MICROBIOLOGICAL AND BIOCHEMICAL CHANGES DURING THE CO-FERMENTATION OF BITTER YAM (*Dioscorea dumetorum*) AND COWPEA (*Vigna unguiculata*) AS INFANT COMPLEMENTARY FOOD

BY

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CERTIFICATION

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DEDICATION

This project work is dedicated to God Almighty, my dear parents and my wonderful siblings, for their support financially, morally and materially. Also to my course mates, friends and well-wishers for their support during the project practical. I love you all. Thank you and God bless you all.

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Abstract

Cowpea grains and bitter yam slices in ratio 7:3w/w and 6:4w/w co-fermented anaerobically for 72 hours at room temperature were studied. During the 72hours of fermentation, the hydrolysis of the carbohydrate and the subsequent conversion of sugars and minerals by the fermenting microbes must have contributed much especially to the increased microbial load. The controls for the samples were the unfermented food samples in the same ratio as the co-fermented samples. The fermenting of food samples were subjected to microbial analysis, biochemical tests, pH, titratable acidity, consistency, sensory evaluation, mineral analysis, proximate analysis, anti-nutritional factors using the standard methods. Pasting properties were determined using the Bostwick consistometer. The pH had rapid reduction at 24h from 6.8 to 6.0 and reduced gradually to 5 by 72h while total titratable acidity increased gradually during this period. . Microbial counts decreased as fermentation progressed to 72hours. Meanwhile, the lactic acid bacteria reduced as the fermentation period increased. The sugar tests carried out on the isolated organisms were all positive. Organoleptically, the fermented food sample had the highest rate for general acceptability by the panelist but the unfermented sample was accepted than the co-fermented sample in terms of colour, aroma, taste and texture. Sample BB was slightly more reduced in pH than AA and as the pH reduces, the TTA increased in all the co-fermented samples. For the consistency at 10g, co-fermented samples was too thick in flow measurement meaning higher viscosity. Sample AA had the highest value of vit. A. Sample DD and CC had the highest value for calcium followed by AA and BB. At 10^5, CC and DD had the highest count with the co-fermented having the lowest.

1.0 INTRODUCTION

Malnutrition often begins at conception and child malnutrition is linked to poverty, low levels of education, and poor access to health services, including reproductive health and family planning. Since all children deserve good care, nutrition and health that encourage their social, emotional, psychological, physical and intellectual growth, there is the urgent need for an enabling environment through well-articulated policies, projects, and programmes/interventions to not only enhance their quality of life, but to also aid the wholesome development of Nigerian children (Ojofeitimi, 2007).

The cost of the standard complementary infant foods being sold in the markets, supermarkets and other places seems too high, therefore making it a bit difficult for less-privileged mothers to afford leaving them with no other option than to improvise and formulate the normal 'ogi' which is less nutritional compared to when other legumes, tubers and cereals are added.

Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide additional nutrients. For an infant, good nutrition is essential for the first year because it's the stage that will determine the rate of his physical, psychological growth due to the quality of the food(s) intake. When developing, infants are fed the appropriate types and amounts of foods, their health is promoted.

This study shows the changes occurring during the microbiological and biochemical stages of the co-fermentation process of bitter yam (*Dioscorea dumetorum)* and cowpea (*Vigna unguiculata*) which should serve as an infant complementary food.

1.0.1 BITTER YAM (*Dioscorea dumetorum)* description

Bitter yam is a member of the ***Dioscorea*** family. It is widely cultivated in West Africa, especially in Nigeria where it is locally called ***Esuru.*** This species is found wild throughout tropical Africa between 15°N and 15°S. Bitter yam is available in different species including: yellow specie and white specie. They may be single or usually cluster in shape, robust and spiny.

Trifoliate yam *Dioscorea dumetorum* is among the underutilized yam species and it is known by various names which include three leaved yam, bitter yam and cluster yam. The tuber is large and coarse; one plant usually produces a cluster of tubers which are bitter due to the presence of alkaloids dioscorine. Trifoliate yam is high yielding compared to other species and the starch grains are smaller, more soluble and more digestible than those of other species. The proteins are however more balanced than those of white yam and it is also rich in vitamins and minerals. The consumption of trifoliate yam is restricted due to the bitter taste, inability to keep for longer time after harvesting and poor binding capacity of the flour.

Trifoliate yam (*Dioscorea dumetorum*) is however a lesser-known yam among the species and underutilized. The tubers are eaten during the time of famine or scarcity and are usually boiled with the peel and eaten as boiled yam. The tubers are eaten during the time of famine or scarcity and are usually boiled with the peel and eaten as boiled yam. Trifoliate yam tubers contain alkaloid dihydrodioscorine which causes paralysis of the nervous system (Degras, 1993). These alkaloids are reported to be water soluble and can be dissipated by soaking and boiling (Eka, 1998). Also trifoliate yam hardens few days after harvest and this leads to reduction in moisture and starch content and increase in sugars and structural polysaccharides (Sefa-Dedeh and Afoakwa, 2001).

1.0.2 Cultivation/planting

A deep well-drained sandy loamy soil is required for optimum yields and a good drainage is essential. Fertilizers are not widely used but there is a wide response to treatment, particularly to the application of phosphorus and potassium. The application of potash alone has given yield increases in Nigeria, and it is also reported to increase the storage life of the tubers, while chlorine in the fertilizer adversely affects the starch content.

Setts are normally used for the planting but satisfactory results have been obtained with vine cuttings. *Araecerus fasciculatus* and *Lepidobregma minuscula* have been identified in Nigeria as infesting insects of tubers of *D. dumetorum* (Purseglove, J. W. 2002).

Most edible yams cannot withstand frost and they make poor growth at temperature below 20°C. Although they are generally considered drought resistant, they require adequate moisture throughout their growing period. Adequate moisture between the 14^{th} and 20^{th} weeks of growth is of great importance for optimum yields. Its growth period is usually between 8-10 months. Ref

D. dumetorum is easily harvested by hand (and could be mechanically harvested were it grown on the large scale). The tubers do not store well, a high proportion becoming hard and inedible within 4 weeks after lifting. Drying of sliced tubers is used as a method of storage (Passam, H. C. 2011).

1.0.3 Nutritional value

A typical analysis of the edible portion of the tuber is:

Water – 79%, Protein – 2.78%, Fat – 0.28%, Carbohydrate – 17%, Fibre – 0.3%, Ash – 0.72%, Calcium – 92mg/100g, Ascorbic acid – 6.6mg/100g. The carbohydrate consists mainly of starch, the granules of which are small, rounded or polyhedral, average size 1-4 microns, with a gelatinisation temperature of 77-85.5°C. Analysis of the starch of *D. dumetorum* has been given as: moisture 13.5%; protein 1.49%; ash 0.39%; amylose 15%; pH 4.4; iod. val. 3.9. Many forms of *D. dumetorum* contain a convulsant alkaloid which is a mixture of stereoisomers of dihydrodioscorine (Rasper, V. and Coursey, D. G. 2007).

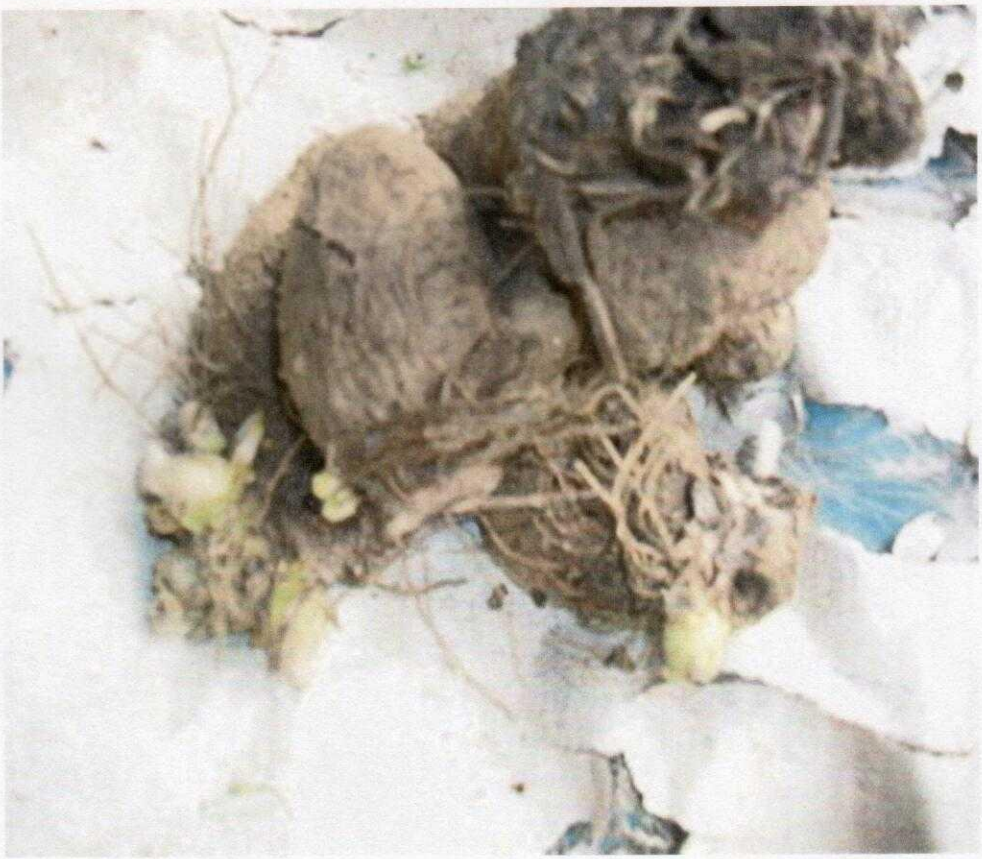


Fig 1: Clustered bitter yam

Source: Wikipedia

1.1 COWPEA (*Vigna unguiculata*) description

Cowpea is an important food legume grown in the semi-arid tropics, covering Africa, Asia, Southern Europe and Central South America (Davis, 2013). It is one of the ancient crops known to man and is cultivated primarily for grain, but also as vegetable (leafy green, green pods, shelled dried peas, and fresh shelled green peas), a fodder and cover crop. Moreover, cowpea forage is significant to animal feeds mainly during the dry season when the demand for the feeds is at its peak. Its ability to replenish soil nitrogen gives it a key position in the modern crop farming system in rotation with other crops, with the view for long term sustainable agriculture development prospect.

According to Tarawali *et al.*, (2002), he noted that cowpea has a wide role in contributing to food security, income generation and sustainable environment for millions of small scale farmers who cultivate it in the region. Cowpea also contains bioactive antioxidants such as vitamin c, carotenoids and phenolic compounds. The compounds represent a crucial group of bio-active elements in foods which prevents development of diseases such as atherosclerosis and cancer (Omae, 2011). Due to the increase in the demand for the crop, arising from the growing population in the country, Nigeria remains the largest producer and consumer of cowpea both in West Africa and in the World (FAO, 2010 and IITA, 2011).

The cowpea (*Vigna unguiculata*) is one of several species of the widely cultivated genus *Vigna*. Most cowpeas are grown on the African continent, particularly in Nigeria and Niger which account for 72% of world cowpea production. It originated and was domesticated in Southern Africa and was later moved to East and West Africa and Asia. More than 5.4 million tons of dried cowpeas are produced worldwide, with Africa producing nearly 5.2 million. Nigeria which

is the largest producer and consumer, accounts for 61% of production in Africa and 58% worldwide. Africa exports and imports negligible amounts.

In Nigeria, cowpea adapts well to wet conditions, as well as by supplementary irrigation or residual moisture along river or lake flood plains during the dry season, in as much as the range of minimum and maximum temperatures is between 28° and 30°C (night and day) during the growing season. Cowpeas are well-adapted to the drier regions of the tropics where other food legumes do not perform well. It is shade tolerant and so it is compatible as an inter-crop with maize, millet, sorghum, sugarcane and cotton.

Cowpea performs well in agro ecological zones where rainfall range is between 500 mm and 1,200 mm/year. However, with the development of extra-early and early maturing cowpea varieties, the crop can thrive in the Sahel where rainfall is less than 500 mm per year. It is tolerant of drought and well adapted to sandy and poor soils. However, best yields are obtained in well-drained sandy loam to clay loam soils with the pH range of between 6 and 7. Cowpea yields in Niger state and Nigeria have been on the increase between 1999 and 2010, rising from 490 kg/ha to 651Kg/Ha in the case of the former and 474Kg/Ha and 687Kg/Ha in the case of the latter, with average growth of 3% and 4% respectively (FMARD, 2011).

1.1.1 Harvesting

The crop can be harvested in three stages: while the pods are young and green, mature and green, and dry. Cowpeas are grown mostly for their edible beans, although the leaves, fresh peas and fresh pea pods can also be consumed, meaning the cowpea can be used as a food source before the dried peas are harvested. Cowpeas thrive in poor dry conditions, growing well in soils up to 85% sand.

1.1.2 Nutritional value

Cowpea contains 20% - 25% of protein, several minerals, vitamins and 64% carbohydrate and has potential for poverty alleviation and malnutrition amongst the poor (Alice, 2010). Cowpeas provide a rich source of proteins and calories. All parts of the cowpea crop are used as all are rich in nutrients and fibre. Cowpeas are rich in potassium with a good amount of calcium, magnesium, and phosphorus. It also has a small amount of iron, sodium, zinc, copper, manganese, and selenium. Cowpeas are rich in vitamin A and C. It has a good amount of thiamin, riboflavin, niacin, vitamin B6, and pantothenic acid. It also has a small amount of folate. 100 grams of Cowpeas have 44 calories. Calories from fat are only 3 K cal (IITA, 2011).

Fig 2: Cowpea

Source: Wikipedia

1.2 FERMENTATION

Fermentation is a metabolic process that converts sugar to acids, gases, and/or alcohol. It occurs in yeast and bacteria, but also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a substrate often with the goal of producing a specific product. A great number of unfermented foods primarily derived from plant materials have been an indispensable dietary element in many parts of the world. In other parts of the world, bacteria and yeasts traditionally dominate the fermentation of locally grown cereal grains or cereal grains supplemented with a locally grown legume or milk product. At a relatively low cost, these traditional processes have converted plant materials into culturally acceptable foods that are palatable, safe, economical and often nutritious (Khandwala, *et al.,* 2005).

1.2.1 Spontaneous fermentation

Many food fermentations rely on spontaneous fermentation by the indigenous microbiota present in the food substrate. This implies that variations in the indigenous biota may affect the composition and activity of the fermenting community. This has a direct effect on product quality and the reproducibility of fermentations. With few exceptions, food fermentations rely on mixed cultures of microorganisms. Most substrates for food fermentations have a highly heterogeneous physicochemical composition which offers the possibility for the simultaneous occupation of multiple niches by "specialized" strains, for instance, through the utilization of different carbon sources. Lactic acid spontaneous fermentation is one of the ancient methods of food processing and preservation especially cereals and legumes.

Lactic acid bacteria (LAB) are the most commonly used microorganisms for preservation of foods and their importance is associated mainly with their safe metabolic activity while growing in foods utilizing available sugar for the production of organic acids and other metabolites. Their common occurrence in foods and feeds coupled with their long-lived use contributes to their natural acceptance as GRAS (Generally Recognized As Safe) for human consumption (Aguirre & Collins, 1993). Many microbiological studies deal with identification of organisms isolated from various fermented foods.

However, there are many kinds of fermented foods in which the dominating processes and end products are contributed by a mixture of endogenous enzymes and other microorganisms like yeast and mould especially where we have multi-components as substrates. Very often, a mixed culture originating from the native microflora of the raw materials is in action in most of the food fermentation processes.

1.2.2 What are fermented foods?

Campbell-Platt (1987) has defined fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. Fermented foods are foods that have been through a process of lacto-fermentation in which natural bacteria feed on the sugar and starch in the food creating lactic acid. This process preserves the food and creates beneficial enzymes, B-vitamins, Omega-3 fatty acids and various strains of probiotics.

Fermented foods also have strong and pungent flavous. Fermented foods contain folic acid which is very important for pregnant women in preventing birth defects. Whichever way in which the

definition is used, foods submitted to the influence of lactic acid producing microorganisms is considered a fermented food. A food is considered fermented when one or more of the constituents of its substrate have been acted upon by selected microorganisms or their enzymes to produce a significantly altered final product desirable for human consumption.

1.2.3 Nutritional value of fermented foods

Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value, and often the content of water-soluble vitamins is generally increased, while the anti-nutritional factors show a decline during fermentation (Paredes-López & Harry, 2006). Fermentation results in a lower proportion of dry matter in the food and the concentrations of vitamins, minerals and protein appear to increase when measured on a dry weight basis (Adams, 2002).

1.2.4 The advantages of fermented foods are as follows:

- Probiotic power (i.e. growth of beneficial microorganisms) which helps to restore the correct balance of bacteria in the gut. According to an article published in the "Journal of Applied Microbiology" in June 2006 noted that eating foods that contain lactic acid bacteria improves intestinal tract health, improves the bioavailability of nutrients, reduces symptoms of lactose intolerance and decreases the prevalence of allergy in susceptible people.

  A fermented food product or live microbial food supplement which has beneficial effects on the host by improving intestinal microbial balance is generally understood to have probiotic effect (Fuller, 2009).

- **Flatulence reducing effect**: based on the research carried out during fermentation of the beans for preparation of tempe, the trypsin inhibitor is inactivated, and the amount of several oligosacharides which usually cause flatulence are significantly reduced (Hesseltine, 2011). Bean flour inoculated with Lactobacillus and fermented with 20% moisture content, showed a reduction of the stachyose content (Duszkiewicz-Reinhard, *et al.* 2008)
- **Anticancerogenic effect:** Apart from this, there are interesting data on anticarcinogenic effect of fermented foods showing potential role of lactobacilli in reducing or eliminating procarcinogens and carcinogens in the alimentary canal (Reddy, *et al.,* 2001; Shahani, 2004; Mital & Garg, 2004). The enzymes β-glucuronidase, azoreductase and nitroreductase, which are present in the intestinal canal, are known to convert procarcinogens to carcinogens (Goldin & Gorbach, 2000). An article published in "Current opinion in Lipidology" in February 2006 noted that there is evidence that fermented milk product have a mildly decreasing effect on abnormally high blood pressure, known as hypertension.

  The processes of fermentation of foods are also reported to reduce the mutagenicity of foods by degrading the mutagenic substances during the process.
- Fermented foods contain folic acid which is very important for pregnant women in preventing birth defects.
- They are also rich in enzymes which are needed for digestion and absorption of food.
- It increases the flavor of food.
- It increases the shelf-life of food.

It has been estimated that more than 13 million infants and children under five years of age die annually in the tropical regions of the world. After respiratory infections, diarrhoea diseases are the commonest illnesses and have the greatest negative impact upon the growth of infants and young children. The causes of diarrhoea have traditionally been ascribed to water supply and sanitation (Motarjemi, *et al.,* 2004). Foods prepared under unhygienic conditions and frequently heavily contaminated with pathogenic organisms play a major role in child mortality through a combination of diarrhoea diseases, nutrient malabsorption, and malnutrition. All food items contain microorganisms of different types and in different amounts. Which microorganisms that will dominate depends on several factors, and sometimes microorganisms initially present in very low numbers in the food, for example lactic acid bacteria (LAB), will outnumber the other organisms inhibiting their growth. In contrast to fermented meat, fish, dairy and cereal products, fermented vegetables have not been recorded as a significant source of microbial food poisoning (Fleming & McFeeters, 2000)

2.0 LITERATURE REVIEW

The purpose and type of processing applied in preparing complementary foods for infants should be for separation and purification of the edible parts of raw material and most importantly it should also improve the nutritional qualities by enrichment and inactivation of the anti-nutritional factors. The low socio-economic status mothers are not adequately knowledgeable about the available nutrients and the anti-nutritional factors in the raw materials (Omoruyi, *et al.*2007). So gruels prepared from fermented cereals are of high viscosity and low in energy density; therefore, they are not potentially nutritious enough to be used as complementary foods. It is therefore desirable to propose a processing method in which cereals and cowpeas can be jointly processed to reduce labour, cost, energy and loss of nutrients that could arise from separate processing of cereals and cowpeas.

Triofoliate yam has been reported to be nutritionally superior to the commonly consumed yams with high protein and mineral content (Martin, *et al.,* 1983). Processing of trifoliate yam tubers into flour have been reported to be a means of adding longer term value to the tubers with a high nutritional potential (Medusa, *et al.,* 2005). The ultimate quantity of yams that can be utilized in a processed form would depend on availability in many end- use forms. Dry snacks made from yam, with or without nutrient fortification, would encourage its increased use (Okaka and Okechukwu, 1987).

Research has been carried out on the nutritional value, biochemical, textural and physicochemical changes in trifoliate yam (Martins, *et al.,* 1983; Sefa-Dedeh and Afoakwa, 2001; and Medusa, *et al.,* 2005). Study needs to be done on the effect of various processing methods on the physico-chemical properties of trifoliate yam flour.

Aderiye and Ogunjobi (1998) reported that the organisms implicated in the fermentation of cooked fermented yam included the species of Pediococcus, Lactobacillus and Pseudomonas, Bacillus subtilis and two other Gram negative coccal cells. They further reported that the decrease in microbial counts at the latter stage of fermentation was due to the high total acidity of the medium of about 2.67 times the initial value of 0.027% lactic acid and that the lactic acid bacteria increased continuously throughout the period of fermentation.

Legumes have been underutilized because of the presence of antinutritional factors such as enzymes (like trypsin, chymotrypsin, α-amylase) inhibitors, phytic acid, flatulence factors, saponins and toxic factors, lectins and the need for prolonged cooking (Lyimo, *et al.,* 1992). Fermentation of common beans has not been extensively studied but it has been shown to produce a reduction in nutritional factors, positive effects on protein digestibility, texture and aroma and an improvement in the biological value of legumes (Salunkhe and Kadam, 2000).

Yam (*Dioscorea* sp.) is one of the most important food crops in West Africa especially Nigeria and is well accepted as a staple food in most homes (Igyor *et al*., 2004). The most cultivated species in Nigeria are the white yam (*D. rotundata*), yellow yam (*D. cayenensis*), water yam (*D. alata*) and trifoliate yam (*D. dumetorum)* (Amusa, *et al.,* 2003). Trifoliate yam (*Dioscorea dumetorum*) is however a lesser-known yam among the species and underutilized. The tubers are eaten during the time of famine or scarcity and are usually boiled with the peel and eaten as boiled yam. Trifoliate yam tubers contain alkaloid dihydrodioscorine which causes paralysis of the nervous system (Degras, 1993). Also trifoliate yam hardens few days after harvest and this

leads to reduction in moisture and starch content and increase in sugars and structural polysaccharides (Sefa-Dedeh and Afoakwa, 2001).

Trifoliate yam *Dioscorea dumetorum* is among the underutilized yam species and it is known by various names which include three leaved yam, bitter yam and cluster yam. The tuber is large and coarse; one plant usually produces a cluster of tubers which are bitter due to the presence of alkaloids dioscorine. Trifoliate yam is high yielding compared to other species and the starch grains are smaller, more soluble and more digestible than those of other species. The proteins are however more balanced than those of white yam and it is also rich in vitamins and minerals. The consumption of trifoliate yam is restricted due to the bitter taste, inability to keep for longer time after harvesting and poor binding capacity of the flour.

According to Oyarekua (2013), cereal-based traditional complementary foods commonly fed to infants are inadequate to meet daily nutrients and energy requirements, while infant formula foods are too expensive for mothers of low socio-economic status.

Cowpea (Vigna unguiculata) is one of the most important food legumes grown throughout the tropics and the black-eyed variety is mostly grown in West Africa. Dry cowpea has little vitamin and more than human requirements of thiamin and nicotinic acid. Methionine is the most limiting amino acid. Cowpea provides a significant level of dietary protein and lysine in regions of chronic protein shortage like Nigeria. Studies have shown several applications of legume proteins which have a potential role as substrates for the development of complementary foods due to the high nutritional value (Pereira, *et al.*, 2009). Various processing methods have been employed to improve the nutritional quality and utilization of cowpeas. Many efforts had been made to improve the nutritive value of cereal by supplementation with legumes.

Fermentation has been reported to improve the nutritive value of cowpea (Fields, 1980). Osundahunsi, and Aworh (2002) observed improved 70:30 mixture of maize/ soybean; Ojofeitimi *et al.,* (1984) studied black-eyed cowpea (ewa) and maize gruel ogi as and reported that babies on ewa-ogi showed disappearance of physical kwashiorkor. (Bressani, 1985). Wannik, *et al.,* 1994) investigated the effect of roasting and fermentation (with natural inocula) on the viscosity of maize-sorghum-soya porridges and reported that natural fermentation of mixed ingredients resulted in lower porridge viscosities when pH was 5.0-5.5, at lower pH viscosity of final porridge increased while roasting of the mixture resulted in higher viscosity. Microbial fermentation provides a way to food preservation, reduction in volume of material to be transported, inhibition of microbial growth, improvement of appearance and taste of some foods and reduce the energy required for preparing food, safer food products and enhancement of nutritive value. (Afoakwa, *et al.* 2007).

The presence of antinutrients and food toxicants limit the full utilization of cereal- legume based food mixtures by humans (Hsu, *et al.* 2006). This is because the foods are unprocessed to reduce food toxicants and other antinutrients to safe levels. Fermentation, dehulling, drying and milling are economic domestic food processing techniques used at homes to improve and increase nutrient density, acceptability, quality, availability, flavour, aroma and palatability (Wang, 1968; Beuchat and Worthington, 1974; Odunfa, 1985; Hotz and Gibson, 2007) . They reduce bulk, viscosity and antinutrients (Nnam, 2002).

Not many studies have been carried out on co-fermentation of bitter yam tuber and legumes by using traditional processing methods of fermentation. Therefore, this study was undertaken to formulate inexpensive processing cofermentation technique that can be carried out at household

level to produce suitable complementary food mixtures with low- cost locally available raw materials that are bio-compatible.

2.1 JUSTIFICATION

Not much has been reported on the co-fermentation of bitter-yam and cowpea. Also in Nigeria bitter-yam and cowpea have not been used as infant complementary food. Reports of *Dioscorea dumetorum* and *Vigna unguiculata* have shown that the two food staples contain protein, carbohydrate, water, vitamins which are of great benefit to the body.

It is noted that the evaluation of *Dioscorea dumetorum* and *Vigna unguiculata* in general is faced with some problems. Firstly, is the bitter taste of the yam which makes it unfit for consumption except it undergoes several processes (Plumbey and Rees, 2001). Secondly, it undergoes very difficult task to process it before becoming fit for consumption. Another problem of this specie of yam is that it is going into extinction, the knowledge of the existence and uses of this yam is limited and this has resulted in less planting and cultivation of the crop (Plumbey and Rees, 2001). Also, *Vigna unguiculata* when soaked for a day becomes smelly talkless of soaking it for 3-4days without decanting the water and now the combination of the two food staples together might give a quiet offensive smell/odour.

This study has become necessary to find out if only the fermented food samples would be good enough for infant complementary food or the unfermented food samples with or without any complications.

2.2 AIM AND OBJECTIVES OF THE STUDY

2.2.1 AIM OF THE STUDY

The aim of this research work is to improve the nutritional status/quality of complementary foods for infant.

2.2.2 OBJECTIVE OF THE STUDY

Not so much work has been done on the co-fermentation of bitter yam and cowpea and cowpea is not usually used for infant's complementary food. Likewise bitter yam.Therefore the objectives of this study is to:

1) To determine the effect of co-fermentation on microbiological and nutritional status of bitter yam and cowpea.
2) To determine the organoleptic acceptability of the product.
3) To examine the microbiological and bio-chemical changes involved during the co-fermentation of bitter yam and cowpea.
4) To evaluate the nutritional and microbiological values of the co-fermented end product.
5) To determine the sensory evaluation using nursing mothers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study was conducted at the laboratory of the Department of Microbiology, Federal University Oye Ekiti, Ekiti State and the Microbiology laboratory of Nigerian Institute of Science and Laboratory Technology, Samonda, Ibadan, also at the laboratory of the Department of Animal production and Health, Nutrition Laboratory, Federal University of Technology Akure, Ondo State.

3.1 Source of food samples

Bitter yam *Dioscorea dumetorum* was gotten from Old secretariat road, Ado Ekiti, Ekiti State while the Cowpea *Vigna unguiculata* was gotten from Oja-oluwa market, Oye. Ekiti State, Nigeria.

3.2 Preparation of food samples

After the distribution of the Mothers' Questionnaire, seeing and comparing how they prepare the normal 'ogi' for their babies. The food samples fermentation was then modified by using distilled water instead of the normal well and tap water been used by the mothers. The fermentation was also carried out in a septic and monitored environment.

Bitter yam *Dioscorea dumetorum* was washed with distilled water to remove the sand, the Cowpea *Vigna unguiculata* was picked to remove pebbles. The *Dioscorea dumetorum* was later peeled and 700g was weighed and fermented with 300g of *Vigna unguiculata* which served as Sample A. 600g of *Dioscorea dumetorum* was also weighed and fermented with 400g of *Vigna unguiculata* which served as Sample B. The weighing balance used for weighing the food

samples was an electronic balance of model XY3000C, number 14092185. A 500ml measuring cylinder was used in measuring the volume of distilled water used.

3.3 Fermentation Process

The two samples in their different proportions was fermented in different covered buckets with distilled water in the ration of 1:3 (i.e. samples= 1000g, water=3000ml) for 72hours.

For the remaining two unfermented samples which served as the control, the bitter yam was washed and peeled before placing it in the oven to reduce the moisture content at 105°C. After the tuber has dried, it was then weighed on the electronic weighing balance in the proportion of 700g and 600g respectively. The cowpea was also picked so as to remove the pebbles and was weighed with the electronic weighing balance in the proportion of 300g and 400g respectively. The dried 700g of bitter yam was mixed and grinded together with 300g of cowpea. The same was also done for 600g of bitter yam and 400g of cowpea. The samples were grinded with the WARING laboratory blender.

The pH and titratable acidity, microbiological analysis including the biochemical tests, proximate analysis, anti-nutritional factors, sensory evaluation, consistency and statistical analysis were determined.

3.4 pH and Titratable Acidity

Procedure for preparing the reagents used for titratable acidity determination

1) 1g of phenolphthalein was dissolved in 100ml of distilled water.
2) Exactly 4g of NaOH was dissolved in 1000ml of distilled water to give 0.1M solution

At the initial start of the fermentation (i.e. at 0hour), the pH of the two samples was taken. 12ml each of the medium from the two samples was taken with a 5ml syringe into two separate test tubes for the pH reading. An absorbent hydrophylic cotton BP was used to clean the pH sensor before and after taking the readings. This procedure was done at the stipulated interval (i.e. 0hour, 24hours, 48hours and 72hours). PHS-3C pH meter with fuse of 0.25A, serial number 600413079098, voltage 220V, power 5Wand frequency 50Hz was used at room temperature of 25^0C.

Below is the procedure for the titre acidity of the samples:

1) 10ml of the sample medium was taken and poured inside a clean beaker.
2) 2 to 3 drops of the indicator (i.e. 1% phenophtalein) was added to the medium in the beaker.
3) 0.1M NaOH in the already set up burette on a retort stand was titrated against the medium in the beaker.
4) When the colour changes to a light pink coloration, the reading was taken.

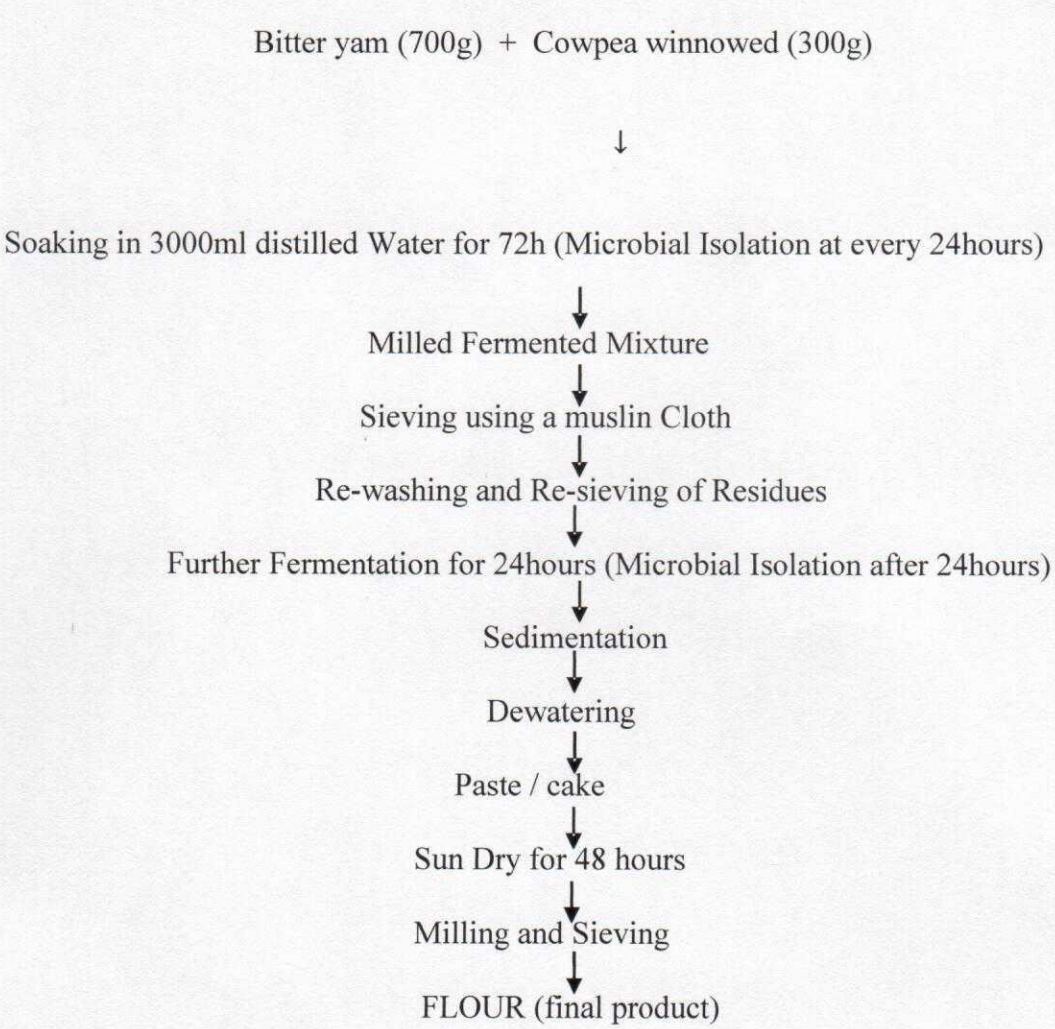


Fig 3: Flowchart for the fermentation proces

3.5 Microbiological Analysis

The microbial status of bitter yam and cowpea in the ratio of 700g:300g, 600g:400g were investigated.

At 0hour of the fermentation, 10ml of the medium was taken which served as the stock and dispensed into a test tube. 1ml of the stock was added to 9ml of distilled water in another test tube, this serial dilution procedure was done till the sixth test tube. The test tubes were labeled as thus: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. 1ml was taken each from test tubes 10^{-2} and 10^{-5} to be poured plated. This procedure was carried out for the other fermentation time intervals (i.e. 24hours, 48hours and 72hours).

1g of the blended control (sample A and sample B unfermented) was taken each from each samples and was mixed with 9ml of distilled water in a test tube which served as the stock. 1ml of the stock was taken and transferred into another test tube containing 9ml of distilled water which was labeled 10^{-1}. The test tube was rocked for even distribution of the transferred sample. 1ml was taken from test tube 10^{-1} and transferred into test tube 10^{-2}, the test tube was also rocked for even distribution before another 1ml was taken from test tube 10^{-2} and transferred into test tube 10^{-3}. This procedure was done till the last testtube which was testtube 10^{-5}. 1ml each was taken from test tubes 10^{-2} and 10^{-5} to be poured plated. This procedure was carried out for the other fermentation time intervals (i.e. 24hours, 48hours and 72hours).

Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) were used for the culturing of microorganisms from 0hour, 24hours and 48hours intervals. De-Man Rogosa and Sharpe Agar (MRS) was used for the culturing of Lactic acid bacteria for 72hours.

3.6 Biochemical Test on Isolated Microorganisms

Different biochemical test analysis was carried out on the samples, both fermented and unfermented sample. This following test include: Sucrose, Lactose, Maltose, Urease, Citrate, Catalase, gelatin and gram reaction test.

3.6.1 Gram reaction

a. A drop of distilled water was put on the center of a free grease sterile slide.
b. A colony was picking from the culture plate and makes a smear, after it was heat fixed with the use of flame from bursen burner filled with gas in order to aid the dryness.
c. Stain with crystal violet was first put on the fixed smear for 60secs, after 60secs it was wash away with the used of running tap water.
d. Iodine was added for another 60secs and washed away with the used of running tap water.
e. Decolorized (ethanol) was added after iodine and allow to stay for 30secs after it is been washed away immediately.
f. Safranin was added for another 60secs and was washed with running tap water.
g. After these procedures the slide sample was air dry and examine under microscope by addition of oil immersion.

3.6.2 Catalase test

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is a potent oxidizing agent that can wreak havoc in a cell; because

of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide.

1) A small amount of growth from sample culture was placed at the center of a clean microscope slide and was smeared.
2) A few drops of H_2O_2 was added onto the smear and was mixed together.
3) Result was observed for effervescence within 1-3 seconds.
4) A positive result is the rapid evolution of O_2 as evidenced by bubbling.
5) A negative result is no bubbles or only a few scattered bubbles.

3.6.3 Gelatin test

Nutrient gelatin is a differential medium that tests the ability of an organism to produce an exo-enzyme, called gelatinase that hydrolyzes gelatin.

1. Gelatin medium was measured and pour into a clean beaker and was mixed with distilled water of about 200ml and heated gently to dissolved, after that 3-5ml of the medium was dispense into a test tube and the mouth was cork with the used of aluminum foil paper and cotton wool and was arranged inside a big beaker and put inside the autoclave at121^{0}c for 15minutes for sterilization.
2. After sterilization the medium was removes and allow cooling in an upright position before use. After that the organism was introduce by start the Bunsen burner, selecting a sterile wire loop and pick an inoculum.
3. Inoculate a heavy inoculum of test bacteria (18- to 24-hour-old) by stabbing 4-5 times (half inch) on the tube containing nutrient gelatin medium.

4. Incubate the inoculated tube along with an uninoculated medium at 35°C, or at the test bacterium's optimal growth temperature, for up to 2 weeks.
5. Remove the tubes daily from the incubator and place in ice bath or refrigerator (4°C) for 15-30 minutes (until control is gelled) every day to check for gelatin liquefaction. (Gelatin normally liquefies at 28°C and above, so to confirm that liquefaction was due to gelatinase activity, the tubes are immersed in an ice bath or kept in refrigerator at 4°C).
6. The tube was tilt to observe if gelatin has been hydrolyzed.
7. Partial or total liquefaction of the inoculated tube (uninoculated control medium must be completely solidified) even after exposure to cold temperature of ice bath or refrigerator (4°C) show that it is positive.
8. Complete solidification of the inoculated tube even after exposure to cold temperature of ice bath or refrigerator (4°C) shows that it is negative.

3.6.4 Sugar fermentation tests (sucrose, lactose and maltose)

A small clean foil paper was put on a weighing balance; 5.6g of broth (Nutrient broth) was weighed and poured inside a clean beaker. 200ml of distilled water was added and allowed to dissolve and settle for some minute. After some minute of the residue was removed and discard. About 2-4 drops of phenol red was added to the broth before it was divided into three (3) and the sugars were added respectively .10ml was dispense inside sterile test tube using 10ml of syringe. The test tube used was labeled according to the name of each test .After dispensing the broth inside each test tube Durham's tube was introduce immediately after dispensing the broth inside test tube, the test tube was cork in the mouth with foil paper and cotton wool. After that it was put inside the autoclave for sterilization, after complete sterilization the sample was remove and

arrange inside test tube rack immediately and allow to cool completely after it as cool completely, microorganism was introduce inside with the used of sterile wire loop on each test, after completing this process the test tubes was arranged inside a beaker and put inside the incubator for 24hrs at 37°c for result analysis.

3.6.5 Urease and citrate tests

A small clean foil paper was put on a weighing balance, 2.27g of urease and 2.2g of Simmons citrate agar was weighed using different foil paper, and it was removed and poured inside clean beaker. 90ml of distilled water was added and allow dissolving for some minute, then few drops of phenol red was also added. It was placed in the autoclave for few minutes because of the hotness of the autoclave so as to dissolve the agar. 10ml of each agar was dispensed respectively inside sterile test tube using 10ml of syringe into the already labeled test tubes. After dispensing the broth inside each test tube the test tube was cork in the mouth with cotton wool and foil paper. After that it was put inside the autoclave for sterilization, after complete sterilization the sample was remove and slant immediately before it gel after it as gel by slanting process, microorganism was introduce by streaking on the urease and citrate test making use of sterile test tube, after completing this process the tubes was arrange inside beaker and put inside the incubator for 24hrs at 37°c for result analysis.

3.7 Consistency

Consistency is the way in which a substance holds together or the degree of viscosity of something. Different grams of the samples were dissolved in different ml of distilled water as illustrated below and were stirred constantly on hot plate at 80°C. At the first appearance of first

bubble, cooking was timed to 5 minutes after which were held for 15 minutes cooled to 45°C. The first compartment was filled with 150ml of each co-fermented and unfermented sample.

At time =0, the trigger was pressed to release the gate of the first compartment to allow gruel flow by gravity to the second compartment. The distance the gruel covered in 30seconds was measured in millimeters as the Consistency reading (i.e. flow in mm/30seconds) and final viscosity was recorded. . Dry matter of gruel was also determined. The dry matter values were compared with consistency and viscosity measurements. The cooked paste viscosity of the slurry was determined using Rapid Visco Analyser (RVA) (Model TM Super-3 Analyzer Newport Scientific PTY Ltd. 1998 ½ -Apollo st., Warriewwood MXW 21- 02 Australia).

The consistency of the food samples were carried out as follow;

I. 2g of the powdered food sample was mixed and cooked with 150ml of distilled water.
II. 6g of the powdered food sample was mixed and cooked with 144ml of distilled water.
III. 9g of the powdered food sample was mixed and cooked with 141ml of distilled water.
IV. 10g of the powdered food sample was mixed and cooked with 138ml of distilled water.
V. 12g of the powdered food sample was mixed and cooked with 135ml of distilled water.

The samples were prepared with a new clean stainless pot, a ladle, an electric cooker and a clean spoon for serving the food in an aseptic way.

3.8 Sensory evaluation

Sensory evaluation was carried out in order to determine the taste, texture, colour, Aroma, and acceptability by making used of 9 hedonic scales using: 1 = dislike extremely, 2 = dislike very much, 3 =dislike moderately, 4= dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 =

like moderately, 8 = like very much and 9 = like extremely as described by Ihekoronye and Ngoddy (1985).

Thirteen (13) mothers were recruited from the University crèche for the sensory evaluation of the food samples. They were asked not to eat any food or take any sweets or chew gum an hour before the evaluation. The mothers evaluated the samples in individual testing booths so as not to see another person's reaction. The cooked food samples were dished in a transparent plate so as not to affect the colour scoring of the food samples. Each mother was provided with a spoon, serviette paper, a sachet of water to rinse their mouths so that the taste of the food samples will not affect another.

The four samples (i.e. samples AA and BB fermented, samples CC and DD unfermented) were given to the mothers for evaluation. Sample AA was first placed in front of the mothers to be assessed and after which it was removed and they rinsed their mouths with the water provided. Sample BB was also placed in front of the mothers for assessment and removed before the remaining other two samples (sample CC and DD) were given to them separately for assessment.

3.9 ANTINUTRITIONAL FACTORS

3.9.1 Phenols

1. 80:20 concentrations of solvent mixture i.e. 0.2% Acetone and formic acid was prepared respectively.
2. 2g of the samples was weighed into 20ml of the prepared solvent mixture in a conical flask.
3. Allow it to stand for 2 minutes and then filter.

4. 2ml of the filtrate was pipetted into a test tube and 0.5ml of folin-Ciocaltean reagent was added.
5. Leave to stand for 20minutes for colour changes.
6. The concentration was read at a wavelength of 650nm.

NOTE: blank is the solvent mixture

3.9.2 Phytate/phytic acid

1. 1g of the samples was weighed into 250ml conical flask.
2. 50ml of 2% HCl was added and allowed to soak for 3hours.
3. It was then filtered through a double layer of hardened filter paper.
4. 25ml of the filtrate was pipetted into a 250ml beaker.
5. 50ml of distilled water was added so as to give proper acidy.
6. 0.3% Ammonium thiocyanate solution (containing 0.00195g iron per ml) was used as the standard.
7. The concentration was read at a wavelength of 510nm.

NOTE: blank is 2% HCl

3.9.3 Tannins

1. 1g of the well-grounded sample was weighed into a test tube containing 10ml of distilled water and agitated.
2. It was left to stand for 30 minutes at room temperature.
3. The content in the test tube was centrifuged at 3000rpm for 10 minutes.

4. 2.5ml of the supernatant was taken out and dispersed into a 50ml volumetric flask.
5. 2.5ml of standard tannin acid solution was also taken and dispersed into 50ml volumetric flask.
6. 1.0ml of folin-dennis reagent was added into the flasks in steps 4 and 5.
7. 2.5ml of saturated Na_2CO_3 solution was added to each flask.
8. Distilled water was used to make up 50ml volumetric flask to the meniscus.
9. It was incubated for 90minutes at room temperature.
10. The absorbance of the sample was taken at 725nm with reagent blank at zero.
11. The tannin percentage present was then calculated.

3.9.4 Cyanide

Standard method of AOAC (1990) was used.

1. 4g of each sample was soaked in a mixture of 40ml distilled water and 2ml of orthophosphoric acid.
2. The sample was thoroughly mixed, covered and left overnight at room temperature so as to set free all the bounded hydrocyanic acid.
3. The resulting mixture was transferred into distillation flask and a drop of paraffin (antifoaming) was added to the broken chips. The flask was fitted to other distillation apparatus and distilled.
4. About 4ml of distilled water containing 0.1g of Sodium hydroxide pellets.
5. The distillate was then transferred into 50ml volumetric flask and made up to mark with distilled water.

6. 20ml of the distillate was collected and then placed in the conical flask and 1.6ml of 5% Potassium iodide solution was added to the flour and titrated against 0.01M Ag $(NO_3)_2$ solution.
7. The blank was also titrated until the end point was indicated by a faint but permanent turbidity.

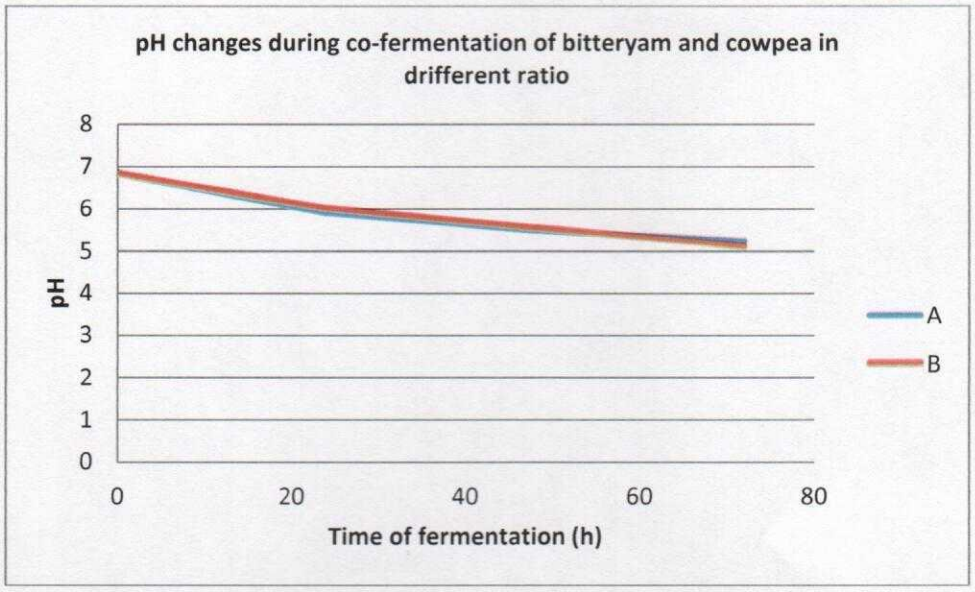
3.9.5 Phytin - Phosphorous

1. 8g of each of the samples was soaked in 200ml of 2% hydrochloric acid and allowed to stand for 3hours.
2. The extract was thereafter through two layers of hardened filter paper.
3. 50ml of the filtrate was pipetted in duplicate into 400ml capacity beakers before the addition of 10ml 0.3% ammonium thiocyanate solution as an indicator and 107ml of distilled water to obtain the proper acidity (pH 4.5).
4. The solution was then titrated with a standard iron chloride ($FeCl_3$) solution containing 0.00195gm Fe/ml until a brownish yellow colour persists for 5 minutes.
5. Phytin-phosphorous was determined and the phytin content was calculated by multiplying the value of phytin- phosphorous by 3.55 (Young and Greaves, 1940). Each milligram of iron is equivalent to 1,19 milligrams of phytin- phosphorous.

4.0 RESULTS AND DISCUSSION

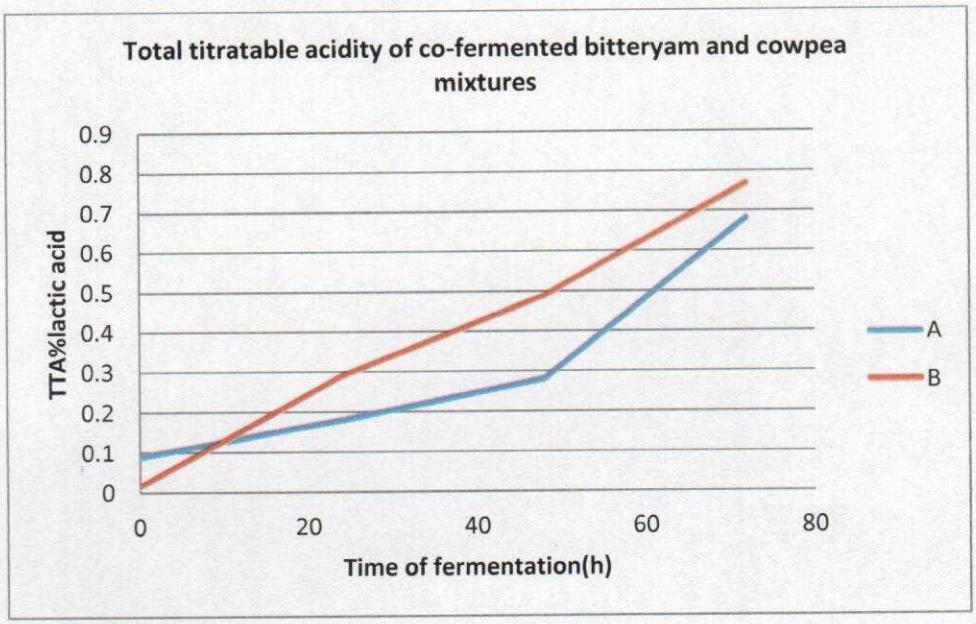
4.1 RESULTS

Fig 4: pH Changes During Co-Fermentation Of Bitter yam And Cowpea In Different Ratio



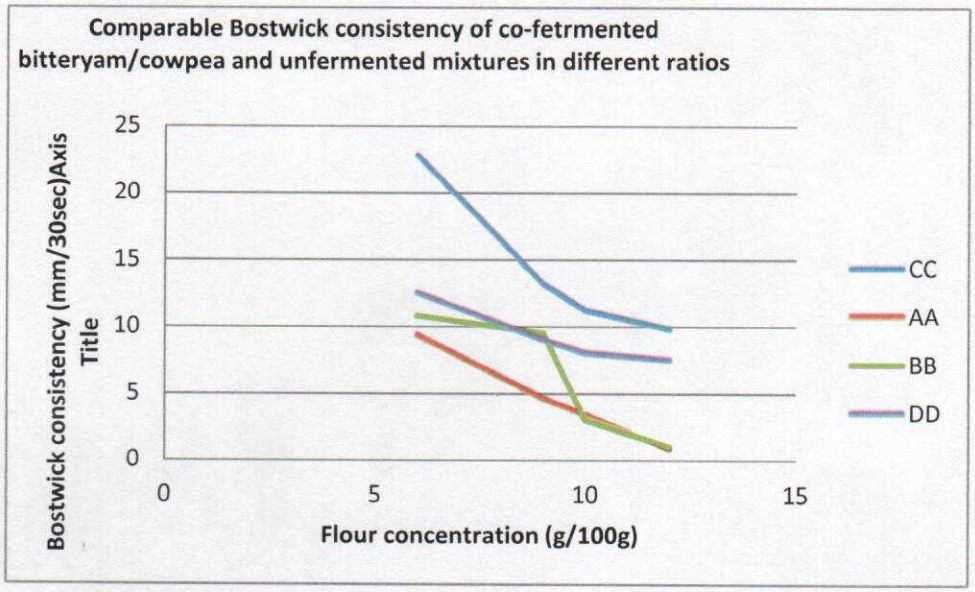
Key: A= Co-Fermented Bitter Yam/ Cowpea 700:300g, B= Co-Fermented Bitter Yam/ Cowpea 600:400g

Fig 5: Total Titratable Acidity of Co-Fermented Bitter Yam and Cowpea Mixtures



Key: A= Co-Fermented Bitter Yam/ Cowpea 700:300g, B= Co-Fermented Bitter Yam/ Cowpea 600:400g

Fig 6: Comparable Bostwick Consistency of Co-Fermented Mixtures and Unfermented Mixtures In Different Ratios



Key: AA= Bitter Yam and Cowpea 700:300, CC= Unfermented Bitteryam/Cowpea 700:300, BB= Bitter Yam / Cowpea 600:400g, DD= Unfermeted Bitter Yam/ Cowpea 600:400

Table 1: Sensory Evaluation of Co-Fermented Bitter Yam/Cowpea In Various Ratio And Their Unfermented Analogues

SAMPLE	COLOUR	TEXTURE	TASTE	AROMA	ACCEPTABILITY
AA	4.40[a]	6.10a	4.11a	3.90b	6.10a
BB	4.40[a]	6.70b	4.90b	3.50a	6.11a
CC	5.11[b]	7.40c	6.70c	5.90c	4.10b
DD	5.10b	7.40c	6.80d	5.90c	4.12b

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g

MICROBIOLOGICAL ANALYSIS

Table 2: BACTERIAL COUNT (CFU/ML)

	FERMENTATION TIME INTERVAL							
Sample I.D	**0 h**		**24 h**		**48 h**		**72h**	
	10^2	10^5	10^2	10^5	10^2	10^5	10^2	10^5
AA	2.8×10^{-4}	N.G	2.7×10^{-7}	1.7×10^{-5}	7.8×10^{-7}	2.8×10^{-5}	5.2×10^{-7}	2.2×10^{-5}
BB	1.3×10^{-4}	N.G	4.4×10^{-4}	N.G	5.6×10^{-7}	1.3×10^{-5}	3.0×10^{-7}	1.2×10^{-5}
CC	1.4×10^{-4}	N.G	1.7×10^{-7}	1.2×10^{-5}	TNTC	1.1×10^{-8}	TNTC	1.0×10^{-8}
DD	2.6×10^{-4}	N.G	4.2×10^{-7}	1.4×10^{-5}	TNTC	1.0×10^{-8}	TNTC	1.0×10^{-8}

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g, N.G= No growth, TNTC= Too numerous to count

Table 3: Yeast Count

	FERMENTATION TIME INTERVAL							
Sample I.D	**0 h**		**24 h**		**48 h**		**72 h**	
	10^2	10^5	10^2	10^5	10^2	10^5	10^2	10^5
AA	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**
BB	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**
CC	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**
DD	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**	**N.G**

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g, N.G: No Growth

Table 4: ISOLATION and BIOCHEMICAL CHARACTERISTICS of *LACTOBACILLUS SPP.*

Sample	Gram rxn	Cat	Suc	Lac	Mal	Ure	Cit	Gelatin Utilization	Probable organism	Isolation Code
AA_1	+ve cocci	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*L. plantarum*	ATCC 14910
AA_2	+ve short rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Lactobacillus Spp.	
BB_1	+ve short rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Lactobacillus Spp.	
BB_2	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*L. planterum*	ATCC 14910
CC_1	+ve long rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Lactobacillus Spp.	

Table 4 (contd)

CC_2	+ve short rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Lactobacillus Spp.	
DD_1	+ve short rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*L. planterum*	ATCC 14910
DD_2	+ve short rod	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Lactobacillus Spp.	

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g, -Ve= Negative, +Ve= Positive. ATCC= American Type Culture Collection (Rockville Maryland). Suc= sucrose, Lac=lactose, Mal=maltose, Cat=catalase, Ure=Urease, Cit=citrate

Table 5: Proximate Composition and Vitamin A Content of Co-Fermented Mixture

S/NO	%Ash	%Mc	%Cp	%Fat	%Fibre	%CHO	(mg/100g) Vit. A
AA	1.01	8.59	18.85	4.46	1.01	66.09	0.06
BB	0.44	9.93	17.42	3.95	0.66	67.60	0.03
CC	1.55	12.87	19.64	3.41	0.51	62.02	0.015
DD	1.76	11.54	17.40	3.09	0.36	65.83	Nd

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g, N.d= not detected, Cp= Crude protein, Mc= Moisture content

Table 6: Anti-nutrients content of Co-Fermented Mixture in Different Ratio and Their Unfermented Analogues

S/NO	Tannin (mg/100g)	Phytate(mg/100 g)	Phytin-P (mg/100g)	Phenol(mg/100g)	Cyanide(mg/Kg)
AA	0.35	5.77	1.62	0.33	34.07
BB	1.40	12.40	3.44	0.36	31.48
CC	0.48	9.35	2.58	0.18	42.41
DD	0.51	7.75	2.12	0.24	48.34

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g

Table 7: Mineral composition of Co-Fermented Mixtures in different Ratio and their unfermented analogues

Sample	Na	K	Ca	Zn	Fe	Mn
AA	188.25	219.92	416.84	2.24	7.63	1.00
BB	202.03	441.12	490.63	6.94	8.52	1.74
CC	325.15	463.21	565.62	2.26	4.25	1.28
DD	438.36	513.72	717.82	2.86	5.76	2.24

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g. Na= Sodium, K= Potassium, Ca= Calcium, Zn= Zinc, Fe= Iron, Mn= Manganese

Table 8: The Mineral Safety Index of co-fermented bitter yam/cowpea in various ratios and their unfermented analogues.

	Ca			Fe			Na			Zn		
Sample I.D	**TV**	**CV**	**D**	**TV**	**CV**	**D**	**TV**	**CV**	**D**	**TV**	**CV**	**D**
AA	**10**	**10.42**	**-0.42**	**6.7**	**5.11**	**1.59**	**4.8**	**2.26**	**2.54**	**33**	**12.32**	**20.63**
BB	**10**	**12.27**	**-2.27**	**6.7**	**5.71**	**0.99**	**4.8**	**2.42**	**2.38**	**33**	**38.17**	**-5.17**
CC	**10**	**14.14**	**-4.14**	**6.7**	**2.85**	**3.85**	**4.8**	**3.90**	**0.90**	**33**	**12.43**	**20.57**
DD	**10**	**17.95**	**-7.95**	**6.7**	**3.86**	**2.84**	**4.8**	**5.26**	**-0.46**	**33**	**15.73**	**17.27**

Key: AA= Co- fermented bitter yam/cowpea 700:300g, BB= Co-fermented bitter yam/cowpea 600:400g, CC= Unfermented bitter yam/cowpea 700:300g DD= Unfermented bitter yam/cowpea 600:400g, TV= Tabulated value; CV= Calculated value; D= Difference between TV and CV.

Minus = overshooting than recommended

If the MSI is less than recommended, then there will not be any minus.

Table 9: MINERAL SAFETY INDEX (Whytney and Houston 1990)

MINERAL	RECOMMENDED INTAKE e.g For infants	MINERAL SAFETY INDEX (tablulated)
Calcium	400	10
Phosphorus	270	10
Magnesium	80	15
Iron	10	6.7
Zinc	6	33
Copper	0.65	33
Sodium	400	4.8

Calculation:

$$\frac{\text{Tabulated Mineral Safety Index}}{\text{Recommended Infant Intake}} \times \text{Value of Mineral in Sample}$$

4.2 DISCUSSION

4.2.1 pH

Fig 4 shows that the pH had rapid reduction at 24h from 6.8 to 6.0 and reduced gradually to 5 by 72h. Lactic acid bacteria are generally grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able to grow. *Leuconostocs* and lactic streptococci generally lower the pH to about 4.0 to 4.5, and some of the lactobacilli and pediococci to about pH 3.5, before inhibiting their own growth. The presence of cowpea must have made the fermenting medium alkaline in nature thus preventing the pH from further reduction to pH 4.0 or below. Preservation of foods by fermentation depends on pH 4.0–4.5; some are active at pH 9.6 and others at pH 3.2. The decrease in the pH of the co-fermented samples might be due to availability of more nutrients probably from microbial proliferation and enhanced metabolic activities. Microbial interactions of combinations of the multi component substrates in the spontaneous fermentation may play an important role in the nutritional, safety and sensory characteristics of the end product.

4.2.2 Total titratable acidity

According to fig 5, the titratable acidity of the fermented mixtures increased as the pH decreased during the fermentation hours.

4.2.3 Biochemical Reaction

Table 6 shows the result for the biochemical test for sucrose, lactose, maltose, urease and citrate showed to be positive which indicates the presence of the sugars in the food samples. There was production of acid and gas which indicates that the organism utilized the sugars.

Table 5 shows the catalase, gelatin and gram reaction test results. The gram reaction result showed that most of the organisms were gram positive rod, just a single dilution of AA was gram positive cocci which indicates the presence of some bacteria in the sample. There may have been the presence of other organisms but *Lactobacillus spp* was mostly detected which indicates that *Lactobacillus* is the main organism present in these sample which has helped in development of acidity, the induced acidity is responsible for inhibiting any spoilage microorganism.

4.2.4 Yeast count

No growth was observed on the yeast plates for all the samples.

4.2.5 Consistency

Fig 6 shows the graph for the consistency flow of the samples. It shows that the flow is inversely proportional to the flour concentration as the flour concentration increased, the consistency reduced. Also a high consistency value means low viscosity. A low viscous infant food is desirable because by implication, it means higher nutrient and energy densities (Oyarekua 2013). The Figure 6 also shows that at 10% flour concentration, CC had a higher consistency value of 11.2/30sec followed by DD and BB while AA had the lowest. This shows that CC gruel at 10% might be easy for the infant to swallow and might contain more nutrient and energy densities than other mixtures at this concentration. This study showed in agreement with Oyarekua (2013); that the incorporation of 30% cowpea was capable of increasing the consistency and by implication reduction in viscosity. High energy density (ED) gruels which depend on the flour concentration and nutrient contents improve infant's energy and nutrient intakes (Golden 2009).

4.2.6 Sensory evaluation

The sensory evaluation results for the fermented samples are presented in Table 4. For flavor, the panelists' scores ranged from 5.90 to 3.90 , on a hedonic scale of 1 to 9, indicating that sample DD was preferable than other samples. The comparative study of the food samples in different ratio indicates that they prefer the fermented sample to unfermented sample.

4.2.7 Microbiological analysis

In Table 6, it was observed that the total count was decreasing from 0hr to 72hrs. This is in agreement with Golshan *et., 2013)* there was presence of *Lactobacillus spp* during the fermentation process mostly in 24hrs and 48hrs, the organism present in co-fermented sample and unfermented sample A is high in number than that of sample B fermented and unfermented. The appearance may be due to its ability to survive the modified acidic environment after about hours of fermentation. At 0hr there was growth in the co-fermented samples (A,B,C,D), under the dilution 10^{-2}, and no growth was observed at dilution 10^{-5}. At 24hrs and 48hrs, the growth increased which indicated that the organisms present were very active in the fermentation process. At 10^{5} CC and DD had the highest count with the co-fermented having the lowest. Some microbes are proteolytic microbe that can utilize protein during its metabolic activities to release amino acids which might neutralize the acidic fermenting medium to become a bit alkaline. Similarly, Adams (1990) suggested that lactic acid bacteria are inhibitory to many other microorganisms when they are cultured together, and this is the basis of the extended shelf life and improved microbiological safety of lactic-fermented foods. *Lactobacillus species* can produce a variety of metabolites, including lactic and acetic acids which lower pH, that are inhibitory to competing bacteria, including Psychritrophic pathogen (Breidt and Fleminf, 1997).

4.2.8 Bacterial count (CFU/ml)

In general, high numbers of viable probiotic bacteria are desired in these products at the moment of consumption. Typically between 5 and 8 logs Colony Forming Unit per gram of product is considered acceptable. At 0hour and 48hours, AA had the highest CFU/ml count. Therefore, the growth, survival, and activity of the probiotic strain in the product environment are of key importance, and these traits are influenced both by specific environmental conditions and by interactions with the starter organisms (Sieuwerts et al 2008).

4.2.9 Anti-nutritional factors

The study of table 11 showed ANF values of the mixtures BB and AA had more total **phenol** than mixture CC and DD, this might be from the cowpea component. This showed that co-fermentation of bitter and cowpea did not effectively or effectively reduce these anti nutrients. A higher **phytic acid** content in BB co-fermented or CC unfermented sample can be attributed to phytate hydrolysis during fermentation by enhanced activity of the enzyme phytase and that of alpha-amylase leading to the release of Ca ion chelated with phytic acid making phytic acid more available. Ca ion is important for the activity of alphaamylase (Oyarekua 2013). As reported by Oyarekua and Bankefa(2015), phytic acid is a strong inhibitor of iron absorption in both infants and adults, inhibits pepsin, amylase and trypsin necessary for hydrolysis of proteins and starch in the small intestine.

The pH of mixture BB and AA by 72hours 5.0 and 5.5 respectively (fig.1) were optimal for phytases activities as reported by Oyarekua and Bankefa (2015)

Cyanide:

Cyanide ions inhibit several enzymes systems; depress growth by interference with certain amino acids as reported by Soetan and Oyewole (2009). DD had the highest value of cyanide followed by CC then AA and lastly BB.

Phytates, and phenols bind important minerals thus resulting to their low availability. Phenols are easily vaporized at 22°C to 26°C therefore substantial part must have evaporated during the fermentation temperature of between 25-27°C (Oyarekua, 2013). Fermentation process can reduce phytate by almost 90%, due to activation of endogeneous phytase in cereal and legume being prosessed (Oyarekua and Bankefa (2015).

However since the toxic effect of anti-nutrients can be avoided if the food is fermented and cooked before consumption; the combination effect of fermentation and cooking may drastically reduce the effect of anti-nutritional factors to safe levels (Oyarekua 2013).

Phenols and tannins complex with iron and zinc inhibit enzymatic activities of pectinase, amylase, lipase, proteolytic enzymes, β-galactosidase and those microbial enzymes involved in fermentation of tubers like bitter yam while tannins in legumes like cowpea reduce ionisable iron absorption by acting as a natural iron chelating agent. Soaking in water prior to cooking has a significant effect on reducing the tannin content (37.5-77.0%), provided the water used for soaking is discarded (Oyarekua and Bankefa, 2015).

4.2.10 Minerals

Table 12 shows the mineral composition of the samples. Mixture DD had the highest Manganese value followed by BB fermented. Manganese: is a component of enzymes that are involved in antioxidant pathways and it helps in proper functioning of the brain and spinal cord. Deficiency can lead to defective growth abnormalities, central nervous systems and disturbances in the way the body handles fats (Nieman, *et al.* 2002).

Calcium helps prevent Colon Cancer, Osteoporosis. Mixtures DD and CC unfermented had the highest value of calcium, making them very rich in calcium. There is a substantial difference between the calcium content of hulled versus dehulled sesame seeds. When the

hulls are removed they will be 60% reduction of calcium content. The calcium found in the hulls is in form of calcium oxalate which is less absorbable than calcium found in the kernels and also might not be beneficial to an oxalate-restricted diet.

4.2.11 Proximate analysis

In table 7, CC and AA had the highest crude protein value which is in accordance to the guide for the nutrient complementary food intake. Sample AA had the highest value of vit. A. Sample DD and CC had the highest ash volume compared to co-fermented sample AA and BB.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

In Nigeria the weaning foods are imported to overcome the problem of malnutrition of infants and babies during weaning periods. But these foods are too expensive for low income peoples/families. So it is important to prepare complementary food from locally available raw materials because commercial weaning food is gradually getting out of the reach for these families. Developing a technology to prepare weaning from low cost materials has drawn lots of attention now in the country. From this research work, it may be concluded that weaning food can be prepared using bitter yam and cowpea, to meet the macro nutritional needs of infants and babies. However, certain aspects like the digestibility and bio-availability of macronutrients as well as micronutrients in the prepared weaning food needs further study to know whether the prepared food needs further modification with or without fortification.Co-fermentation of bitter yam and cowpea increased the mineral value of the samples. The β- carotenoid level improved in co-fermented mixtures. Through the fermentation process, the anti-nutritional factors of the samples reduced which indicates that fermentation helps reduce ANF in food samples.

RECOMMENDATION

Further research should be carried out on other mineral analysis of the samples and locally fermented infant complementary food should be encouraged among mothers because fermented foods aids digestion and easy bowel movement.

REFERENCES

- Adams M.R (2002): Topical aspects of fermented foods. *Trends in Food Science & Technology 1*, 141-144.
- Afoakwa, E.O., Sefa-Dedeh, S. Simpson Budu, S. Sakyi-Dawson. A.E. and Asomaning J (2007). Some Quality Characteristics of Maize Based Cowpea-fortified Nixtamalized Foods. *African Journal of Food Agric Nutrition and Development 17: 1 Assessed Feb 4th 2010:http.www.guidelines.gov/summary.aspx?doc_i d=8*
- Aguirre M & Collins MD (1993): Lactic acid bacteria and human clinical infection. *Journal of Applied Bacteriology 75, 95-107.*
- Alice, J. (2010). An Economic Analysis of Cowpea Production among Women Farmers in Askira-Uba, Borno State. *African Journal of General Agriculture, 6 (1), p8.*
- Bressani, R (1985). *Nutritive value of cowpea. In: Cowpea Research,Production and Utilization . Edited by S.R. Singh and K. O. Rachie. John Wiley & Sons Ltd. New York.*
- Campbell-Platt G (1987). *Fermented foods of the world - a dictionary and guide.* London, Butterworths. ISBN: 0-407-00313-4.
- Davis, D.W., Oelke E. A., Oplinger E. S., Hanson C.V. & Putnam D.H. (2013). *Alternative Field Crop Manual*. Department of Horticultural Science, Agronomy and Plant Genetics: Center for Alternative Plant and Animal Products Minnesota Extension Service, University of Minnesota, U.S.A.

- Degras, L., (1993). *The Yam: A Tropical Root Crop*. 2nd Edition., Macmillan Press, London, pp: 121-138.
- Duszkiewicz-Reinhard W, Gujska E & Khan K (2008): Reduction of stachyose in legume flours by lactic acid bacteria. *Journal of Food Science 59, 115-117.*
- Eka, O.U., 1998. *Root and Tubers. In: Nutritional Quality of Plant Foods*, Osagie, A.U. and O.U. Eka (Eds.). Macmillian Press, London, pp: 1-31.
- Federal Ministry of Agriculture and Rural Development (FMARD), (2011). Agricultural Production Survey Data (1999-2010). FCT, Abuja, Nigeria.
- Fields, M.L (1980).Improvement in Nutritive quality of Cereals and legumes by Fermentation and Germination. Bioletin Tenico LABAL 1 (4): 14-16.
- Fleming HP & McFeeters RF (2000): Use of microbial cultures: vegetable products. *Food Technology 35, 84-88.*
- Fuller R (2009): Probiotica in man and animals. *Journal of Applied Bacteriology 66.*
- Goldin BR & Gorbach SL (2000): The effect of oral administration on *Lactobacillus* and antibiotics on intestinal bacterial activity and chemical induction of large bowel tumors. *Developments in Industrial Microbiology 25, 139-150.*
- Golshan T.A., Peighambarboust, S.H and Hejazi N.A. (2013): Biochemical characterization and technological properties of predominant *Lactobacilli* isolated from East-Azarbaijan sour doughs (Iran).

International Food Research Journal 20 (6), pp 3293-3298.

- Hesseltine CW (2011): The future of fermented foods. *Nutrition Reviews 41, 293-301.*
- International Institute of Tropical Agriculture (IITA), (2011). Agency Report, Cowpea Driving Silent Economic Revolution in Nigeria.
- Ihekoronye A.I., Ngoddy P.O (1985) Integrated Food Science and Technology. Macmillan Publishers pp.129-130. *Microbiological and Nutrition Quality of Retail and Laboratory 'ikpan' (mushroommeloncake) a local snack- Internet Journal of Nutrition and Wellness (8)2.*
- Khandwala, P.K., Ambegaokar, S.M., Patel and Radhakrishna Rao, M.V. (2005). Studies in fermented foods: Part 1. *Nutritive value of idli. J. Sci. Ind. Res. 21C: 275-278.*
- Martin G.S, Treche L., Noubi T., Agor E. and Gwangwa A. (1983): Introduction of flour from Dioscorea dumetorum in a rural area. In: Terry E.R., Doku E.V., Arene O.B and Mahungu N.M (eds): Tropical Root Crops: Production and uses in Africa. *Proceeding of the second triennial Symposium of the International Society for Tropical Root Crops Africa, Doaula, Cameroon.* Pp 161-163.
- Medoua, G.N., I.L. Mbome, T. Agbor-Egbe and G.M.F. Mbofung, 2005. Physicochemical changes occurring during post-harvest hardening of trifoliate yam (*Dioscorea dumetorum*). Food Chemistry, 90: 597-601.

- Mital B.K & Garg S.K (2004): Anticarcinogenic, hypoholesterolemic and antagonistic activities ofLactobacillus acidophilus. *CRC Critic. Rev. Microbiol. 21, 175-214.*
- Motarjemi Y, Käferstein F, Moy G & Quevedo F (2004): Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization 71, 79-92.*
- Nieman, D.C., Butterworth, D.E and Nieman, C.N (2002). *Nutrition. Revised First Edition pp 273-308.* Publisher wim.C. Brown Publisher.
- Odunfa, S. A., (1985). Africa fermented foods. In: *Microbiology of fermented foods.* Vol. 2. Wood, B.J. (Ed.) 155–191 (London) Elsevier Applied Science Publisher, London and New York.
- Ojofeitimi, E.O., Afolabi, O.A and Fapojuwo, O.O (1984).The Use of Black-eyed Cowpea- Maize Gruel Mixture "Ewa- 'ogi'" in the Prevention of Infantile Protein Malnutrition. *Nutrition Reports International. 30 (4): 841-852.*
- Ojofeitimi,E.O (2007). Malnutrition and Approaches to Improving the Nutritional Status of Children In Low Income Settings. Department of Community Health,College of Health Sciences, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.
- Okaka, J.C. and P.E. Okechukwu, 1987. Yam Processing: Problems and Prospects. In: Advances in Yam Research, Osuji, G. (Ed.). Biochemical Society of Nigeria/Anambra State University of Technology, Nigeria, pp: 22.

- Omae, H., Saidou, A. K., Ajeigbe, H. A. & Satoshi, T. (2011). Potentials of Cowpea for Dry Season Seed and Fodder Production in Sahelian Sandy Soil of Niger. *American- Eurasian Journal on Agriculture and Environmental Science; 11 (1): 71.*
- Omoruyi FO, Ashworth L and Asemote HN (2007). *Nutrition and Food Science 37(1):8-15.ISSN:0034-6659*. Emerald Group Publishing.
- Osundahunsi, O.F and Aworh, A.C (2002). A Preliminary Study on the use of Tempe-Based Formula-Plant Foods and Human Nutrition . 57 (3-4):365-376.
- Oyarekua, M. A (2013) Studies on the Nutritional quality, anti-nutritional factors, amino acid profileand functional properties of co-fermented wheat/cowpea. *Elixir Food Science 54 : 12360-12364*
- Oyarekua M.A and Bankefa, E. O (2015) The Effect of Cooking and Fermentation on the Functionaland Nutritional Properties of Walnut and Maize International Journal of Nutrition and Food Sciences; 4(3):332342.Published online April 23, 2015 (http://www.sciencepublishinggroup.com/j/ijnfs) ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)
- Passam, H. C. (2011). Dormancy of yams in relation to storage. Yams: Ignames (Mi, J. and Lyonga, S. N., eds), pp. 285-293. Oxford: Oxford University Press, 411 pp.
- Paredes-López O & Harry GI (2006): Food biotechnology review: traditional solid-state fermentations of plant raw materials –

application, nutritional significance and future prospects. *CRC Critical Reviews in Food Science and Nutrition 27, 159-187.*

- Purseglove, J. W. (2002). Dioscorea dumetorum (Kunth) Pax. African bitter or cluster yam. Tropical crops: Monocotyledons 1, p. 106. London: Longman Group Ltd, 334 pp.
- Rasper, V. and Coursey, D. G. (2007). Properties of starches of some West African yams. *Journal of the Science of Food and Agriculture, 18, 240-244.*
- Reddy BS, Ekelund G, Bohe M, Engle A & Domellöf L (2001): Metabolic epidemiology of colon cancer: Dietary pattern and fecal sterol concentrations of three populations. Nutr Cancer 5, 34-40.
- Sefa-dedeh, S.K and Afoakwa E.O (2001): Biochemical and textural changes in trifoliate yam (*Dioscorea dumetorum)* tubers after harvest. IFT Annual meeting- New Orleans. Louisiana.
- Shahani KM (2004). Nutritional impact of lactobacillic fermented foods. *Nutrition and the Intestinal Flora. ed. B. Hallgren ISBN 91 22005935.*
- Tarawali, S.A., Singh, B.B., Gupta, S.C., Tabo, R., Harris, F., Nokoe, S., Fernandez-Rivera, S., Bationo, A., Manyong, V.M., Makinde, K. and Odion, E.C. (2002). Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa. *In Challenges and Opportunities for Enhancing Sustainable Cowpea Production.*
- Wannik, J. F., Vilet-T. Van,. Nout, M.J.R and Van Vilet, T (1994). Effect

of Roasting and Fermentation on Viscosity of Cereals –Legume Based Food Formulas. *Plant Foods for Human Nurition. 46 (2): 117-126 .*

- Whitney, E.N., Hamilton, E.M.N and Rolfes, R.F (1990). *Understanding Nutrition pp. 430-453.publ.* West Publishing Company. St. Paul, N. York, Los Angeles and San Francisco.

APPENDIX

Estimated Nutrient Needs from Complementary Foods by level of breast milk intake.

Nutrients	6-8 months			9-11 months			12-23 months		
	Breast-milk intake			Breast-milk Intake			Breast-milk Intake		
	Low	Avg	High	Low	Avg	High	Low	High	Low
Protein(g/d)	5.2	2	0	6.7	3.1	0	9.1	5	12
Vitamin µgRE/d	164	13	0	214	42	0	313	126	0
Folate	0	0	0	9	0	0	35	3	0
Niacin mg/d	3	3	3	5	4	4	8	7	7
Panthothenic(Mg/d)	1.0	0.5	0	1.2	0.6	0	1.4	0.7	0
Riboflavinmg/d	0.3	0.2	0.1	0.3	0.2	0.1	0.5	0.4	0.3
Thiamin mg/d	0.1	0.1	0	0.2	0.2	0.1	0.5	0.4	0.5
Vitamin B_6mg/d	0	0	0	0	0	0	0	0	0
Vitamin B_{12}µ/d	0	0	0	0.1	0	0	0.3	0	0
Vitamin C mg/d	10	0	0	14	0	0	23	8	0
Vitamin Dµg/d	6.8	6.6	6.5	6.9	6.7	6.5	7.0	6.7	6.4
Vitamin Kµg/d	9.2	9	8	9.4	9	8	9.6	9	8
Calcium mg/d	421	336	252	449	353	256	301	196	92
Chloride mg/d	344	217	90	386	241	97	727	569	412
Copper mg/d	0.2	0.1	0.1	0.2	0.1	0.1	0.4	0.3	0.2
Fluorideµg/d	0	0	0	0	0	0	0	0	0
Iodineµg/d	19	0	0	30	0	0	51	10	0
Iron mg/d low	20.9	20.8	20.7	20.9	20.8	20.7	11.9	11.8	11.7
Med	10.9	10.8	10.7	10.9	10.8	10.7	5.9	5.8	5.7
High	6.9	6.8	6.7	6.9	6.8	6.7	3.9	3.8	3.7
Mg mg/d	62	51	41	70	58	46	79	66	53
Mn µg/d	14	12	10	14	12	10	15	13	10
Phos mg/d	348	306	263	362	314	266	246	193	141
Potassiummg/d	506	346	188	557	377	196	708	512	315
Selenium µg/d	3	0	0	5	0	0	11	4	0
Sodium mg/d	253	199	144	301	239	177	469	401	334
Zinc mg/d	4.6	4.2	3.8	4.7	4.3	3.8	6.3	5.8	5.4
Zinc mg/d	2.5	2.2	1.9	2.6	2.3	2.1	2.7	2.4	2.1

[a]The categories Low,Avg and High correspond to breast-milk intake (g/d) being -2SD.

[b,c] Zinc needs from complementary foods have been estimatedin 2 ways indicated by superscripts d and c respectively as follows:

[b]Based on amount of zinc in breast milk from Institute of Medicine (1991) and zinc requirement from British DRV(Department of Health, 1991)

[c]Based on amount of zinc in breast milk from krebs et al., (1995) and estimated zinc requirements using data from Krebs & Hambidge (1986) and Krebs et al., (1993)

PRODUCTS SENSORY EVALUATION FORM

Name .....Date.....

1. **Look closely at these samples and check how you like or dislike the color**

	Sample.....	Sample.....	Sample	Sample .....
Like Extremely	.....	.....	.....	.....
Like very much	.....	.....	.....	.....
Like moderately	.....	.....	.....	.....
Like Slightly	.....	.....	.....	.....
Neither like nor dislike	.....	.....	.....	.....
Dislike slightly	.....	.....	.....	.....
Dislike moderately	.....	.....	.....	.....
Dislike very much	.....	.....	.....	.....
Dislike extremely	.....	.....	.....	.....

2. **Feel these samples and check how you like or dislike the texture**

Sample..... Sample..... Sample ..... Sample .....

Like Extremely.....	.....	.....	.....
Like very much	.....	.....	.....
Like moderately	.....	.....	.....
Like Slightly	.....	.....	.....
Neither like nor dislike.....	.....	.....	.....
Dislike slightly	.....	.....	.....
Dislike moderately	.....	.....	.....
Dislike very much	.....	.....	.....
Dislike extremely	.....	.....	.....

3. **Taste these samples and check how you like or dislike each one you, may RINSE your mouth after each sample.**

	Sample.....	Sample.....	Sample	Sample .....
Like Extremely	.....	.....	.....	.....
Like very much	.....	.....	.....	.....
Like moderately	.....	.....	.....	.....
Like Slightly	.....	.....	.....	.....
Neither like nor dislike	.....	.....	.....	.....
Dislike slightly	.....	.....	.....	.....
Dislike moderately	.....	.....	.....	.....
Dislike very much	.....	.....	.....	.....
Dislike extremely	.....	.....	.....	.....

4. **Perceive the aroma of these samples and check how you like or dislike each one.**

	Sample.....	Sample.....	Sample	Sample .....
Like Extremely.....	.....	.....	.....	
Like very much	.....	.....	.....	
Like moderately	.....	.....	.....	
Like Slightly	.....	.....	.....	
Neither like nor dislike	.....	.....	.....	
Dislike slightly	.....	.....	.....	
Dislike moderately	.....	.....	.....	
Dislike very much	.....	.....	.....	
Dislike extremely	.....	.....	.....	

5. **General Acceptability**

Assess all the samples and indicate your level of acceptability of each.

I would eat this at every opportunity I have……..1

I would eat this very often……2

I would frequently eat this……3

I like this and would eat it now and then……..4

I would eat this if available but would not go out of my way……..5

I don't like this but would eat it in an occasion……6

I would hardly ever eat this……7

I would eat this if there were no other food choices……8

I would eat this only if I were forced to……..9

PRODUCT	ACCEPTABILITY
SAMPLE.....	.....
SAMPLE.....	.....
SAMPLE.....	.....
SAMPLE.....	.....

COMMENTS.....

.....

.....

.....

.....

.....

.....

APPENDIX 2
TRADITIONAL PROCESSING OF COMPLEMENTARY FOODS

MOTHERS QUESTIONAIRE

State..... LGA.....

Name of facility (where interview is conducted).....

Name of mother.....

Occupation of mother.....

Educational status (highest attained).....

Age of mother.....

Name of infant... Age of infant

Sex male..... female.....

CHILD FEEDING PRACTICES

How do you normally feed the infant.....

What kind of food have you been giving the infant apart from breastmilk.....

Do you give maize ogi ?..... Sorghum ogi.....or Millet ogi.....

How do you prepare the ogi ?

-Soak with cold/ warm/ hot water.....

-Soak for how many days? 1 .....2.....3.....4.....

-Grind, sieve with washing, allow to settle..... for how long?..... ... .

Decant.....

Others.....

Do you mix ogi cake with water before boiling?.....

Do you add the ogi cake to boiling water?.....

What source of water do you use in the food preparation?(1) Tap.....(2) Stream.....(3) Well.....
Do you mix any other ingredients like milk, sugar, crayfish with the ogi?.....

Do you use separate utensils for preparing baby's food?.....

Is the cooked ogi spoonable or drinkable?.....

How do you feed the baby? Force feeding.....Spoon feeding.....or baby feeds himself or herself.....

What is the appropriate volume

Tea cup.....

Small feeding bottle.....

Glass cup.....

Big feeding bottle.....

Number of meals per day:1.....
2.....
3.....
4.....
5.....
6.... .....