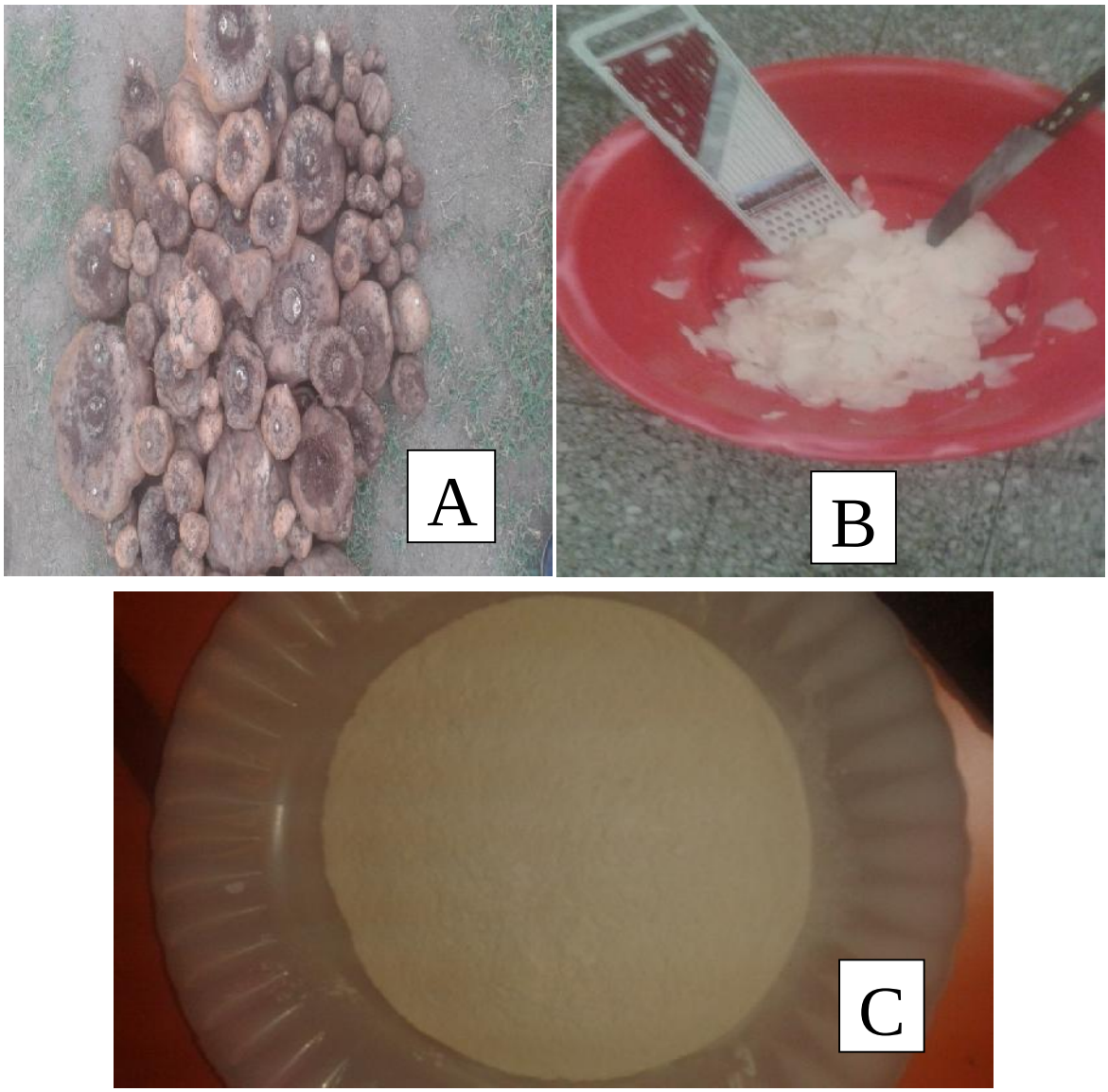
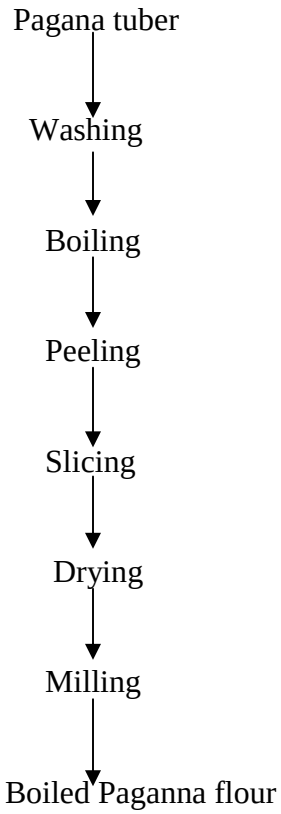


Figure 3.3 Preparation of flour from raw Pagana tubers

A. Washed Pagana tuber
B. Sliced pagana
C. Flour prepared from raw Pagana

3.2.2. Flour preparation from boiled of paganna tuber

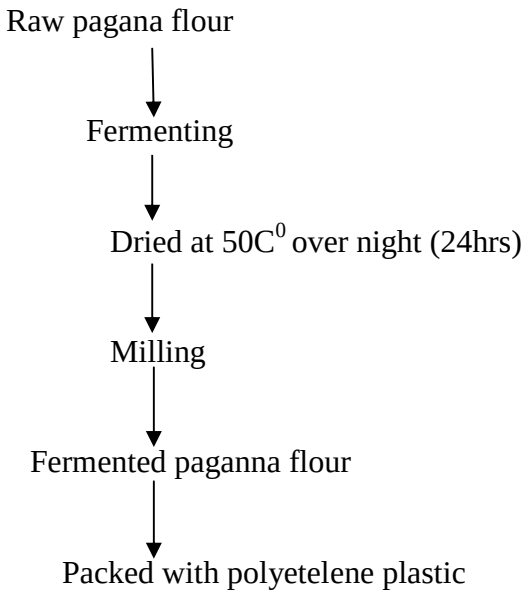
Pagana Corms were thoroughly cleaned using distilled water. About 500g of cleaned and washed samples were placed in a cooking utensil and 1500ml of distilled water was added to it. Samples were boiled for 2 hours in a range of 92 – 95C^0. Then, the tubers were hand peeled and sliced into approximately 0.5mm thick and placed on a stainless steel tray and allowed to dry in an oven at 50℃ overnight. The dried pagana chips were converted to flour using a miller and sieved to pass through 60 mesh sieve.



3.2. Flow diagram of preparing boiled paganna flour

3.2.3. Flour preparation from fermented of pagana tuber

About 100g of Pagana flour was mixed with 300ml of distilled water in 1000ml conical flask and the flask was covered with aluminum foil and allowed to ferment naturally (spontaneously) at room temperature for 24, 48, 72 and 72 hours. Finally, the supernatant was discarded and the slurry was transferred into glass bowls and placed in oven to dry overnight at$50C^0$ to a constant weigh and was then milled.



3.3. Flow diagram of preparing fermented Paganna flour

3.3. Determination of proximate composition

3.3.1. Moisture

Moisture was determined according to [68]. A clean dried and covered flat aluminum dishes were weighed and about 5gm of the sample were transferred to the dish. The dish then

placed in the oven at 105^0C for 3hrs and then cooled in desiccators and re-weighed. Then, the moisture content was estimated by the formula:-

$$\%Mc = (\frac{W2-W3}{W2-W3}) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq(1)$$

Where W1=weight of crucible

W2=weight of crucible and sample

W3=weight of crucible and sample after dried

Mc=Moisture content

3.3.2. Crude protein

Protein content was determined according to [68].A digestion flask containing about 0.5 g of sample, to which 6 ml of acid mixture (conc. sulphuric acid and about 3g of catalyst mixture (K_2SO_4 and Cupper) were added and exposed to about 370^0C in order to allow digestion. Then, distillation took place in Kjeltec by adding 25 ml of 40% NaOH and using 25 ml of boric acid with 10 drops of indicator solution. Finally, the distillate was titrated with standardized 0.1N hydrochloric acid to a reddish color. The crude protein content was estimated using the formula:-

$$\%N = \frac{(V2-V1)\times VHcl\times 14\times 100}{W\times 1000} \ldots\ldots\ldots\ldots\ldots eq(2)$$

Where

V1=volume in ml of the standard hydrochloric acid used in the titration for the blank determination

V2=volume in ml of the standard hydrochloric acid solution used in the titration of the test material

N= normality of the standard hydrochloric acid

W = weight in grams of test material

The conversion factor is 6.25, which is obtained from food composition table of EPHI.

Crude protein content (%) = total nitrogen (%) × 6.25……eq(3)

3.3.3. Crude fat

A clean and dried thimble containing about 2g of dried sample and covered with fat free cotton at the bottom and top was placed in the extraction chamber in petroleum ether. Then the petroleum was evaporated from the extraction flask .Then, extraction took place for at least 4 hrs according to official method [68].The amount of fat was quantified gravimetrically and calculated from the difference in weight of extraction flak before and after extraction as percentage. The crude fat content was determined by the formula:-

$$\%\text{of Crued fat} = \frac{M2-M1}{M} X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{eq(4).}$$

Where M1=mass of dried flask

M2=mass of dried flask and extracted oil

M= mass of the sample

3.3.4. Total ash

The porcelain dish used for the analysis was washed by dilute hydrochloric acid on boiling. And it was washed with distilled and de-mineralized water respectively. Then dried at $105C^0$ in an oven and ignited at $550C^0$ in Muffle furnace for 30 minute. The dish was then removed from furnace and cooled in desiccators. The mass of the dish was measured using analytical balance (M1). About 2.5 gm of sample powder was weighed into the porcelain dish (M2).The sample was charred at $20C^0$ on hot plate until the whole content becomes carbonized. Then the sample was placed in a furnace at $550C^0$ until whitish color appears for 5hours.Then the sample was removed from the furnace and placed in desiccators. Finally the mass was weighed as (M3).

$$\%\text{ash} = \frac{M3-M1}{M2-M1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{eq(5)}$$

Where M1=mass of the crucible

M2=mass of crucible and sample

M3=mass of crucible and sample after ashing

3.3.5. Crude fiber

Crude fiber analysis was conducted using the method of [68]. About 1.6g weighed sample was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml 28% KOH was added and boiled gently again for another 30 minutes. Subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. After, filtering it was then dried in an electric oven for 2hrs. Furthermore, it was cooled at room temperature for 30 minutes in a desiccators and weighed, then transferred the crucibles to muffle furnace for 30 minute ashing at $550C^0$. Finally, it was cooled again in desiccators and reweighed. The crude fiber content was determined by using the formula:-

$$\%\text{Crude fiber} = \frac{W2-w3}{W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{eq(6).}$$

Where W1=Crucible weight after drying

W2=crucible weight after ash,

W3=dry weight

3.3.6. Utilizable carbohydrate determination

The total utilizable carbohydrate was calculated by difference with the exclusion of crude fiber.

Total carbohydrate (%) = 100 - (crude fat + crude fiber + crude protein + ash) ……..eq(7).

3.3.7. Total energy in kilo calories

The gross energy (GE) content in each sample was determined mathematically using the following formulae:

Gross energy (Kcal) = (9 x crude fat) + (4 x crude protein) + (4 x utilizable carbohydrate).

3.4. Minerals analysis

Minerals content was determined according to the method by Association of Official analytical Chemists [68]. About 2.5gm of the sample was charred to remove organic matter, then ash in muffle furnace at $550C^0$ for 5hours for the determination of the total ash content. Three drops of 1M HNO_3 acid and few drops of deionized water added to the sample in each of the crucibles. The ash was digested by using 3N and 6N hydrochloric acid .The digested sample was filtered in to sample bottles each using the Whatmann filter paper (42mm) prior to analysis after filtration made up the volume to 50ml with deionized water. For calcium determination 2.5ml of 10%$Lacl_3$ should added. The Fe, Zn, Mg, Ca, Mn and Cu content in the sample was determined using Atomic Absorption Spectrophotometer (AAS) using air acetylene flame. Using AAS a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g for all of the above minerals. Thus reading was taken from the graph which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula:-

$$\text{Metal content } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{(A-B)}{10W} \times V \quad \text{.....eq(8).}$$

Where A=concentration of sample solution in ppm

B=concentration of blank solution from curve in ppm

W=weight of the sample,

V= volume of extract

3.4.1. Total phosphorous

The sample solutions prepared for mineral determination was used for phosphorous determination. Phosphorus was determined calorimetrically by the method of [69]. Phytate phosphorus was obtained by using the formulae [70] .As shown below non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus. The clear extracts 150µl were diluted into 10ml with deionized water. Five ml of the sample solution was added into test tube, and 5ml of ammonium molybdate and

Metavanadate solution was added into the test tube (sample solution) and mixed thoroughly step by step. The solution was allowed to stand for 10-30 minutes. Then after 10-30minutes the absorbance was read at 460nm using a spectrophotometer model (UV-7804C ultraviolet-visible spectrophotometer). The total phosphorus was calculated using the formulae:

$$P(ppm) = \frac{C \times V1 \times V2 \times Mc}{W \times A} \quad \text{.....eq(9).}$$

Where C=phosphorus concentration in sample digest read from the curve

V1=volume of the digest (50ml)

V2= volume of dilution

A= aliquot (5ml)

Mc=moisture correction factor

The phytate phosphorus could also calculated using the formulae below

$$\text{Phytate phosphorus (mg)} = \frac{A \times 28.18}{100} \quad \text{.....eq(10).}$$

where A = the phytate content (mg),

Non phytate phosphorus was calculated as a difference of total phosphorus and phytate phosphorus.

3.5. Determination of Anti nutritional factors

3.5.1. Tannins

Tannins were determined using the method by [71].About 1.5gm of Pagana flour was weighed in a screw capped test tube and 10ml of 1% HCl in methanol was added to each test tube containing the samples, then the tubes were put on mechanical shaker for 24 hours at room temperature. After 24 hour of shaking, the tubes were centrifuged using (DYNAC II centrifuge, Clay Adams division of Becton and Dikinson Company, USA) for 5 min. One ml of the clear supernatant was taken and mixed with 5ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol).

In another test tube and this mixture was allowed to stand for 20 min to complete the reaction. D-Catechin was used as standard for condensed tannin determination. A 40mg of D-Catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A bout 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and

the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. About 5ml of vanillin HCl reagent was added into each test tube. After 20 min the absorbance was read at 500nm by spectrophotometer (UV-7804C, ultraviolet-visible spectrophotometer). 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. The standard curve was shown in index1.

$$\text{Tannin}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{(\text{As}-\text{Ab})-\text{int})\times 10}{\text{S}\times\text{D}\times\text{W}} \quad \text{.....eq(13).}$$

Where As=absorbance of sample solution

Ab=absorbance of blank solution

S=slope of the absorbance equation

D=density of the solvent

W= weight of the sample

3.5.2. Oxalate

The Oxalate Contents of both raw and processed Pagana flours was determined using the method of [72]. This method involves the following three steps: digestion, oxalate precipitation and permanganate titration.

- **Digestion**

At this step about 2gm dry base of pagana flour was suspended in 190ml of distilled water contained in 250-ml conical (Erlenmeyer) flask; 10ml of 6M HCl is added and the suspension was then digested at 100℃ for 1 hour, this was followed by cooling, and then solution was made up to 250mL before filtration using distilled water.

- **Oxalate precipitation**

Duplicate portions of 125 ml of the filtrate was measured into a beaker and four drops of methyl red indicator was added, 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 9O℃, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90°C and 10 ml of 5% CaC_{l2}, solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The solution was then centrifuged at a speed of 2500 rev/min for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

- **Permanganate titration**

At this point, the total filtrate resulting from digestion of 2 gm of flour was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near-boiling at $90C^0$ and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink color which persisted for 30 s. The calcium oxalate content was calculated using the following formula:

$$\text{Oxalates}\left(\frac{\text{mg}}{\text{100g}}\right) = \text{T x}\frac{(\text{Vme})(\text{DF})\ \text{x}\mathbf{10^5}}{(\text{ME})\text{x mf}} \quad \text{.....eq(11).}$$

where *T* is the titer of $KMnO_4$, (ml), Vme is the volume-mass equivalent in which 1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), DF is the dilution factor VTA *(*2.4, where VT is the total volume of filtrate (300ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction. and mf is the mass of flour used.

3.5.3. Phytate

The phytate content was determined according to the method described and later modified by [73, 74]. About 0.075 grams of dried Pagana flour was extracted with 10mL 2.4% HCl for 1 hour at ambient temperature and centrifuged at (3000 rpm/ 30 min). The clear supernatant was used for the phytate estimation, two ml of Wade reagent (0.03% solution of $FeC1_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3mL of the sample solution and the mixture was centrifuged. The absorbance at 500nm was measured using UV-VIS spectrophotometer (UV-7804C). The phytate concentration was calculated from the difference between the absorbance of the control (3mL of water+2mL Wade reagent) and that of assayed sample. To prepare the phytic acid standard curve, a series of standard solution were prepared containing 5–40 mg/ml phytic acid in water. The concentration of standards were (5, 9, 18 ,

27, 36)ppm. About 3ml of the standard was pipette into15ml centrifuge tubes with 3ml of water used as a zero level. To each about 2ml of the wade reagent was added and the solution was mixed on a vortex mixer for 5s. The mixture was centrifuged for 10 minutes and the supernatant read at 500ηm using UV spectrometer was read by using water as a blank. The concentration of phytate was calculated using phytic acid standard curve and results were expressed as of phytic acids in mg per 100 g dry weight. A standard curve was shown in ape ndex 1.

$$\text{phytate}(\mu g/100g) = \frac{(As-Ab)-int)\times 10}{w\times 3} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq(12).}$$

Where As=absorbance of the sample

Ab=absorbance of the blank

Int=intercept from the absorbance equation curve

W=weight of the sample

3.6. Determination of antioxidant activities

3.6.1. Sample extraction

Samples were extracted based on the procedures as outline by [75].The powdered Pagana samples were homogenized and weight in 5g was then extracted by 25ml of methanol at 25^0C at 150rpm for 24hour using an incubator shaker (ZHWY-103) and then filtered through what man No.1 filter paper. The residue was then extracted with additional 25ml of methanol as described above. The combined methanolic extracts were evaporated at 40^0c dryness using rotary evaporator and re dissolved in methanol at concentration of 50mg/ml and stored at 4^0c for further use.

3.6.2. Determination of free radical scavenging activities by DPPH

DPPH scavenging activity of the tuber methanolic extract was measured according to the method of [75]. IC50 values of the extracts and concentration of the extracts necessary to decrease the initial concentration of DPPH by 50% were calculated. The hydrogen donation ability of the corresponding extracts and some pure compound was measured from the bleaching purpled colored methanol solution of DPPH. The effect of methanolic extract on

DPPH radical was be estimated according to [76] 4ml 0.004% solution of DPPH radical solution in methanol was mixed with 1ml of various concentration (2-12mg/ml) of extracts in methanol with vortex mixer .Incubate the sample for 30min in dark at room temperature Ascorbic acid standards prepared by dissolving 0.3mg into 1ml of methanol (or 3mg in 10ml methanol).Scavenging capacity was read Spectrophotometrically by monitoring the decrease in absorbance at 517 nm by (UV-7804C, UV-Vis spectrophotometer. Inhibition of free radical DPPH in percent (I%) was calculated in following way.

$$I\% = \frac{Ac - As}{Ac} \times 100 \quad \text{.....eq(14).}$$

Where:

Ac=Is the absorbance of control reaction without test sample

As =Is the absorbance of the test sample (containing all reagents except the test sample) Ascorbic acid

%I= Percent of inhibition

The concentration of scavenging activities at IC50 was calculated using the %I from the absorbance of control and absorbance sample solution.

3.6.3. Total polyphenol content

The total phenol content was determined by the method described by [77]. Aliquots (100 µl) of each extracts were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Rapidly after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 765nm against reagent blank using UV-Vis (UV-7804C ultraviolet visible spectrometer. Gallic acid was used to construct the standard curve, and the result was expressed mean± standard deviation expressed as milligram of gallic acid (GAE) equivalent of extract. All determinations were carried out in triplicate. 0.05g of gallic acid in 1ml methanol and then dilute to 10ml with deionized water (5g/L) final stock. Dissolve 0.1, 0.2, 0.5and 1ml with water to create standards with 50, 100, 250, and 500mg/L(ppm) concentration respectively.

The total phenolic compound in the extract in gallic acid equivalent (GAE) was calculated using the formulae:

$$\text{Total phenolic content}(C) = \frac{GAEc \times V}{W} \ldots\ldots\ldots \text{eq(15)}$$

Where, GAEc=concentration of gallic acid equivalent (milligram/ml) from curve

V= Total volume of the extract (ml)

W= sample weight (gm).

3.6.4. Total flavonoid

Flavonoid contents were determined according to the method of [78]. An aliquot (150 µl) of each extract or standard solution was mixed with 1.25 ml of deionized water and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with double distilled water. Prepare 10-1000µL quercetin standard in methanol from 1mg/ ml stock by dissolving (10mg/10ml). Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm using (UV-7804C, ultraviolet visible spectrometer). The results were expressed in mg Catechin equivalent (CE).

$$\text{Flavonoid content} = \frac{DE \times V}{W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq(16).}$$

Where DE= D-Catechin equivalent (mg/ml)

V= total volume of the sample (ml)

W= sample weight (mg)

3.7. Determination of physicochemical properties

3.7.1. Tuber size determination

Sizes of the tubers were measured using a calibrated balance by directly placing the tubers on the analytical balance after adjusting the balance to zero within a 72 hours of harvesting.

3.7.2. pH value

The pH of the raw and processed samples was determined according to the method of[79].About 10 g of the samples were weighed in triplicates in 250ml beaker and mixed with 50 ml of distilled water and stirred for 10 min. The pH of the sample was determined by dipping the electrode of the Jenway pH meter (Jenway 3510 pH meter) in the mixture .The pH meter was calibrated using pH 4.0 and 7.0 buffers prior to determination of the pH of the samples.

3.7.3. Titratable acidity

The total titratable acidity of Pagana flour samples was determined by method [80]. About 5 g of the flour sample was macerated for 30 minutes in a beaker with 15 ml of distilled water as 1 part of the flour to three parts of the water (w/v) ratio. A known volume of water is used for further dilution in order to hydrolyze all the acids in the sample. Before titration of the sample, the water that is used for dilution purpose was titrated to be used as a blank. Three drops of 1% alcoholic phenolphthalein indicator was added to water extract of the sample (dispersion). The dispersion was then titrated with standard base (0.1N NaOH) to phenolphthalein end point. The result of determination was reported as percentage lactic acid consuming definite volume of 0.1 N NaOH. The end point of the titration was reached when the white dispersion changed from a clear white solution to a faint violet colored turbid solution. Triplicate determinations were made in all cases. Finally it was given that the amount of lactic acid in the sample was determined from the relation (1ml 0.1 N NaOH = 0.009008mg Lactic acid ($C_3H_6O_3$).

3.8 Determination of functional properties

3.8.1. Water and oil absorption capacities

About one gram of Pagana flour was mixed with 10 ml distilled water or refined palm oil (frytol) in a pre-weighed 20 ml centrifuge tube. The slurry were agitated for 2 min, allowed to stand at 28^0C for 30 min and then centrifuged at 2000 rpm for 20 min. The clear supernatant

were decanted and discarded. The adhering drops of water or oil in the centrifuge tube was removed with cotton wool and the tube will be weighed, the weight of water or oil absorbed by about 1g of flour were calculated and expressed as water or fat absorption capacity [81].The oil or water absorption index was determined using the formula;

$$\text{WAI}\left(\frac{g}{100g}\right) = \frac{(W2-W1)\times 100}{W3} \quad \text{.....eq(17).}$$

Where W1=weight of test tube

W2=weight of test tube and residue after centrifuge

W_3=weight of sample

WAI= water absorption index

$$\text{WSI} = \frac{(W2-W1)\times 100}{W3} \quad \text{.....eq(18).}$$

Where WSI=water solubility index

W_1=weight of crucible

W_2=weight of crucible and sample

W_3=weight of sample

3.8.2. Bulk density

Using the procedure of [82] 50g of Pagana flour were put into a 100 ml measuring cylinder and tapped to a constant volume and the bulk density (gcm-3) were calculated using the formula:

$$\text{Bulk Density} = \frac{\text{wt of flour(g)}}{\text{flour volume(cm3)}} \quad \text{.....eq(19).}$$

3.8.3. Foam capacity and stability

The foam capacity was determined using the method of [83] the flour (2g) was suspended in distilled water (100 ml) and stirred at room temperature for 5 min using a magnetic stirrer at 10 Ruhrer speed. The contents along with the foam were immediately poured into a 250 ml measuring cylinder. Volume of foam (ml) after mixing was expressed as the foam capacity

and then volume after 60 min as foam stability. The foam capacity is determined by the formulae;

$$\text{Foaming capacity}(\%FC) = \frac{(V2-V1)\times 100}{V1} \quad \text{.....eq(20)}$$

Where: V1=initial volume of solution

V2=final volume after mixing

The foam stability also determined using the formulae:

$$\text{Foaming Stability } (\%FS) = \frac{(VR2-V)\times 100}{V1} \quad \text{.....eq(21)}$$

Where:V1=initial volume of the solution

VR2=foam volume remained after 60minute

3.8.4. Swelling power and solubility

Swelling power and solubility determinations was carried out in the temperature range of 60-90°C (using the method of [84]. About one gram of Pagana flour sample was accurately weighed and quantitatively transferred in to a clear dried test tube and weighed. About 10 ml of distilled water were added and mixed gently at low speed for 5 min. The slurry was heated in a thermo stated water bath, at 80°C for 30 min with mixing the suspension intermittently. The test tube was cooled with its content rapidly to 20^0C. During heating, the slurry was stirred gently to prevent lumps forming in the flour. Then the cool paste was centrifuge at 2200rpm for 15 min. The supernatant was decanted immediately after centrifuging into a pre-weighed evaporating can and dried at 100°C to constant weight approximately for 4 hours. The weight of the sediment was taken and recorded or swollen mass. Swelling power was determined using the formulae:

$$\text{Swellig power} = \frac{\text{Weight of sediment}}{\text{weight of sample}-\text{wt soluble}} \quad \text{.....eq(22).}$$

The solubility index is also determined by the formulae

$$\text{Solubility index}(\%) = \frac{\text{Soluble wt}}{\text{Sample wt}} \quad \text{.....eq(23)}$$

3.9. Experimental design and statistical analysis of data

One-way analysis of variance (ANOVA) was conducted on each of processing methods and Least Significant Difference (LSD) test at significant level of $p < 0.05$ was performed using SPSS version 20 software for windows to compare the difference between treatment means. The results was expressed as mean ± standard deviation of three separate determinations.

CHAPTER FOUR

4. RESULT AND DISCUSSION

In this chapter proximate composition, mineral composition, antinutritional factors, antioxidant activities, physicochemical properties and functional properties of the raw and processed Pagana flours of samples are discussed. Tables show the results of proximate composition, mineral composition, levels of antinutritional factors, antioxidant activities, physicochemical and functional properties for raw and processed Pagana flour samples. All results were based on dry weight basis of Pagana flour.

Figure 4.1 Pagana *(A. gomboczianus)* plant

Figure 4.2 Pagana (*A. gomboczianus)* corms

4.1. Proximate composition of Pagana flour

The proximate composition of raw and processed Pagana sample were presented in (Table4.1).

4.1.1. Moisture

The moisture contents of raw and processed flours on dry bases was ranged from 6.2% to 11.2% (Table 4.1).The maximum moisture content observed for 24 hrs fermented and minimum moisture for raw sample. The determinations of moisture for the two processing methods, fermentation and boiling had a significantly ($p<0.05$) increased the moisture content of Pagana sample as comparing to the raw sample. The increase of moisture on boiling compared with raw sample was due to the water absorption capacity of fibers and other natural chemical components during heat treatment [85]. Fermentation also increased the moisture contents of sample due to absorption of water during fermentation proceed.

4.1.2. Crude protein

The protein content of raw and processed Pagana flour samples collected from Konso ranged from 7.43% to 9.36% (Table4.1). The fermented samples had highest protein value (9.36 %) and the minimum corresponds to boiled samples of Pagana (7.43%). The protein content of the raw Pagana was different from the finding of Getachew (5.8%)[2].The difference might be due to environmental condition like, soil fertility and soil type from where samples were collected. Boiling had significantly ($p<0.05$) decreased protein content. The decreased of protein during boiling was due to leaching of soluble components into cooking water and denaturation of protein [86].The results also showed that fermentation significantly ($P < 0.05$) increased the protein content of the Pagana sample by 21.6%. The increase in the protein content of Pagana flour during fermentation might be due to some microorganisms such as microfungi, which might degraded Pagana flour readily, could have secreted some extracellular enzymes (protein) [87]. The decrease of carbohydrate by fermentation and its increment of carbohydrate by boiling contributes proportionally to the increase and decrease of protein by fermentation and boiling respectively as the total utilizable carbohydrate(CHO) was computed out by difference. It is known that fermentation may enrich foods in protein by removing part of the fermentable carbohydrate as documented in fermented foods made from cassava [88].From the result, it was shown that fermented sample had higher protein content when compared to the raw Pagana sample.

4.1.3. Crude Fat

The crude fat content of raw and processed flour samples are reported given in(Table 4.1). The results showed fat content of raw and processed Pagana flour samples were in the range of 0.45% to 0.506% . Maximum value was recorded for the fermented and minimum for boiled flour sample. Fermentation had a significantly ($P < 0.05$) increased the fat content of samples. An increased in fat content during fermentation for the fermented samples could be attributed to the possibility that, the fermenting organisms could secrete microbial oil [89]. Boiling significantly ($p<0.05$) reduced the crude fat contents. The observed reduction in the fat content of Pagana flour during boiling was due to the leaching into boiling water[90].The observed results was comparable with finding of Getachew for raw sample(0.4%).

4.1.4. Crude ash

Ash values of raw and processed Pagana samples ranges from 6.46 % to 5.08% (Table 4.1). The maximum value was for raw sample and minimum for processed sample. Boiling had no significant effect ($P >0.05$) on the ash content. Fermentation on the other hand had a significantly ($P <0.05$) decreased the ash contents. This was due to attributed to leaching of minerals to the fermentation liquids which is usually discarded. The mean ash content of raw Pagana sample was comparable with findings of [2]. Additionally the ash percentage of boiled sample was less than raw. The reduction of total ash during boiling was attributed due to leaching of mineral compounds and water absorption during boiling [91].

4.1.5. Crude fiber

The crude fiber contents of raw and processed Pagana flour sample were shown in table 4.1, which were between 2.1% to 2.73%.The maximum and minimum observed for raw and minimum value for fermented samples respectively. These values showed that boiled samples had no significant ($P >0.05$) difference in crude fiber content from raw samples. In contrast fermentation significantly ($P < 0.05$) reduced the fiber contents of the Pagana flour.The reduction of fiber by fermentation was 63%.The decreased fiber content during fermentation might be attributed to that fermenting microorganisms ability to degraded the fiber into short

chain hydrolysable carbohydrates [89].These enzymes are capable of hydrolyzing crude fiber into simple sugars, which the organism could use as its carbon source and change it to other macromolecules or metabolites such as protein and fat [92].

4.1.6. Utilizable carbohydrate

From the results of the study it can be observed that Pagana is a very good source of carbohydrate (CHO). The carbohydrate content of the raw and processed Pagana samples ranged from 82.62% to 84.29% (Table 4.1). Boiling and fermentation significantly ($P < 0.05$) increased the carbohydrate content. The slight increment of carbohydrate content by fermentation was about 0.29%.The result indicate that carbohydrate slightly increased by fermentation. There is also the possibilities that an increased of protein content and decreased of carbohydrate. Upon fermentation on current study, both protein and carbohydrate contents were increased. This might be due to the decreasing of ash content caused increased of carbohydrate in both processed samples. Boiling has increased the carbohydrate content to some extent which might be resulted from solubilization of starch which makes it much more available and increased in the carbohydrate content of both samples [43].The possible cause for decrease in the carbohydrate of fermented flour of samples would not be far from the possibility that micro-organisms could secrete hydrolytic enzymes .These enzymes are capable of hydrolyzing carbohydrates into simple sugars, such as maltose which the organism could use as its carbon source and changes it to other macromolecules or metabolites such as protein and fat [92]

Table 4.1: Proximate composition of raw and processed Pagana Four

Sample type	%Moisture	%rude protein	%Crude fat	%Crude ash	%Crude fiber	% Utilizable carbohydrate	Energy (Kcal/100g)
raw	7±0.00^{d}	7.7±0.17^{b}	0.49±0.1^{b}	6.46± 1.1^{a}	2.73±0.0^{a}	82.62^{c}	365.69^{c}
boiled	8.49±0.16^{c}	7.43±0.8^{c}	0.40±0.01^{ab}	5.42±0.1^{ab}	2.71±0.0^{a}	84.04^{a}	369.48^{b}
F24	11.2±0.00^{a}	8.92±0.46^{a}	0.50±0.00^{ab}	5.08±0.0^{b}	1.87±0.8^{ab}	83.63^{ab}	374.70^{ab}
F48	7.2±0.00^{d}	9.10±0.30^{a}	0.50±0.00^{ab}	5.68±0.1^{ab}	1.72±0.1^{b}	83.00^{b}	372.90^{b}
F72	9.2±0.00^{b}	9.36±0.00^{a}	0.51±0.01^{a}	5.82±0.2^{ab}	1.2±0.3^{b}	83.11^{b}	361.79^{d}
F96	6.2±0.20^{e}	9.36±0.00^{a}	0.51±0.01^{a}	5.63±0.3^{a}	1.01±0.1^{b}	83.49^{ab}	375.99^{a}

All values were the mean of triplicate ± SD

Means with the same superscript letters within a column are not significantly different (P>0.05)
All values are expressed in dry weight basis of edible portions of pagana
Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b > c > d….
Key;F24-,fermeted for 24 hours,F48-fermented for 48 hours, F72- fermented for 72 hours, F96-fermented for 96 hours. SD-standard deviation

4.2. Minerals

The results of the mineral analysis of the Pagana tubers are presented in Table 4.2. The results showed that potassium as the most abundant mineral, ranging from 1750.6mg/100g to 2446mg/100g of dry weight.

4.2.1. Calcium

The values of calcium was presents in Table (4.1), Calcium content of Pagana was ranged from 260.535mg/100g to 334.57mg/100g of dry weight .Fermentation significantly ($p< 0.05$), increased calcium content. The maximum increment of calcium content was observed at 96 hour fermented. The increased in the total calcium content with fermentation period would be consistent with the period of maximum increased in the number of microorganisms involved in fermentation which may contribute to the total calcium content of the samples [93].where in contrast boiling a significantly ($p<0.05$) decreased the calcium content compared with the value in the raw Pagana. This could be due to the fact that a substantial amount of solubilized calcium may have been lost in the water used in boiling.

4.2.2. Iron

As shown in Table 4.2 the iron contents in the raw and processed Pagana tuber ranged from (1.59 to 7.71)mg/100g.The maximum value was for raw and lower values had recorded for processed samples fermented for 96 hrs. The iron content of raw Pagana sample which comparable with finding of Getachew (8.72mg/100g)[2]. Iron content significantly ($p<0.05$) decreased by fermentation period reaching the minimum level after fermented to 96 hours, when compared with raw samples. This could be due the fact that a substantial amount of solubilized iron may have been lost in water used in fermentation. Boiling also had significantetly ($p<0.05$) decreased the iron content when compared with raw samples. This might be due to the fact that solubilized iron had been removed with boiling water [94].

4.2.3. Magnesium

Magnesium content of Pagana was ranged from (47.96 to 49.14)mg/100g of dry basis as in (Table 4.2).Total magnesium content decreased with increasing period of fermentation with a loss of 2.22%. Fermentation had a significant effect ($p<0.05$) on magnesium reduction after 72 hour fermentation. There was observation that magnesium exists in a soluble form around the pH of fermentation, this might probably account for the loss. Boiling had also a significant effect ($p<0.05$) on reduced magnesium content with loss of 2.4%. The reduction of magnesium by boiling might be due to the leaching of soluble magnesium by water [95].

4.2.4. Manganese

The amount of Mn in raw and processed Pagana flour were ranged between (0.59 to 1.02)mg/100g on dry basis as present in (Table 4.2).Fermentation significantly ($p< 0.05$) reduced manganese content of the samples with loss of 42.33%.Maximum reduction of manganese was observed after 96hrs fermented. This reduction was due to the fact that a substantial amount of solubilized manganese may have been lost in the water used for fermentation [96]. Boiling also had a significant effect($p<0.05$) on reducing the manganese content with loss of 40.19%. Reduction of manganese by boiling might be due to the binding nature of manganese [96].

4.2.5. Potassium

The results in Table 4.2 showed that the K contents of Pagana were ranged between 1746.0 mg/100g to 2443.66mg/100g.The minimum K content corresponds to fermented Pagana for 96 hours and maximum belongs to raw sample. The current study showed that potassium was the highest of all the minerals analyzed in the Pagana samples. Both fermentation and boiling had significantly ($p<0.05$) decreased the content of potassium from the raw tuber. The decreased of potassium during processing might be due to that highly soluble capacity of potassium salt found in the tuber and leaching out with the supernatant of fermented sample and boiling water [97].

4.2.6. Sodium

The mean sodium content of the raw and processed Pagana samples ranged between 17.59mg/100g to 31.2mg/100g.The maximum increment of Sodium was found in the fermented Pagana after 72hrs. Both processing methods significantly ($p<0.05$) increased sodium content of Pagana, when compared with raw Pagana samples. The increased in potassium content during processing might be due to decomposition of anti nutritional factors like oxalate and phytate that have capacity or chelating minerals and release minerals while decomposed and increases the availability of minerals [97].

High dietary sodium intake is associated with an increased risk of hypertension, which is a risk factor for the development of cardiovascular disease [98]. The World Health Organization (WHO) recommends that adults consume less than 2000 mg of sodium daily (5 grams of salt) [99]; however, average global intake exceeds this level by far. Impact modeling shows that realistic reformulation of food products to lower sodium levels could decrease sodium intake by up to 30% [100]. Population-based studies have also demonstrated that dietary sodium reduction towards the WHO guidelines could lead to a significant reduction in blood pressure and cardiovascular disease risk [100]. Currently, potassium chloride is one of the most commonly used sodium chloride replacers as it has a good ability to convey the perception of a saltiness taste in food products [101]. Potassium chloride is a naturally occurring mineral salt, which is obtained from rock and sea salts in a manner similar to the extraction of sodium

chloride. Dietary intake of potassium is associated with a decreased risk of hypertension, the opposite effect to sodium [102]. While the intake of sodium is too high, the average global intake of potassium is below the WHO guidelines (at least 3510 mg of potassium daily [103].This makes potassium chloride an interesting salt replacer from a consumer, production, and, potentially, also from a health point of view. Therefore from the current study, Paganna had large amount of potassium content that is important for our healthy and less content of sodium that had impact to our health if it is in excess.

4.2.7. Zinc

Zinc content of the raw and processed Pagana samples ranged between 3.1mg/100g to 1.97mg/100g .Both fermentation and boiling had significantly ($p<0.05$) reduced zinc content (Table4.2). However, fermentation for three days, effectively reduced zinc content with loss of about 35.16% reduction. The decreasing of zinc by fermentation was due to microbial growth that degrades the flour and increases the solubility of mineral loss during discarding of fermentation supernatant [94]. Boiling also decreased the zinc. The decreased of zinc during boiling with the hot water which might be due to leach out and the soluble minerals removed out of the sample during discarding the water.

Table 4. 2. Mineral contents of raw and processed pagana flour profile in mg/100g of the dry basis

Type of sample	Type of minerals						
	K	Na	Ca	Fe	Mg	Mn	Zn
R	2443.66±2.51^{a}	17.56±0.45^{d}	260.54±1.2^{c}	7.71±0.07^{a}	49.14±0.58^{a}	1.02±1.30^{a}	3.1±0.12^{a}
B	2035.67±4.84^{c}	21.19±0.3^{c}	258.25±1.97^{d}	6.84±0.78^{b}	47.96±0.1^{c}	0.61±0.60^{b}	2.04±0.15^{b}
F24	2382.37±2.11^{ab}	25.59±0.4^{b}	266.01±0.62^{b}	4.86±0.54^{c}	48.72±.40^{ab}	0.75±0.59^{b}	2.11±00^{b}
F48	2290.95±4.11^{b}	29.23±0.32^{ab}	268.84±4.89^{bc}	4.12±0.02^{c}	48.64±0.14^{ab}	0.9±0.90^{b}	2.02±0.04^{b}
F72	1853.42±5.00^{d}	31.19±0.26^{ad}	262.56±1.48^{bc}	2.09±0.37^{d}	48.05±0.08^{c}	0.73±0.73^{b}	1.97±0.10^{b}
F96	1853.42±5.00^{d}	24.52±4.74^{c}	334.57±2.71^{a}	1.58±0.01^{d}	48.84±0.72^{ab}	0.59±0.75^{b}	2.01±0.14^{b}

*All values are means of triplicate analysis ±standard deviation

Values which are followed by different letters of superscripts in the same column are significantly ($p<0.05$) different

Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b > c > d….

Values are expressed in mg/100g of dry weight basis.

Where F24=fermented for 24hrs, F48=Fermented for 72hrs, F96= Fermented for 96hrs

Table 4.2.1 Comparison of analyzed mineral with RDA of Food and Nutrition Board of the Institute of Medicine, *National Academy of Sciences*, 1997-2001, in a collaboration between the US and Canada in (mg),[104].

Sample type	Ca	Fe	Mg	Mn	K	Na	Zn
raw	260.54	7.71	49.14	1.02	2443.66	17.56	3.1
boiled	258.25	6.84	47.96	0.61	2035.67	21.19	2.04
F24	266.01	4.86	48.72	0.75	2382.37	25.59	2.11
F48	268.84	4.12	48.64	0.9	2290.95	29.23	2.02
F72	262.56	2.09	48.05	0.73	1853.42	31.19	1.97
F96	334.57	1.58	48.84	0.59	1746.00	24.52	2.01
RDA	1200	8	320	1.8	4700	1300	8

According to the values shown in the above table all minerals analyzed from raw and processed Pagana samples were less than the recommended daily allowance of US,Canada. Food table, therefore mixing Pagana with mineral in reach food could improve amount of minerals.

4.2.8. Phosphorus

The total phosphorus content for raw and processed Pagana samples were ranged between 484.09 mg/100g to 654.56mg/. Both the processing methods significantly ($p<0.05$) decreased both total phosphorus and phytate phosphorus. The decreased in total phosphorus as well as the decreased in phytic acid was due to hydrolysis of phytic acid to Inositol and orthophosphate by phytase enzyme present in fermented tuber. There is three reasons why phytate was slowly hydrolysed: Presence of metal ions, and other salts, which form stable salts resistant to enzyme hydrolysis, the existence of phytate P in the form of metals salt and/or in combination with proteins in seeds and these forms may not be available for enzyme attack and Presence of high concentration of phytate P in the medium and which inhibits phytase action [105].

Table 4.3 Phosphorus and phytate phosphorus contents of raw and processed pagana flour

	Total phosphorus(ppm)	Phytate phosphorus(ppm)	Non phytate phosphorus(ppm)
raw	654.56±48.48[a]	0.79±0.00[a]	653.76±4.48[a]
boiled	533.36±0.00[ab]	0.78±0.00[b]	532.61±0.00[ab]
F24	618.69±2.12[a]	0.78±0.00[b]	617.91±4.97[a]
F48	594.69±3.36[ab]	0.75±0.00[c]	593.94±3.36[ab]
F72	569.63±2.11[ab]	0.74±0.00[d]	568.88±2.11[ab]
F96	484.88±4.97[b]	0.74±0.01[d]	484.13±2.12[b]

*All values are means of triplicate analysis ±standard deviation

Values which are followed by different Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b > c > d letters of superscripts in the same column are significantly different

Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b >c>d>e>f

Values are expressed in ppm of dry weight basis

4.3. Determination of anti nutritional factors of Pagana flour

4.3.1. Tannin

The results of tannin contents for processed and raw Pagana were ranged between 1.64mg/100g to 0.31mg/100g (Table4.4).Fermentation significantly (P< 0.05) decreased the tannin contents of raw samples with increased fermentation period. Fermenting Pagana for 96 hour was reduced the tannin content by 34.8% while compared with raw samples. Reduction in tannin contents upon fermentation might be caused by the activity of polyphenol oxidase or tanniase of fermenting microflora on tannins and due to their binding with cotyledon endosperm that are usually undetected by routine method due to their insolubility in solvent [106-110].Boiling also had significantetly (p<0.05) decreased the levels of tannin. Boiling

reduced tannin content than fermentation compared with raw samples. The decreased during boiling might be due to thermal degradation and denaturation of the antinutrients as well as the formation of insoluble complexes and leaching out of hydrolysable tannin in the boiling water [110]. In contrast to this study, tannin content was reported to increase with increasing period of fermentation which might be attributed to hydrolysis of condensed tannins such as asproanthocyanidins [111].

4.3.2. Oxalate

The results in (Table 4.4) showed that the two traditional processing methods reduced oxalate content in the raw Pagana tuber. The values of raw and processed pagana samples ranged between 39.15mg/100g to 13.5mg/100g.Both processing methods had significantly ($p<0.05$) reduced the oxalate content compared with the raw sample. The maximum reduction was observed at 96hrs and 72 hrs of fermentation with loss of 65.51%. The observed marked reduction caused by fermentation was due to the effect of leaching and enzyme/acid hydrolysis of the starch granule during fermentation [112].Boiling had also significantly ($p<0.05$) reduced oxalate while comparing with raw (41.37%) .The reduction during boiling might be due to leaching, because some oxalate fractions are water-soluble (e.g. at room temperature) [105-106]. On the whole, the results from boiling were indicative that calcium oxalate has a hydrothermal lability. The observed marked reduction caused by boiling might be attributed due to the dual effects of leaching and thermal degradation. They are similar to results from aqueous boiling of cocoyams reported that when boiled for 15 min there was a considerable reduction in irritant effect. The current study showed that fermentation decreased the amount of oxalate by 65.15% while comparing its level in Pagana this is the possible reason for reduction of irritation. Therefore fermentation of Pagana is the best method among the two to decrease the effect of oxalate that causes kidney stone. According to the society information the irritability/ burning sensation of Pagana is increasing with increasing of age/ripeness of corms. This might be related with oxalate contents increased with increasing maturity of corms.

4.3.3. Phytate

Processing decreased anti nutritional factors and increased the quality of foods. The phytate contents of analyzed Pagana samples were ranged between 2.83mg/100g to 2.65mg/100g, decreased by 6.4% while compared to raw (Table4.4), the maximum reduction was observed at 96hrsfermentation.During fermentation of Pagana flour, phytic acid contents was significantetly ($p<0.05$) decreased. Fermentation decreased in phytic acid contents, due to microbial phytase activity on phytic acid. Boiling also had a significant effect ($p<0.05$) to decreased the phytate level. On boiling of unpeeled tubers, also reduce phytic content (Table 4.4). The differences in the loss of phytic acid contents during boiling could probably be explained on the basis that phytase activity at a temperature of 40–55C^0 may degraded inositol hexaphosphate to the pentaphosphate or lower molecular weight forms [113]. Phytic acid content decreased because insoluble complexes between phytate and other components were formed during boiling [114].

Table 4.4 Anti nutritional factors contents of raw and processed pagana flour

Type of Sample	Phytate (mg /100 g)	Tannin (mg/100g|)	Oxalate (mg/100g)
Raw	2.83±4.89^a	1.64±0.00^a	39.15±4.59^a
Boiled	2.80±2.22^b	0.31±0.31^c	22.95±2.02^b
F24	2.77±3.66^c	1.24±0.08^b	15.21±1.71^c
F48	2.68±0.00^d	1.89±0.05^a	14.62±1.12^c
F72	2.68±4.44^d	1.32±0.25^b	13.5 ± 0.00^c
F96	2.65±1.78^e	1.07± 0.00^b	13.5±0.00^c

All values were means of triplicate± standard deviation

Values which are followed by different letters of superscripts in the same column are significantly different

Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b > c > d>e

Values are expressed in mg/100g of dry weight basis

Key; F24-fermented for 24 hours, F48- fermented for 48 hours, F72-fermented for 72 hours

F96- fermented for 96 hours.

4.4. Antioxidant activities of Pagana sample

4.4.1. Yield of extract

The percentage of yields of extract from the Pagana dry sample by methanol from raw and processed sample were ranged from 1.4% to 8.2%(Table 4.5): From the yield of extract the free radical scavenging capacity by DPPH, total phenol and flavonoid was determined.

Table 4.5 Percent of extract extracted by methanol from raw and processed Pagana

Sample type	Raw	Boiled	F24	F48	F72	F96
% of extract	8.2	1.4	5.4	10	7.8	8

Where:F24-fermented for 24 hrs, F48-fermented for 48 hrs, f72-fermented for 72hrs, F96-fermented for 96 hours.

4.4.2. Determination of radicals scavenging activity of Pagana by DPPH

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. DPPH is stable free radical at room temperature and accepts an electron / hydrogen radical to become a stable diamagnetic molecule [115]. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 5l7 nm, induced by antioxidants. The decreased in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity [116].The ability of Pagana extracts to quench reactive species by hydrogen donation was determined by the DPPH radical scavenging activity. The antioxidant of the sample can react with DPPH free radical, a violet color is converted in to yellow color of α ,α-diphenil-β-picryllhydrazine. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517nm, which indicates the radical scavenging ability of the antioxidant. The concentration of

antioxidant of the sample at IC50 was determined from the graph of %inhibition vs concentration.

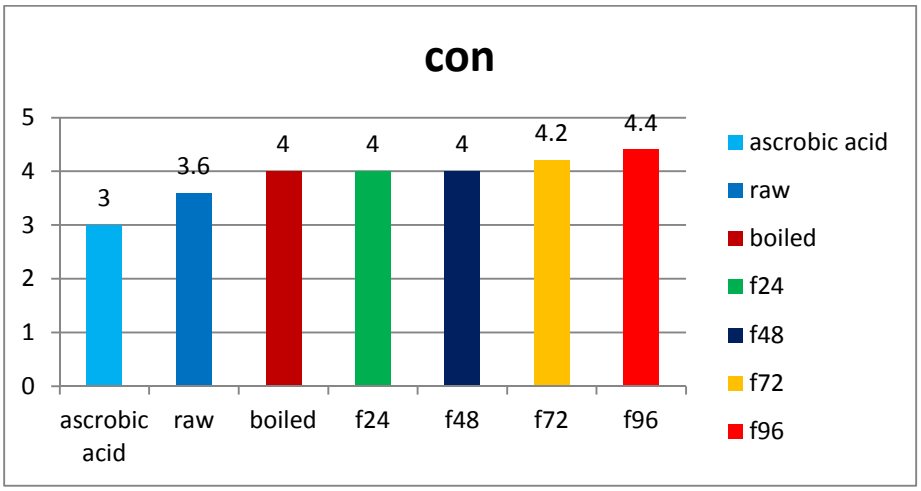


Figure 4. 3 Percentage inhibition verese concntration of samples and ascobic acid at IC50 by DPPH

At the concentration 50mg/ml, the scavanging effect of ascrobic acid and Pagana extract on the DPPH radical scavenging decreased in the order of Ascrobic acid>raw >(boiled=f24) >F48>F72>F96.From the above figure the concentration of the raw and processed Pagana that scavenged free radical ranged from 3mg/ml to 4.4mg/ml, which means the minimum value was for ascorbic acid that had the largest antioxidizing activity and the maximum value was for boiled Pagana which had less oxidizing activity. The reduction of antioxidant of the boiled sample was due to degradation of some particles of the sample by heat. As concentration increased from 3mg/ml to 4.4 mg/ml, the antioxidant activities decreased.

Table; 4.6. Concentration of ascorbic acid and samples at IC50

Sample type	Ascorbic acid	Raw	Boiled	F24	F48	F72	F96
Concentration From graph(mg/ml)	3	3.6	4	4	4	4.2	4.4

Key; 24hour ferment, F48-48hour ferment, F72-72 hour ferment, F96-96hour ferment

4.4.3. Determination of total phenol

Significant ($p<0.05$) differences were found in total phenolic content (TPC) among raw and fermented flour ranging between 67.12±17.48 to 620.42±6.26 mg GAE/g extract (Table 4.5). The highest values was 620.42±6.26 mg GAE/g obtained from 96hr fermented flour in methanolic extract. The phenolic content of fermented flour was higher than that of the raw form in each extract. This shows an increased in phenolic content during fermentation. Fermentation process causes release of microbial enzyme which in turn produces more freely available form of plant chemicals like flavonoid, tannin, alkaloid and phenyl propanoids [117].The presence of lactic acid bacteria in fermentation contributes to the simple phenolic conversion and the depolymerization of high molecular weight phenolic compounds also reported that plant parts have an increase in total phenols after fermentation [118]. Methanol was more efficient to extract polyphenolic compounds. Phenolic compounds are generally more soluble in polar organic solvents. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction [119].

4.4.4. Determination of total Flavonoid

The result of total flavonoid content from extracts of Pagana flour was given in Table 4.5. The total flavonoid content varied from 32.29±1.91 to 80.53±9.97mg DE/g. Methanolic extract from boiled flour had the highest flavonoid contents (80.53±9.97 mg DE/g). There were significant differences ($P<0.05$) in total flavonoid content among raw and fermented methanolic extract, and boiling for methanolic extract had also a significant difference ($P<0.05$) increased the total flavonoid. The TFC from methanolic extract increased from 32.29±1.91 to 80.53±9.97 mg DE/g after boiling, and fermentation increased from32.29±1.91 to 42.18±1.23DE/g. Fermentation increased the flavonoid content might be due to microbial enzymes, such as glucosidase, amylase, cellulase, tannase, esterase, invertase or lipase produced during fermentation can hydrolyse glucosides, and break down plant cell walls or starch. These enzymes play a role in disintegrating the plant cell wall matrix and consequently facilitating the flavonoids extraction [120].Another mechanism is along fermentation the β-glucosidases of microbial origin could also be used to hydrolyze the phenolics and flavonoids.

Table 4.7 Total phenol and flavonoid contents of raw and processed Pagana flour

Sample type	Total phenol	Total flavonoid
raw	67.12±4.4^{c}	32.29±1.91^{d}
boiled	41.69±2.58^{d}	80.53±4.97^{a}
F24	72.21±3.90^{c}	31.87±.022^{d}
F48	142.79±3.77^{b}	34.07±1.65^{cd}
F72	58.61±2.26^{c}	42.18±1.23^{b}
F96	620.42±2.25^{a}	40.41±1.29^{bc}

All values are means of triplicate± standard deviation

Values which are followed by different letters of superscripts in the same column are significantly different

Key: a, b, c, d, e …. are superscripts given to show the significant difference between means, a > b > c > d>e

4.5. Physicochemical property of Pagana samples

The physicochemical properties of raw and processed paganna flours were presented in (Table 4.6). All results were based on dry basis of edible portions of paganna.

4.5.1. Tuber size

The size of pagana tuber is depends on the maturity and growth length of Pagana. According to my personal contact with local community of Konso revealed that Pagana tuber can stay more than two years under soil. During this period the size of corms/tuber increases.

The size of tuber collected for study ranged from 50g to 1334.83g.The average weight of tuber collected for analysis was 345g.

4.5.2. pH value

Table 4.6 shows the results of P^{H} values of the raw and processed samples. The P^{H} values for raw and processed Pagana sample ranged from 6.34 to 3.62. Fermentation had significantly ($P < 0.05$) reduced the P^{H} value of Pagana samples compared with raw. Boiling had no

significant effect (P > 0.05) on the P^H values of sample. Fermentation reduced the P^H values of sample at maximum fermented for time after three day by 42.90%; this was due to the fact that during fermentation some of the sugar present in Paganna was converted to organic acids. Acid production during Pagana fermentation might be attributed to the activities of the lactic acid bacteria on the carbohydrates of the Paganna tuber that results in the formation of lactic acid and formic acid where the formation of these acids in turn increases the rate of acid tolerant microorganisms such as *Geotriccum Candida* that converts lactic acid in to aldehydes and esters developing a product of good aroma and flavor due to the formation of these organic compounds [121].

4.5.3. Titratable acidity

The titratable acidity values of the raw and processed samples, which were expressed as percentage of lactic acid in the sample, ranged from 0.01 to 0.05, where the lower value belonged to the boiled Pagana sample and the higher for the fermented samples. The two processes had a significant effect (P < 0.05) on increasing titratable acidity (Table 4.5). Fermentation had a more significant effect on titratable acidity than boiling methods, which is evident that fermented foods have higher lactic acid content and lower P^H values.

Table 4.8.Titratable acidity of raw and processed pagana samples

Sample type	P^H	Titratable acidity
Raw	6.34 ± 0.18^a	0.025 ± 0.00^e
Boiled	6.30 ± 0.10^a	0.011 ± 0.00^d
F24	5.52 ± 0.03^b	0.031 ± 0.00^c
F48	4.66 ± 0.05^c	0.050 ± 0.00^a
F72	3.62 ± 0.05^d	0.036 ± 0.00^b
F96	3.62 ± 0.05^d	0.051 ± 0.00^a

All values of are the triplicate mean of ±standard deviation, n=3
The same subscript letter with column are not significant at (p>0.05)
Key; a, b, c, d ,e, are subscript give to show the significant difference between a>b>c>d>e

4.6. Functional properties

Functional properties of both raw and processed pagana flour were determined. These are bulk density, water and oil absorption capacity, foaming capacity, foaming stability, swelling power and solubility index.

4.6.1. Water absorption capacity

Water absorption capacity (WAC) of processed and raw Pagana sample was shown in (Table 4.6).The values ranged between 216 to 253.66. Boiling significantetly ($p<0.05$) increased water absorption of the sample compared to the raw Pagana . The increased water absorption capacity during boiling , was due to the loose structure of starch and removal of fat exposes the water binding sites of amino acids .Significant($p<0.05$) effect was observed during fermentation decreased water absorption capacity as fermentation time proceed [122]. The decreased of water absorption during fermentation could be attributed to the increment in fat content, as fat block the hydrophilic sites of amino acids and carbohydrate. Water absorption capacity describes flour water association ability under limited water supply. Water binding capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations [123].

4.6.1.1. Oil absorption capacity

Oil absorption capacity is another important functional property since it plays an important role in enhancing the mouth feel while retaining the flavor of food products [124].It has been reported that variations in the presence of non-polar side chains, which bind the hydrocarbon side chains of oil among the flours, explain differences in the oil binding capacity of the flours [122].The results of oil absorption capacity of the sample was shown in (Table 4.6). Boiling and fermentation has a significant effect ($P<0.05$) on the reduction of oil absorption capacity. The oil absorption capacity of samples ranged from 0.80 to 1.23. This study indicates that

boiling reduced oil absorption than fermentation. Oil absorption is an important property in food formulations because fats improve the flavor and mouth feel of foods [125].

4.6.2. Bulk density

The results of the bulk density for the raw and processed samples were given in (Table 4.6.) The bulk density values for Pagana flour samples were, ranged from(0.833 to 0.75)g/ml. These values shows that boiling had a significant effect on ($P < 0.05$) increased the bulk densities of the sample and fermentation had also a significant effect ($P < 0.05$) on bulk density compared to raw sample. Generally Pagana boiled sample had higher bulk density than flours fermented. Having higher bulk density is an advantage because it takes less packaging materials and hence less cost of packaging [126]. Bulk density is influenced by the structure of the starch polymers and loose structure of the starch polymers could result in low bulk density. Bulk density is very important in determining the packaging requirement, materials handling and application in wet processing in the food industry [127].

4.6.3. Foaming capacity

The results showed that the foaming capacity of Pagana were ranged from 8.66 to 14.33 (Table4.9). The maximum values were found to 72and 96hrs fermented Pagana and while the minimum values found in raw sample. Foaming ability was related to the amount of solubilized protein and the amount of polar and non-polar lipids in a sample [128].It is also dependent on the configuration of protein molecules [129].Boiling had a significant effect ($P < 0.05$) on reducing the foaming capacity. The reason for the reduction of foaming capacity during boiling was due to the fact that proteins were denatured irreversibly that resulted the lower protein content which in turn reduce foaming capacity. Foam formation and stability were a function of the type of protein, pH, processing methods, viscosity and surface tension [130].The increase and decrease of the foam capacity is due to the increase and decrease of the protein content due to processing. The foam ability of flours has been shown to be related to the amount of native protein. Native protein gives higher foam stability than the denatured protein. It was related to the amount of solubilized protein [126]. Soluble proteins in general play an important role in the formation of foam and this probably justify why legumes exhibit

higher foaming capacity [59].Fermentation significantetly ($p<0.05$) increased the foam capacities compared to the raw samples. For increased protein and foam capacity, the most probable reason for an increase in the foaming capacity of flour was due to an increase in the percentage of protein content by fermentation process. The low foam capacity may be attributed to the low protein content of the flour since foam ability was related to the amount of solubilized protein and the amount of polar and non-polar lipids in a sample [128].

4.6.3.1. Foam stability

The foaming stabilities of Pagana flours were presented in (Table 4.6) and the values ranged from 3.00 to 9.83ml/g of sample. The maximum determination was obtained for fermented to 96hrs and the minimum determination was found to raw sample. From the result, it was shown that fermentation significantly ($P < 0.05$) increased the foam stability. Boiling significantly ($P < 0.05$) reduced foaming stability in sample. Foam stability is important since the usefulness of whipping agents depend on their ability to maintain the whip as long as possible [131].

4.6.4. Swelling power

The swelling power of the processed and raw Pagana was ranged from (12.89 to 36.26)ml/g.Here the maximum swelling power was observed for boiled sample and the minimum value for 72hr fermented sample. The results of swelling power of the sample were shown in the (Table 4.6).Fermentation significantetly ($p<0.05$) reduced the swelling power at maximum fermentation time to 96 hour. Boiling also significant effect ($p<0.05$) on swelling power reduced when compared to raw sample. The swelling power of flour samples is often related to their protein and starch contents [132]. A higher protein content in flour may cause the starch granules to be embedded within a stiff protein matrix, which subsequently limits the access of the starch to water and restricts the swelling power. In addition to protein content, a higher concentration of phosphorous may increase hydration and swelling power by weakening the extent of bonding within the crystalline domain [133].Furthermore, the amylopectin is primarily responsible for granule swelling, thus higher amylose content would reduce the swelling factor of starch [134].The variation in the swelling power indicates the

degree of exposure of the internal structure of the starch present in the flour to the action of water [135].Boiling enhances the WAC of the flours and hence increased the swelling powers, as swelling power of starch based flour is related WAC of the flour during heating [136].

4.6.4.1. Solubility index

The results of the solubility index for the samples were shown in (Table 4.6). From the table it was observed that the solubility indexes for the Pagana sample were between 7.83 to 17.66.The maximum was absorbed to the raw sample and the minimum to boiled sample. From the results it observed that both process had a significant effect ($P < 0.05$) on reduction of solubility index compared with raw sample. Boiling and fermentation reduced the solubility index of Paganna flour. As a direct result of flour swelling, there is a parallel increase in the solubility of flour. High solubility implies high leaching. The high water solubility of any sample analyzed may be attributed to the degree of swelling power and swelling power and solubility of the flour provide evidence of non-covalent bonding between molecules within the flour [137].

Table 4.9 Functional properties of raw and processed pagana flour

Sample type	BD(g/ml)	FC(ml/g)	FS(ml/g)	WAC(ml/g)	SI (%)	OAC(ml/g)	SP(g/g)
raw	**0.83±00^{b}**	**8.66±0.57^{b}**	**3.0±0.10^{d}**	**253.66±0.57^{b}**	**17.66±0.57^{a}**	**1.23±0.00^{a}**	**17.19±0.00^{c}**
boiled	**1.02±0.28^{a}**	**5.83±0.28^{c}**	**2.03±0.28^{e}**	**370.00±2.08^{a}**	**7.83±5.48^{b}**	**0.80±0.10^{c}**	**36.26±0.55^{a}**
F24	0.77±0.02^{c}	9.33±0.57^{b}	8.00±0.10^{b}	240.66±0.57^{c}	12.33±0.57^{ab}	1.00±0.00^{b}	19.34±0.30^{b}
F48	0.83±0.03^{b}	13.83±0.76^{a}	9.83±0.28^{a}	226.66±0.57^{e}	9.50±4.94^{b}	1.00±0.00^{b}	12.12±0.55^{d}
F72	0.75±0.00^{c}	14.00±0.76^{a}	8.5±0.05^{b}	216.00±1.00^{f}	14.66±0.57^{ab}	1.00±0.00^{b}	12.89±0.57^{e}
F96	0.78±0.01^{bc}	14.33±0.28^{a}	6.50±0.05^{c}	231.66±0.57^{d}	12.66±0.57^{ab}	1.00±0.00^{b}	17.29±0.25^{c}

All values are the means of triplicates ± standard deviation n=3
Means with the same superscript letters within a column are not significantly different at (P>0.05)
a ,b, c ,d are subscript to show significance difference between means a>b>c>d

key: BD=bulk density, FC=foam capacity, FS=foam stability, WAC=water absorption capacity, SI=solubility index, OAC=oil absorption capacity, SP=swelling power F24=fermented for 24 hours, F48=fermented for 48 hours F72 fermented for 72 hours F96=fermented for 96hrs

Summary of comparison of chemical composition of *Amorphophallus konjac* with *Amorphophallus gomboczianus*

Tuber type	%protein	%carbohydrate	%mineral	%ash
A.*gombozianas*(Paganna)	7.7	82.62	1.2-7.7	6.46
A. Konjac	5-14	40-60	2.6-7	3.4-5.3

Note: *Amorphophallus konjac* have potential as a nutraceutical, or pharmaceutical product for use in the treatment of obesity, obesity-related dyslipidemia diabetes, hyperlipidaemia and hypercholesterolemia, and diabetes [17,18].With these similar chemical composition the above (Table) and the same family with *A. konjac, Amorphophallus gomboczianus* (Paganna) might have the same pharmaceutical properties, therefore further study is necessary on Pagana for these interesting pharmaceutical property.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Wild and semi-wild edible plants are an integral part of the diet in Konso communities. This study attempted to investigate the effect of two processing methods (boiling, and natural fermentation) on nutritional composition, antinutritional factors, antioxidant activities, physicochemical and functional properties of raw and processed Pagana flour. From the results of the present study it was understood that Pagana contains appreciable quantity of carbohydrate, crude fiber, crude protein, calcium, potassium and iron. The results of this study also showed that Pagana contains low levels of antinutrients (phytate and tannin) except oxalate when compared to other root and tuber crops. Moreover, there were further reductions of the antinutritional factors during processing. Additionally it was observed in the study that functional property of Pagana flour is remarkably higher. High water absorption capacity, swelling power, foaming capacity and foam stability than other root crops flour was recorded. But the solubility index and oil absorption capacity was in the range. Reduction of titratable acidity of the samples also observed. Evaluation of the two processing methods in terms of antinutrients reduction and nutrients enrichment indicated that all the two processing method was found to be effective in the reduction of antinutrients but, effect of natural fermentation was found to be highest in the reduction of antinutritional factors. In relation to nutritional profile, the low protein content of Pagana flour sample was observed to be increased by fermentation and total phenol and flavonoid contents also increased by fermentation. Natural fermentation of Pagana flour is a more acceptable process as it is inexpensive, fuel efficient method and environmentally friendly by which people can obtain good quality food and this process can only be performed at their own homes. However, boiling was found to decreased most of the functional properties of pagana flour. Fermentation increased oil absorption and reduced most of functional properties of pagana samples.Therefore among the two traditional applied processing methods natural fermentation for 72hrs without starter culture is recommended process.

5.2. Recommendations

Due to the low attention given to wild edible plant in Ethiopia and limitation of Pagana in specific region and yet no research on it, Pagana cannot known as functional food

- Encourage public awareness on the importance of wild edible Pagana and community based management of these plant resources through avoiding antinutritional factors by processing.
- Encourage research on this plant, especially for its potential as a nutraceutical, or pharmaceutical product for use in the treatment of obesity, obesity-related dyslipidemia diabetes, hyperlipidaemia and hypercholesterolemia, because *Amorphophallus konjac* with the same family have these function
- Encourage farmers to cultivate and conserve edible wild Pagana that grow in their farmlands to be used in times of drought.
- Analysis should be done for the vitamins and other minerals content of Pagana
- Investigation on more specific properties, such as baking property, of these composite flours should be undertaken in the future to fully investigate their specific application.
- Pagana is an excellent source of carbohydrate. Therefore it can be used for industrial raw material for starch production.
- Functional property and physicochemical properties of Pagana is relatively higher than other tuber plants, therefore it is a good input for product formulation with other crop
- The starch characteristic should be studied how much amylose/amylopectin, gelatinization.
- Sensory evaluation of the product developed

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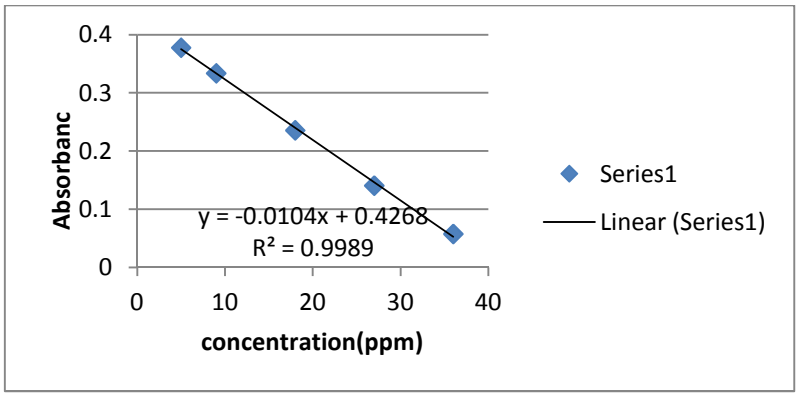
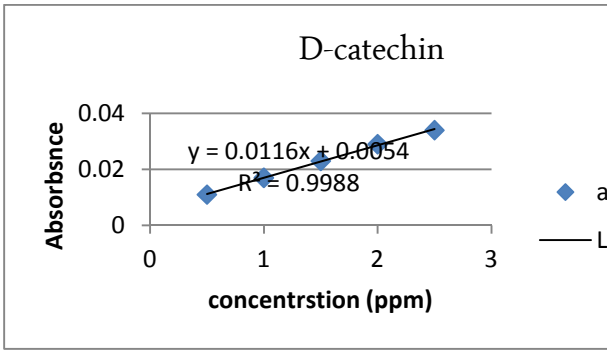
Appendix-1

1. Standard curve of concentration verses absorbance

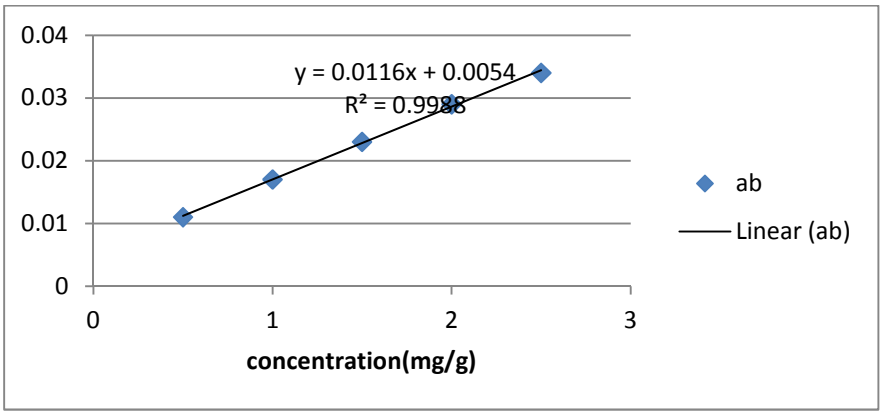
1.1.Phytic acid curve



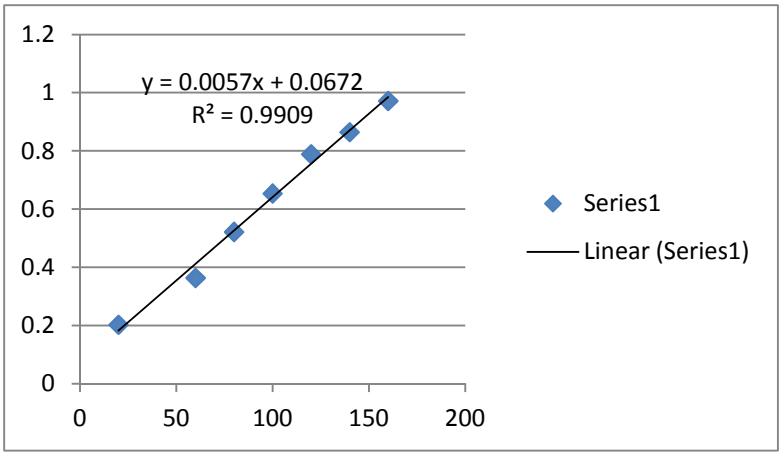
1.2. D-Catechin curve



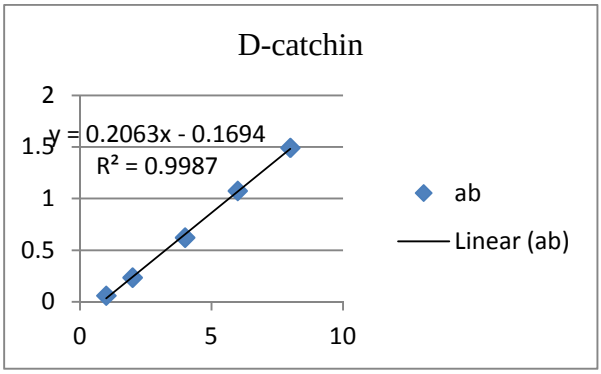
1.3 . Standard curve for phosphorus



1.3. Standard curve for phenol analysis



1.4. Standard curve for flavonoid



con(g/l)	ab
1	0.0605
2	0.2355
4	0.6225
6	1.0745
8	1.4915