# COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY AND SENSORY PROPERTIES OF COFERMENTED MAIZE, CARROT, PIGEON PEA AND MILLET, SWEETPOTATO, PIGEON PEA.


BY

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## CERTIFICATION

This is to certify that this research work was carried out by AJAYI FRANCISCA BOSEDE with the Matriculation Number MCB/11/0325 of the Department of Microbiology, Faculty of Science. Federal University Oye Ekiti under the supervision of DR. (Mrs.) OYAREKUA.

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# DEDICATION

My dedication goes to Almighty God, the source of my progress and the keeper of my dreams, whose hand has brought me this far through his favour and grace and who has also give me the privilege to witness this research work (project), my dedication also goes to my loving and caring parent Mr. and Mrs. Ajayi G.O.

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## TABLE OF CONTENT

# LIST OF TABLES

# LIST OF FIGURE

# ABSTRACT

In Nigeria low-socio economic mothers use fermented cereals like maize (*Zea mays*) and Millet (*Pennisetum americanum*) as infants' complementary food. Pigeon peas *(Cajanus cajan* L.) are underutilized legumes in Nigeria. In study of maize, carrot/pigeon pea and millet, sweet potato/ pigeon pea were co-fermented in ratio 6:2:2 (w/w) and the fermented products were compared. Nutritional, viscosity and microbial status of co-fermented cereal, legumes, tubers and vegetable were studied. Products were analyzed for vitamins, reducing sugar, trypsin inhibitory activity, pH and titratable acidity using standard methods. The predominant microorganisms isolated from both samples during fermentation period were *Lactobacillus* spp. Consistency properties were determined using Bostwick Consistometer. Vitamin B3 content was higher in the samples ($p > 0.05$) which was higher than recommended value. Vitamin B6 (Pyridoxine) and B1 (Thiamine) values were comparable to recommended values while some values were lower. In terms of organoleptic properties of the product co-fermented sample A (maize, carrot/ pigeon pea) was most preferred by the panelist with regards to color, taste, texture and aroma than sample that are not co-fermented. Co-fermentation of maize, carrot/pigeon pea and millet, sweet potato/pigeon pea resulted in a product of improved nutritional quality than fermented maize.

# CHAPTER 1

## INTRODUCTION

In Nigeria cereal, grains and tubers are the most widely cultivated and consumed, it provides a major food resource for man. They are the major source of energy and protein in the diet of many people. Nigeria National Food Consumption and Nutrition Status Survey (NFCNS) (2004) showed that 43% of children under 5 years of age were stunted, 10% wasted, 36% are underweight and 29.5% vitamin A deficient (VAD). 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. Complementary foods must have ease of preparation affordable price and meet the Infant's nutritional requirements (Wieringa *et al.,* 2003). Socio-economic status mothers still see vitamin supplements as pharmaceuticals, extra-labor and cost. Nigeria is rich in cereals, but vitamin A activity of these foods in form of provitamin (β-carotene) is low (Shamin *et al.,* 2003).

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 from infectious especially diarrhea diseases which has the greatest negative impact upon the growth of infants and young children. The sources of diarrhea have been ascribed to be from water supply and sanitation and preparation of foods under unhygienic conditions as reported by Motarjemi *et al.*, (1993). The microorganisms that will dominate during and after fermentation depend on several factors, sometimes microorganisms initially present in very low numbers in the f d, for example lactic acid bacteria (LAB), will outnumber the other organisms inhibiting their growth.

Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide nutrients. Good nutrition is essential for the growth and development that occurs during an infant's first year of life. When developing infants are fed the appropriate types and amounts of foods, their health is promoted. Positive and supportive feeding attitudes and techniques demonstrated by the caregiver help infants develop healthy attitudes toward foods, themselves, and others. For proper growth and development, an infant must obtain an adequate amount of essential nutrients by consuming appropriate quantities and types of foods. During infancy, a period of rapid growth, nutrient requirements per pound of body weight are proportionally higher than at any other time in the life cycle. Although there are many nutrients known to be needed by humans, requirements have been estimated for only a limited number of these.

**1.1 Cereals** are the most widely cultivated and consumed crops on globally. In Nigeria, specifically in the Northern part and southwest of the country, cereal provides a major food resource for man. It is the major source of energy and protein in the diet of many people especially to infant. Maize is the second most important cereal crop in Nigeria ranking behind sorghum in the number of people it feeds. Estimated annual production of maize is about 5.6 million tones. (Central Bank of Nigeria report 1993).Maize is a multipurpose crop, providing food and fuel for human being and feed for animals (poultry and livestock). Its grain has great nutritional value and can be used as raw material for manufacturing many industrial products. (Afzal *et al.*, 2009).Due to nutritional composition of maize, it serves as a good.

**1.2 Maize** (*Zea mays*) is the second most important cereal crop in Nigeria ranking behind sorghum in the number of people it feeds. Estimated annual production of maize is about 5.6 million tones. (Central Bank of Nigeria report 1992).Maize is a multipurpose crop, providing food and fuel for human being and feed for animals (poultry and livestock). Its grain has nutritional value and can be used as raw material for manufacturing many industrial products. (Afzal *et al.*, 2009).Due to nutritional composition of maize, it serves as a good.

Maize (*Zea mays L., Poaceae*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Osagie and Eka, 1998). The name maize is derived from the South American Indian Arawak-Carib word 'mahiz'. It is also known as Indian corn or corn in America (Kochhar, 1986; Purse glove, 1985). It was introduced into Nigeria probably in the 16th century by the Portuguese (Osagie and Eka, 1998). In Nigeria, maize is known and called by different vernacular names depending on locality like 'agbado', 'igbado' or 'yangan' (Yoruba); 'masara' or 'dawar masara' (Hausa); 'ogbado' or 'oka' (Ibo); 'apaapa' (Ibira); 'oka' (Bini and Isha); 'ibokpot' or 'ibokpot union' (Efik) and 'igumapa' (Yala) (Abdulrahaman and Kolawole, 2006). Maize (*Zea mays L.)* commonly referred to as corn is used as staple food in Nigeria. The most probable origin of maize is Mexico (Central America), from where it spread to other parts of the world. In Teotihuacán (Mexico), the maize was found about 7000 years ago. It is reported that maize has been introduced to other counties in 1400s' where it has been cultivated in spring and winter seasons. Presently the maize is cultivated throughout the year in almost every part of the world. The potential yield of maize per unit land area is highly dependent upon fertility levels, plant population, management practices, and inherent potential of the variety adapted to that area. Present day maize hybrids show better response towards maximization of fertilizers. Higher yields are achieved from hybrid maize because it uses

nutrients more efficiently than synthetic varieties. Maize is a cereal crop that grows across a range of agro-ecological zones in Nigeria, through it is grown slightly more in the northern part of the country. Two type of maize are grown in Nigeria, the yellow and white variety and due to its rate of adaptability. Maize is a warm-season crop that germinates and grows poorly during cool weather, and should only be planted when there is no more danger of frost. When harvesting maize crop, the crop must not be left on the ground or on bare soil, where fungal spores develop. It must be properly clean and sieve to remove broken kernels, foreign matter, and diseased and rotten grains. These attract moisture and pests leading to spoilage and/or fungal Growth. Maize crop should be properly dried immediately after harvesting. Drying will not reverse the effect of poison in contaminated grains, but it may inhibit further growth of molds.

**1.3 Nutritional Quality of Maize**

The importance of maize grains to the nutrition of millions of people around the world is widely recognized. Because they make up such a large part of diets in Nigeria, maize grains cannot be considered only as a source of energy, as they provide significant amounts of protein as well. It is also recognized that cereal grains have a low protein concentration and that protein quality is limited by deficiencies in some essential amino acids, mainly lysine Much less appreciated, however, is the fact that some cereal grains contain an excess of certain essential amino acids that influence the efficiency of protein utilization. Maize, being popular as a food item, is enjoyed by people in various forms, like, whole corn, corn flour, cornstarch, corn gluten, corn syrup, cornmeal, corn oil, popcorn, cornflakes, etc. Apart from satisfying the taste buds of its users, maize is also a good source of vitamins, minerals and dietary fiber.

**1.4 Millet** (*Pennisetum americanum*) is one of the cereals produced extensively in Nigeria. Nigeria produces 21% of the world's total millet (FAO, 2002). Millet contains about 67% carbohydrate, and 12% protein. The seed is high in ash, iron, and phosphorus and is an important source of the B group of vitamins (FAO, 2005). The essential amino acid profile of millet indicates that it contains more lysine, threonine, methionine and cysteine than sorghum (FAO, 2005). Despite the rich nutrient content of millet, its use in Nigeria is limited to the production of household porridge-type breakfast gruel (akamu dawa) and dough (fura).

Millets are some of the oldest of cultivated crops. The term millet is applied to various grass crops whose seeds are harvested for food or feed. Millet is thought to have originated in North, specifically in Ethiopia, where it has been consumed since prehistoric times in Africa countries. The five millet species of commercial importance are proso, foxtail, barnyard, and brown top and pearl. Millet is tiny in size and round in shape and can be white, gray, yellow or red. Generally, the millets are small-grained, annual, warm-weather cereals belonging to grass family. They are highly tolerant to extreme weather conditions such as drought and are nutritious compared to the major cereals such as rice and wheat. They contain low phytic acid and are rich in dietary fiber, iron, calcium, and B vitamins. Moreover, these millets release sugar slowly in the blood and also diminish the glucose absorption. These properties of the minor millets made the present consumers attracted to the consumption of millet.

However, after the grain is harvested and threshed, it must be de-hulled for human consumption. The hulls of millet seeds are variable in color, and can even be striped. Millet should be cleaned of weed seeds and broken kernels, then stored at no higher than 13% moisture. For human consumption, millet can be de-hulled using compressed a[illegible] or impact dehullers, and used as is or ground into flour. Millet has a bland flavor, which some describe as slightly nutty.

Gene Logsdon, author of Small-Scale Grain Raising, asserts that this nutty flavor can be further brought out if the millet seeds are lightly toasted before using them.

### 1.5 Nutritional Quality of Millet

Millets are found to have high nutritive value and comparable to that of major cereals such as wheat and rice (Parameswaran and Sadasivam 2003). It has also been reported that millet proteins are good sources of essential amino acids except lysine and threonine but are relatively high in methionine. Millets are also rich sources of phytochemicals and micronutrients (Mal *et al.,* 2010; Singh *et al.,* 2012).

For example, pearl millet was found significantly rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants (Ragaee and others 2006). It contains about 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali *et al.,* 2003). Also, foxtail millet protein characterization showed that its protein concentrate is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Mohamed and others 2009).

Finger millet also is known to have several potential health benefits and some of the health benefits are attributed to its polyphenol contents (Chethan and Malleshi 2007). It has a carbohydrate content of 81.5%, protein 9.8%, crude fiber 4.3% and mineral 2.7% that is comparable to other cereals and millets. Its crude fiber and mineral contents are markedly higher than those of wheat (1.2% fiber, 1.5% minerals) and rice (0.2% fiber, 0.6% minerals); its protein

is relatively better balanced; it contains more lysine, threonine, and valine than other millets (Ravindran 1991; Sripriya and others 1997). In addition, black finger millet contains 8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein (Glew and others 2008). Kodo millet and little millet were also reported to have 37% to 38% of dietary fiber, which is the highest among the cereals; and the fat has higher polyunsaturated fatty acids ( Hegde and Chandra 2005). The protein content of proso millet (11.6% of dry matter) was found to be comparable with that of wheat and the grain of proso millet was significantly richer in essential amino acids (leucine, isoleucine, and methionine) than wheat protein (Kalinova and Moudry 2006). Thus, the presence of all the required nutrients in millets makes them suitable for large-scale utilization in the manufacture of food products such as baby foods, snack foods, and dietary food and, increasingly, more millet products have entered into the daily lives of people, including millet porridge, millet wine, and millet nutrition powder from both grain and flour form (Subramanian and Viswanathan 2007; Liu and others 2012).

**1.6 Pigeon Pea** (*Cajanus cajan*) cultivated widely in Nigeria is known as 'otili' in southwest Nigeria, is one of the most drought –tolerant and widely grown legumes in tropical and sub-tropical regions. Its cultivation has been reported in more than seven countries including Nigeria (Aiyeloja and Bello, 2006, Odenyi 2007). However, pigeon pea is still underutilized as food due to its tough texture, long cooking duration and lack of education on its nutritional potentials. (Oyarekua 2012) levels high levels of cysteine, methionine, and lysine

It is drought –tolerant and Nigeria is one of the major producers (Odenyi 2007, Aiyeloja and Bello, 2006). Although pigeon pea is reported to contain 20- 22% protein, 1.2% fat, 65%

carbohydrate and 3.8% ash with mineral content and high level cysteine and methionine 30%, and lysine (6.8%) when compared with soybeans except in methionine content (FAO, 2002), it is high in soluble sugars. The lipid content includes triglyceride and phospholipids. The legume has flatulence-causing Oligosaccharides, anti-nutritional factors like protease (Trypsin and Chymotrypsin) inhibitors, amylase inhibitors and polyphenols, in smaller amounts than most legumes. (Singh, 1988).

However, It is still under-utilized as food in Nigeria (Eneche, 2009), where it is mainly resorted to during the hunger period (before harvest season) when it will be boiled for several hours to soften the hard seed and then eaten alone or in combination with starchy staples. (Obizoba, 1983). The long cook-ability consumes time and fuel, and there is generally lack of education on its nutritional potentials. (Faseyiro et al, 2009 and Amaefule and Nwagbara, 2004, Odeny 2007). In Nigeria, Legume fermentation is rarely used for food but only as condiments such as like 'iru' or 'dawadawa'. Fermentation can increase the soluble phenolic content of pigeon pea and consequently enhance the antioxidant activities of the legume (Oboh, Ademiluyi and Akindahunsi 2009). It can be cooked whole until tender then mixed with maize, freshly cooked sweet potatoes in addition to vegetables, palm oil, salt, pepper and other spices (Enwere, 2005). Virtually all products obtained from soya bean can be produced from pigeon pea Odeny (2007).

Pigeon pea can be grown in a wide range of soil textures, from sandy soils to heavy clays. It grows best at a soil pH of 5.0–7.0 but tolerates a wider range (4.5–8.4). It does well in low fertility soils, making it a favorite among subsistence farmers. As with most legumes, it does not tolerate waterlogged or flooded conditions for very long. Pigeon pea is very heat-tolerant and grows well in hot, humid climates; it thrives under annual rainfall between 24 and 40 inches (600–1000 mm). It is generally grown where the temperatures are in the range of 64–85oF (18–

30C), but under moist soil conditions it can withstand temperatures of 95oF (35oC) or more. Once established, it is one of the most droughts tolerant of the legumes, and it can be grown in rain fed conditions or with minimal irrigation. Pigeon pea is widely known for its use as food. Immature pods, immature seeds, and the mature seeds can be consumed. The seeds are used whole, dehulled, or ground to a flour. In the Caribbean, people often eat the seed as the green (immature) pea, but it is mostly processed into a dried split-pea ("dahl").*Bradyrhizobium* and *Rhizobium* strains. Pigeon pea are cultivated in more than 25 tropical and sub-tropical countries,either as a sole crop or intermixed with cereals such as sorghum (*Sorchum bicolor*), pearl millet (*Pennisetium glaucum*), or maize (*Zea mays*) or with other legumes.

Storage of food grains is an important aspect of Harvest Technology. Food grains requirement of any country remains constant throughout the year regardless of price and season. Supply of food grains has to be maintained by proper stage throughout the year. Storage losses are considerably higher in pulses than in cereals. Pigeon pea is usually stored for long periods to ensure availability of whole seed at the time of sowing, and as a dhal to meet consumer requirement.

**1.7 Nutritional Quality of Pigeon Pea**

Pigeon pea seeds are made up of 85% cotyledons, 14% seed coat, and about 1% embryo, and contain a variety of dietary nutrients. The cotyledons are rich in carbohydrates (66.7%) while a major proportion (about 50%) of seed protein is located in embryo. About one-third of seed coat is made up of fiber. The quantities of important sulfur-containing a. ino acids such as methionine and cystine range around 1% and they are pre-sent in cotyledons and embryo; while

calcium is pre-dominantly present in seed coat and embryo. In general, pigeon pea is not rated superior for sulfur-containing amino acid, but it is not linked in pigeon pea seed the proportion of prola-min is low while sugars such as stachyose and verbas-cose are high. Pigeon pea significantly contributes to meet the dietary requirements of crude fiber, ash, fat, magnesium, manganese, and copper (Faris and Singh 2006). Pigeon pea contains high amounts of vitamin B, carotene, and ascorbic acid (Miller *et al.,* 2002). These are deficient in cereals; therefore, pigeon pea has a good supplemental value of cereal-based diet. Pigeon pea is a rich source of lysine but deficient in the sulfur-containing amino acids—methionine and cysteine. Cereal grains contain sufficient levels of methionine and cysteine. Faris and Singh (1990) reported that pigeon pea improves the amino acid score for lysine in rice- and wheat-based diets, and for threonine, leucine, and isoleucine in wheat-based diet when used in a 70:30 cereal: pigeon pea ratio. Pigeon pea may be sprouted by keeping them damp—either in a bowl of water or a wet towel—for one to several days. Sprouting increases the digestibility of pigeon peas and changes the flavor. Pigeon peas may also be ground into flour. Immature pigeon peas are edible, but generally have less nutritional value than mature peas.

**1.8 Sweet Potato** *(Ipomean batatas)*, another major staple Nigeria food also grows best in sub-humid regions with rainfall between 1000-2000 mm and temperature of 25-32oC. Sweet potato roots are bulky and are easily perishable unless cured. This limits the distance over which sweet potato can be transported economically (Katan and De Roos, 2004; FAO, 2011). It is consumed as boiled or fried chips.

The **sweet potato** is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are a vegetable. The young leaves and shoots are sometimes eaten as greens. *Ipomoea batatas* is native to the tropical regions in America of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *I. batatas* is the only crop plant of major importance—some others are used locally, but many are poisonous. The sweet potato is only distantly related to the potato (*Solanum tuberosum*) and does not belong to the nightshade family.

Production is highly seasonal in most countries leading to marked variation in the quantity and quality of roots in markets and associated price swings. Most especially, in Africa, there is commercial processing into chips or flour, which could be stored for year round consumption for use such as in bread and cakes, or processing into fermented and dried products like fufu.

Sweet potato consumption has declined as incomes rise - a change often linked with urbanization, partly because it is perceived as a "poor man's food" but mostly because of the lack of post-harvest processing or storage (FAOSTAT, 2008; Centro International de la Papa, 2009). A sensible approach to achieve the goal of sweet potato product development would be to increase the nutritional content of this highly consumed crop.

It is among the world's most important and under-exploited food crops (Scott and Maldonado, 2007; Grant, 2003). With more than 133 million metric tons in annual production, sweet potato currently ranks as the fifth most important food crop on a fresh-weight basis in developing countries after rice, wheat, maize, and cassava (Scott and Maldonado, 2007; Grant, 2003).

Sweet potato is a very efficient food crop and produces more dry matter, protein and minerals per unit area in comparison to cereals (Woolfe, 1992). Apart from being a rich source of starch,

sweet potatoes contain good quantity of secondary metabolites and small molecules which play an important role in a number of processes (Friedman, 1997). Many of the compounds present in sweet potato are important because of their beneficial effects on health, therefore, are highly desirable in the human diet and functions as a functional food (Katan and De Roos, 2004).

Sweet potato can, and does, play a multitude of varied roles in the human diets being either supplemental or a luxury food besides being a staple crop for some parts of the world (Sosinski *et al.,* 2001).

Sweet potato is an herbaceous perennial in the family Convolvulaceae grown for its edible storage roots. Sweet potato is a plant grown for its tuberous roots in tropical, subtropical and warm-temperate regions. Sweet potato tubers are a staple food or an alternative food in many countries and part of the production is used for animal feeding. The plant has enlarged roots called tubers which act as an energy store for the plant. The fruits are round, 1-4 seeded pods containing flattened seeds (Ecocrop, 2010; Duke, 1983). The tubers can be variable in shape and can be red, yellow, brown, white or purple in color

### 1.9 Nutritional Quality of Sweet potatoes

Depending on varieties and growing conditions, sweet potato leaves are comparable to spinach in nutrient content. Sweet potatoes also are high in minerals such as potassium, calcium, magnesium, sodium, phosphorus, and iron (USDA, 2009). The average mineral and vitamin content in a recently developed cultivar, is 117 mg calcium, 1.8 mg iron, 3.5 mg carotene, 7.2 mg vitamin C, 1.6 mg vitamin E and 0.56 mg vitamin K/100 g fresh w ht of leaves. Levels of iron, calcium and carotene rank among the top, as compared with other major vegetables. Sweet potato leaves are also rich in vitamin B, ß carotene, iron, calcium, zinc, protein and as well as

high fiber. The content of these nutrients differs according to harvesting period and variety. They are also rich in dietary fiber and have high water content and also provide 359 kJ energy with low total lipid content, which is only about 0.05 g per 100 g. In addition, Because of the various roles that sweet potatoes play around the world, the concept of nutritional quality and its contribution must transform to meet specific roles in human diet especially in infant nutrition. Similarly, supplemental types of sweet potato must have many of the same characters as staple types in terms of nutritional components. However, as they will not be major food component, the level of components may be more flexible and good.

As a crop, sweet potato is more tolerant of diseases, pests and high moisture than many other leafy vegetables grown in the tropics. Sweet potato leaves are an excellent source of antioxidative polyphenolics, among them anthocyanins and phenolic acids such as caffeic, monocaffeoylquinic (chlorogenic), dicaffeoylquinic and tricaffeoylquinic acids, and are superior in this regard to other commercial vegetables (Ishiguro *et al.,* 2004).

**1.10 Carrot:** This member of the parsley family has lacy green foliage and long, slender, edible orange roots. Carrot has been renowned for over 2,000 years for their health-giving properties and high vitamins content in Nigeria. Although carrots are available throughout the year-round, making them a highly popular vegetable. Locally grown carrots are in season in the summer and fall when they are the freshest and most flavorful. Carrots belong to the *Umbelliferae* family, named after the umbrella-like flower clusters that plants in this family produce. As such, carrots are related to parsnips, fennel, anise, caraway, cumin and dill.

Carrot roots have a crunchy texture and a sweet and minty aromatic taste, while the greens are fresh tasting and slightly bitter., carrots can actually be found in a host of other colors including white, yellow, red, or purple. In fact, purple, yellow and red carrots were the only color varieties of carrots to be cultivated before the 15th or 16th century.

Most carrots can be harvested in less than three months. They can be picked anytime they reach a usable size. The largest carrots will have the darkest, greenest tops, but don't leave the roots in the ground too long or they will be tough. Most are at their prime when about 2.5cm in diameter at the crown. The tops of the carrots should be trimmed right after harvesting. Trimmed carrots should be stored in the refrigerator and wrapped in plastic and used within 1-2 weeks after harvesting. Set refrigerator temperature level below 35 degree F and high humidity to maintain vitality. Carrot are rarely bothered by pests

During the first five months of storage, carrots will actually increase their vitamin A content; and, if protected from heat or light, can hold their nutrient content for another two or three months. Since carrots are rich in beta carotene, steaming them makes this nutrient more readily availability to the body as heat breaks down the tough cellular walls that encase the nutrient. The crisp texture of carrots is the result of the cell walls being stiffened with the indigestible food fibers cellulose, hemicellulose, and lignin.

### 1.11 Nutritional Quality of Carrot

Carrot are rich in antioxidants Beta Carotene, Alpha Carotene, Phytochemicals and Glutathione, Calcium and Potassium, and vitamins A, B1, B2, C, and E, which are also considered

antioxidants, protecting as well as nourishing the skin. They contain a form of calcium easily absorbed by the body. Finally they also contain Copper, Iron, Magnesium, Manganese, Phosphorous and Sulphur - better than a wonder drug!!

Carrot can enhance the quality of breast milk. It can improve the appearance of the skin, hair and nails especially in infant. When taken daily it can lower cholesterol and blood pressure. Raw carrot contains beta-carotene, a strong antioxidant that can prevent cancer. Carrot juice when taken every day prevents bodily infections and is claimed to be valuable for the adrenal glands (the small endocrine glands situated above the kidneys). Carrot can help improve eye health. Carrot can help increase menstrual flow. Carrot can regulate blood sugar. Carrot can promote colon health, because it is rich in fiber.

Carrot is also helpful in the following cases: Obesity, poisoning of the blood, gum disease, insomnia, inflamed kidney, liver, gallbladder, Alzheimer's disease, colitis, ulcer and painful urination. Carrots are one of the richest sources of Vitamin A. Carotene present in this vegetable gets converted into Vitamin A by our body. It is indeed amazing that a mere 100grams of carrot supplies around 11,000 milligrams of vitamin A.

Other major minerals present in carrot include sulphur, phosphorous and magnesium. The three minerals calcium, phosphorus and magnesium are essential for ensuring the strength of bones. Phosphorus is essential for the health of skin, hair and nerves. The vital magnesium content present in fresh carrot enables mental development, digestion of fats and the metabolism of mineral salts such as calcium, phosphorus, sodium, and potassium. ılphur also forms a major ingredient of insulin, the hormonal function of which is to convert carbohydrates into energy. Chlorine can be present in carrot from the processing method, this element is vital for the proper

functioning of liver. Provides a cleansing and antiseptic effect on the digestive and circulatory systems, but can of course be obtained from other sources.

Another nutrient in carrot is Vitamin E, the muscle vitamin. It promotes the efficiency of the entire muscular system by the effective utilization of oxygen.

**1.12 Fermentation**

Fermentation is an age long method of processing cereals, grains, tubers and vegetable. It modifies some physical characteristics of cereals, grains, tubers and vegetable increases the level of some nutrients, digestibility and bioavailability (WHO, 1998), decreases levels of anti-nutrients, increases nutrient density and imparts some antimicrobial property. According to Quinn et al. (1975), fermentation of grains and oil seeds results in increased nutritional value and wholesomeness over the starting material and it may also lead to changes in vitamin Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some underutilized plant foods like maize, millet, pigeon pea, sweet potato, and carrot as infant food. Microorganisms in food products are diverse due to intrinsic and extrinsic factors which affect the functional properties, processing, storage and consumption (Dullon 2004). Complementary foods are the first nutrients providing foods given to infants in addition to breast milk after six months of age and are consumed by over 90% of infants over 6 months of age in Nigeria. Prevalence of under nutrition and micronutrients deficiency is high among infants and young children of 6-23 months old (Oyarekua, 2011). During c nplementary feeding, infant is very vulnerable nutritionally therefore; introduction of semi-solid foods at the expense of breast milk must provide adequate nutrients for the rapid phase of growth and development.

Complementary foods are specially prepared to meet the particular nutrient and physiological needs of the infant (FAO/WHO, 2005). A complementary food must have a high calorie and micronutrient density, be in a "low bulk" or drinkable form, free of bacterial contamination, and must be of a quantity that can be consumed at one feeding. In Nigeria, the first complementary food is usually a thin cereal porridge made from fermented cereals (Oyarekua 2011). This porridge is called ogi it and provides 20-26 kcal/kg per day and Nigerian infants have an average energy density of 0.26 kcal/g (Oyarekua 2011). Most of these cereals contain some nutritional factors and minerals like carbohydrate, protein lipids, fat, water and ash. In their natural form whole grain cereals are also significant contributor of vitamins, minerals like manganese, zinc, copper and magnesium and considerable iron but its bio- availability is low. The results in incidence of iron deficiency anemia, however processes like fermentation has improved the chemical bio- availability of iron. (Dada 2002).

### 1.13 Lactic acid spontaneous fermentation

Lactic acid spontaneous fermentation is one of the ancient methods of food processing and preservation especially cereals and legumes. By tradition, lactic acid bacteria (LAB) are the most commonly used microorganisms for preservation of foods. 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).

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 micro flora of the raw materials is in action in most of the food fermentation processes.

During fermentation, there is significant increase in the soluble fraction, water soluble vitamins of the food substrate. The quantity as well as quality of the food proteins as expressed by biological value and decrease in the anti-nutritional factors. (Paredes-López & Harry, 1988).On dry matter basis, fermentation decrease dry matter of the food and increase the concentrations of vitamins, minerals and protein. (Khetarpaul & Chauhan, 1990), reported that single or mixed culture fermentation of pearl millet flour with yeast and lactobacilli significantly increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content. Fermentation leads to appreciation of fermented food for pleasant flavor, aroma and texture. Enzymes and microbes indigenous to the raw material play major roles in enhancing these attributes. Fermentation also improves cooking and processing properties and average storability where refrigeration is unavailable. Combination of cooking and fermentation have been reported to improve the nutrient quality of all tested sorghum seeds and reduced the content of anti-nutritional factors to a safe level in comparison with other methods of processing (Obizoba & Atii, 1991). Fermentation process is reported to increase the digestibility of starch in Bengal gram, cowpea and green gram and cooking of these fermented legumes further increased the starch digestibility (Urooj & Puttaraj, 1994).

Foods like i*njera* from tef, and *kisra* from sorghum are commonly made after fermenting dough for two or three days with or without starter. *hama-natto* which is a soybean paste made of

groundnut press cake, or soybean press cake used as a relish, *Kimchi* is a popular fermented food made mainly of vegetables in Korea. Pickled fruits and vegetable are common in many countries and *sauerkraut* is a well-known product made by fermenting cabbage.

Nigerian *ogi* a smooth-textured, sour porridge is made by lactic acid fermentation of corn, sorghum, or millet. Optimum pH for *ogi* is 3.6 to 3.7. The concentration of lactic acids may reach 0.65 percent and that of acetic acid 0.11 percent during fermentation. If the pH falls to 3.5, it is less acceptable. *Ogi* is a naturally fermented product. A wide variety of molds, yeasts, and bacteria are present initially. *Lactobacillus plantarum* appears to be the essential microorganism in the fermentation. Following depletion of the fermentable sugars, it is able to utilize dextrins from the corn. *Saccharomyces cerevisiae* and *Candida mycoderma* contribute to the pleasant flavor.

The lactic acid fermentation in the production of Indonesian *tempe* a vegetable (soybean) protein meat substitute, is by mycelium of *Rhizopus oligosporus*. The metabolic activity of *lactobacilli* occurs during the initial soaking leads to decrease in pH falls from about 6.5 to between 4.5 and 5 .0. The lower pH facilitates growth of the mold and prevents development of pathogenic bacteria that might spoil the *tempe*.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Traditional complementary foods based on cereals have inadequate nutrients required by infants. Gruel of fermented cereal is called 'ogi' in Yoruba native language of south-west Nigeria.

In many West African countries, exclusive breastfeeding is usually adequate up to three to four months of age, but after this period it may become increasingly inadequate to support the nutritional demands of the growing infant. Thus, in a weaning process there is always the need to introduce soft, easily swallowed foods to supplement the infant's feeding early in life. The process may be gradual, lasting for months until the infant is finally introduced to the family diet. On the other hand, in abrupt weaning, the infant is introduced straight into the family menu. This latter option creates a problem, as the child may not be able to eat enough of the adult diet to meet his or her nutritional needs.

In West African countries, weaning can be a period of problems and vulnerability for the survival of a child. In Nigeria the usual first fermented food given to infant is called pap, *akamu, ogi,* or *koko* and is made from maize (*Zea mays*) and millet (*Pennisetum americanum*). In most states mothers introduce the thin gruel at three to six months of age. The baby is fed on demand with a spoon or a cup, although in certain parts of the country, a few mothers use the traditional forced hand-feeding method.

Much research work has observed that the role of fermentation i food processing cannot be overemphasized. Olukoya, Tichaxzek, , Butsch, Vogel, and Atammes, ( 1993a) observed that

microorganisms involved in the fermentation process helps to enhance the nutritive value, eliminate toxic component, produces flavor and removes antinutritional factors.

According to Oboh, Ademiluyi, and Akindahunsi, (2009), f fermentation can increase the soluble phenolic content of pigeon pea and consequently enhance the antioxidant activities of the legume Adebayo-Tayo ET AL. C (2009) worked on the microbiological and nutritional Quality of Retail and Laboratory 'Ikpan' (mushroom-melon cake) a local Snack-

Amuna *et al.,* (2000) reported that cooked carrots due to release of antioxidants by heat contain *more* of antioxidants than raw carrots and that Sweet potatoes (*Ipomea batatas*) Sweet potato's root (tuber) is rich in raffinose and high percentage of anti-nutritional factors (which can inhibit with calcium absorption (Amuna *et al.,* 2000). Eneche, (2009) reported that traditional lactic acid spontaneous fermentation reduce anti nutritional factors (ANF), increase nutrient density giving a product of pleasant flavor, aroma and texture due to the metabolic activities of enzymes and microflora of the raw materials (Eneche, 2009). Much work has been done on composite blends of fermented maize with other legumes to improve nutritional quality of maize (Agu and Aluya, 2004). Phillippy (2003) suggested that combination of cereals and legumes or tubers with fruits, vegetables food rather than the single diets, can better support growth and development.

Much work has been done by using composite blends of raw, malted and fermented maize with other legumes as a way of improving nutritional quality (Agu and Aluya, 2004). The uses of supplements surpass current recommendation for energy and protein intakes therefore supplements can be growth-limiting due to nutrient deficiencies other than energy or protein. Most supplementations last only by 2-3 months which is long enough to treat anemia but not

long enough to detect improved linear growth. (WHO/FAO/UNU98). Poor combination of staples has partly contributed to the poor performance of traditional complementary foods. A number of researches (Oyarekua, 2013) have shown that a combination of cereals and legumes or tubers with fruits, vegetables and animal sourced food rather than the single diets, can better support growth and development. Since the consumption of the cereals based flour food is very popular among weanlings in Nigeria; it is necessary to produce co-fermented cereal/legume/tuber and vegetable as means of improving the nutritive value of cereal- based gruels using affordable time and energy saving co-fermentation technology. Acidic foods like fermented maize or co-fermented cereals/cowpea slows gastric emptying and decrease absorption rate. So also viscous food with soluble fibers, protein also delays gastric emptying. Besides being a rich source of vitamin and mineral, and other valuable nutrients roots like carrots and sweet potatoes also contain high amount of carotene and antioxidants such as polyphenolic compounds that have nutritional benefits if used as infant complementary food Therefore, it is important to include these roots or their products in complementary food preparation.

Many microbiological studies deal with identification of organisms isolated from various fermented foods. Lactic acid bacteria isolated from tomatoes that were naturally fermented under partial anaerobic conditions were found to *be Leuconostoc mesenteroides, Lactobacillus brevis* and *Streptococcus* sp. (Moktsm and sarkar 2010). In Asia mainly moulds of the genera *Aspergillus, Rhizopus, Mucor, Actinomucor, Amylomyces, Neurospora* and *Monascus* are used in the manufacture of fermented foods. In Europe, mould-ripened foods are primarily cheeses and meats, usually using a *Penicillium*-species. Gari made by fermentir cassava slurry was found to contain *Bacillus, Aspergillus* and *Penicillium* spp. as the Predominant organisms (Dullon 2004). The micro-organisms present in a 13 fermented food made in Ghana called dawadawa after 24 h

of fermentation, predominantly were *Bacillus* sp. with small numbers of (0,3%) *Staphylococcus* sp., after 36 h 60% *Bacillus* sp., 34% *Staphylococcus* sp. and after 48 h 56% *Bacillus* sp. and 42% *Staphylococcus* sp. Indonesian tapé ketan, a sweet, sour and alcoholic rice product, is produced using a starter culture containing moulds, yeasts and bacteria. After 72 h of fermentation, the pH was 3.5 while the biomass of the hyphae of the moulds was 15.3 mg/g and of the yeast 3.3 mg/g.

## 2.1 JUSTIFICATION

Cultivation of grains, legumes, and vegetable and tuber crops in Nigeria as in most African countries is threatened by the low prices of the crops and their products. With the rising cost of labour and transportation, rural farmers can hardly sustain their farming systems considering the meager returns from their harvest. It is therefore advantageous to diversify the use of root crops beyond those of the traditional food adult consumption to infant complementary food. Because maize, millet, carrot, sweet potato, pigeon pea surpasses other root crops in terms of agronomic potentials, diversification into infant foods uses will increase demand, ensure attractive prices and consequently encourage farmers to sustain and expand their root crop farming units. Before this can be done, it is critical to have a detailed documentation of the production, utilization and marketing of this crop in Nigeria so that effective interventions to develop products that will suit the economic limits and socio-cultural habits of the populace can be put in place. The existence of technologies will accelerate development of such suitable products as soon as bottlenecks to their production, utilization and marketing are identified.

Fermentation process is mostly popular in maize and millet, but fermentation process has never been done on carrot, pigeon pea and sweet potato, this following food are been consumed through heating or cooking process.

Most people believed that pigeon pea is a proteinous food which takes many hours on cooking for consumption and it is cultivated world- wide and is produced extensively in Nigeria. Carrot as a vegetable and as a fruit crop is consumed mostly by infant and adult, it serve as food which aid digestibility and produce B-carotenoid for infant, it has never been fermented before or co-fermented with another food sample. Likewise sweet potato a tuber crops, fermentation is not popular in sweet potato.

## 2.2 AIM AND OBJECTIVE:

- This study was carried out to produce co-fermented product using cereal/legume/tuber/ vegetable to improve the nutritive value of cereal- based gruels as infants complementary food using affordable time and energy saving co-fermentation technology
- To isolate microorganism from co-fermented product
- To determine the organoleptic property of the product.
- To determine the sensory evaluation using nursing mother.

# CHAPTER 3

## 3.0 MATERIALS AND METHODS

Dry grains of maize, millet, pigeon pea, orange-fleshed tubers of sweet potatoes and fresh carrot were obtained from a local market in Ado-Ekiti, south-western Nigeria and Oye Ekiti. The research analyses were performed at the laboratories of Federal university Oye Ekiti, Nigeria Institute of Science Laboratory Technology, Ibadan. Department of Animal Production and Health Nutrition Laboratory, Akure.

### 3.1 Preparation of food samples

Questionnaire was distributed to about 15 mother's in federal university of Oye Ekiti in order to see 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. Before the fermentation process commence sweet potato whish serve as tuber crop was peeled us clean sterile knife and was washed using distilled water after wish it was weigh for further process.

### 3.2 Fermentation Process

Maize grain, carrot, pigeon pea grain were sorted, winnowed and weighed 600g of maize, 200g of carrot and 200g of pigeon pea were mixed together in a cover peal using 3000ml of distilled water for soaking at 72hours at a room temperature. For sample B, millet grain, sweet potato

tuber and pigeon pea grain were sorted and weighed, 600g of millet, 200g of sweet potato and 200g of pigeon pea were mixed together in a small cover peal using 3000ml of distilled water for soaking at 72hours at a room temperature, after 72h the mixture was wet milled and wet sieved using a muscling cloth. The sieved mixture was rewashed and re- sieved and the shaft was discarded. The mixture was allowed to settle in the steeping water for 24 hours after which the sediment was dewatered, oven dried, milled and packed for further analysis. 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.

The two samples fermentation process was carried in aseptic and conducive environment, before the experiment commence the work bench used was disinfected with the use of 75% ethanol.

For the control of the two samples, for sample A, maize grain Maize, carrot, pigeon pea grain were sorted and weighed. 60g of maize, 20g of carrot and 20g of pigeon pea were mixed together without fermenting them, they were blend together inside a blander (Warring Blender) after it has blend completely, they were sieved and the residue was discard and the sieved ones was put inside a small cover plate for further analysis. This same procedure was also carried out on the second sample which was sample B millet grain, sweet potato tuber and pigeon pea grain. Further analysis like pH and titre acidity, microbiological analysis including the biochemical tests, vitamins analysis, sensory evaluation, consistency, reducing sugar, trypsin inhibiting activity analysis and statistical analysis were carried out.

**Sample (A), (B) fermentation process 1000g (600:200:400)**

↓

Soaking in distilled Water for 72h (Microbial Isolation at every 24hours)

↓

Milled Fermented Mixture

↓

Sieving using a Cheese-Cloth

↓

Re-washing and Re-sieving of Residues

↓

Further Fermentation for 24 (Microbial Isolation after 24hours)

↓

Decantation

↓

Slurry

↓

Dewatering

↓

Paste

↓

Sun Dry for 48 hours

↓

Milling and Sieving

↓

FLOUR

**Figure 1: Flow chart diagram for fermentation process**

### 3.3 pH and Titratable Acidity (TA)

pH and TA of fermenting samples were monitored / carried out at 0hr, 24 hrs. till 72hrs of fermentation. pH was done by making use of pH meter in which 12ml of fermented aliquot were put in side a test tube by the use of 5ml syringe, the pH was checked at room temperation of 25°c after which the pH experiment was done. PHS-3C pH meter with fuse of 0.25A, serial number 600413079098, voltage 220V, power 5Wand frequency 50Hz was used at room temperature of $25^0$C.

For titratable acidity: 0.1 molar of NaOH was put inside an already set up burette on a retort stand, 10ml of the fermented sample was put inside a beaker and serve as acid, 2 or 3 drops of indicator was added with 10ml of the fermented sample (1% phenolphthalein). In TA determination, 10 ml of fermenting aliquot was titrated against 0.1M sodium hydroxide to Phenolphthalein end point. When the colour changes to a light pink coloration, the reading was taken.

#### 3.3.1Procedure for preparing the reagents used for the titre acidity

a) 1g of phenolphthalein was dissolved in 100ml of distilled water.

b) Four gram (4g) of NaOH was dissolved in 1000ml of distilled water to give 0.1M.

### 3.4 Microbiological Evaluations

The microbial status of maize, carrot, pigeon pea and millet, Swe potato, pigeon pea *ogi* (600:200:200w/w), were investigated.

**Isolation procedure:** Steep liquor (10ml portions of fermented sample) at 0hours was aseptically removed and put inside a test tube; five test tubes were put inside a test tube rack each test tube were labeled $10^1$, $10^2$, $10^3$, $10^4$, and $10^5$. 9ml of distilled water was dispense inside each tube using different sterile 1.0ml pipette , 1ml of steep liquor (stock) was dispense inside the first test tube containing 9ml of distilled water and the serial dilution was carried out on the other 4 test tube, when the dilution get to the last test tube which is the $5^{th}$ test tube the 1ml that was dispense to it from the test tube 4 which makes it 10ml, after it has been dispense inside it, 1ml was been removed from the $5^{th}$ test tube and was discard. The serial dilution was done aseptically at 0hour, 24 hours, and 48hours till 72hour period. 1ml was removed from test tube $10^2$ and $10^5$ inside a Petri dish plates for poured plate process.

For control analysis: 1g was taking from sample A and B control (Unfermented sample) and was pour inside a test tube with addition of 9ml of distilled water which serve as the stock for sample A control and sample B control. 5 test tube was arranged inside a tube rack for each sample and was labeled $10^1$, $10^2$, $10^3$, $10^4$, and $10^5$ 9ml of distilled water was dispense inside each tube using different sterile 1.0ml pipette , 1ml of steep liquor (stock) was dispense inside the first test tube containing 9ml of distilled water and the serial dilution was carried out on the other 4 test tube, when the dilution get to the last test tube which is the $5^{th}$ test tube the 1ml that was dispense to it from the test tube 4 which makes it 10ml, after it has been dispense inside it, 1ml was been removed from the $5^{th}$ test tube and was discard. The serial dilution was done aseptically at 0hour, 24 hours, and 48hours till 72hour period. 1ml was removed from test tube $10^2$ and $10^5$ inside a Petri dish plates for poured plate process. Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) were used for the culturing of microorgan ms from 0hour, 24hours and

48hours intervals. De-Man Rogossa and Sharpe Agar (MRS) was used for the culturing of microorganisms for 72hours.

### 3.5 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.13 sensory questionnaire was distributed for each one of them for them to record their result after each analysis of each sample. They were made to abstain from eating or drinking one hour before the assessment. Each mother's conducted an independent assessment in separate sensory booths of a fluorescent light laboratory. The cooked food samples were dished in a transparent plate so as not to affect the colour of the food samples. Each mother was provided with a spoon, serviette paper, a sachet of water to rinse their mouths before and after tasting each food sample so that the taste of the food samples will not affect one another.

The four samples (i.e. samples A and B fermented, samples AC and BC unfermented) were given to the mothers for evaluation. Sample A 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 B was also placed in front of the mothers for assessment and removed before the other two samples (sample AC and BC) were given to them separately for assessment.

### 3.6 Bostwick Consistency Measurements

A different grams sample was dissolved in different ml of distilled water as was illustrated bellow, were mixed and stirred constantly on hot plate at 80$^{o}$C. At the first appearance of first bubble, cooking was timed to 5 minutes after which were held for 15 minutes cooled to 45$^{o}$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 Bostwick Consistency measurment (Model TM Super-3 Analyzer Newport Scientific PTY Ltd. 1998 ½ -Apollo st., Warriewwood MXW 21- 02 Australia).

The consistency procedure was carried out on clean sterile work bench, food samples were prepared using a new clean stainless pot, a starrier, an electric cooker, a new clean small plates and a clean spoon for serving the food.

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

### 3.7 Reducing sugar

**Test for lactose and maltose sugar**

Lane and Eynon's method is used for carrying out reducing sugar analysis.

Reagents:

a) Fehlings solution: 69.278g of hydrated copper sulphate ($CUSO_4$, $^{5H}2^{O}$) was dissolved in water and make up to 1 litre. The strength of this solution was adjusted to contain 17.636mg copper per ml by standardizing by the following method.

10ml of the solution was pipetted into a 250ml flask and was diluted to about 50ml. 1g of potassium iodide was added and liberated iodine was titrated with 0.1N sodium thiosulphate solution, using starch near the end point, as an indicator.

1ml 0.1N sodium thiosulphate = 0.006354g$^{c}$u

10ml of Fehling solution; 1 should require 27. 75ml

0.1N thiosulphate.

b) Fehlings solution: 100g of sodium hydroxide and 346g of sodium potassium tartrate was dissolved in water and make up to 1litre.

c) Methylene blue solution: 1% aqueous solution.

d). Sugar solution: Preparation of sugar of a neutral solution of sugar clarifies with zinc acetate and potassium ferrocyanide solution. Aliquot of the sugar solution was diluted so that the titration is between 10ml and 25ml. This is achieved by diluting the sugar solution so that it contains 0.1% to0.3% sugar when 10ml of mixed Fehlings solution is used and 0, 25% sugar when 25ml is used.

e). Mixed Fehlings solution for the titration: 5ml (or 12.5ml) of Fe..ngs solution was pipetted into a 250ml titration flask. 5ml (or 12.5ml) of Fehlings solution was measure into the same flask

and mixed. In the alternative, equal volume of Fehlings solution may be mixed just prior to used and 10ml (or 25ml) pipetted into the titration flask.

### 3.7.1 Procedure

Add from the burette, 15ml or the sugar solution into the titration flask containing the Fehlings solution. The solution was boiled on an asbestos covered gauge, and the sugar solution was added. 1ml at a time at about 10 second intervals to the boiling liquid, until the blue colour is nearly discharged. 4 drops of the methylene blue solution was added and continue the titration until the blue colour of the indicator is completely discharged and the supernatant liquid becomes orange-red in colour.

The titration was repeated in another titration flask containing a similar quality of Fehlings solution. Before heating, (v-2) ml of the sugar solution was added. It was boiled gently for 2minutes. 4 drops of methylene blue solution was added and the titration was completed in one further minute so that the total boiling time is within 3 minutes. At the end point, the entire blue colour was been discharged and the liquid should appear orange-red.

**Determination of sucrose:**

For present of sucrose; an aliquot of the solution contain about 0.25g sucrose was put into 100ml volumetric flask to about 70ml with water.

Inversion of the sugar solution. 7ml of concentrated hydrochloric acid was added to the solution in the 100ml flask, it was mixed and the flask was immerse in a water bath maintained at 60°c for 12minutes, the flask was rotating for the first 3minutes. At the end of 12mintes the flask was removed and immerse in cold water for 15mintes. Neutralize of the contents of the flask was

removed to phenolphthalein with 5N sodium hydroxide. Make up to the mark with distilled water.

The Lane and Eynon titration with the inverted solution was perform using 10ml of Fehling's solution and calculated as invert sugar.

### 3.8 Trypsin inhibitor activity Test

a. 1g of sample was weigh into 50ml of 0.5M NaCl solution
b. The mixture was stirred for 30mins at room temperature
c. After, it was centrifuge at 1500rpm for 5min.
d. After 5mins, the supernatant was filter and use used for the assay
e. The concentration was read at 410nm.
f. 10ml of the same substrate was used as blank (i.e. 0.5M NaCl solution)

### 3.9 Biochemical Test

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.

#### 3.9.1 Procedure for urease and citrate

A small clean aluminum foil paper was put on a weighing balance, 1g of urease and citrate broth was weigh using different foil paper and was poured inside clean beaker. 90ml of distilled water was added with 2-3 drop of phenol red and allow dissolving for some minute. After some minute, 10ml was dispensing 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 the test tube was cork in the mouth with aluminum foil paper and cotton wool. After that it was put inside the autoclave for sterilization at $121^0$c for 15minutes, after complete the 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 loop, after completing this process the tubes was arrange inside beaker and put inside the incubator for 24hrs at $37^o$c for result analysis.

### 3.9.2 Procedure for sugar fermentation.

A small clean foil paper was put on a weighing balance, 5.6g of sucrose, lactose and maltose broth (Nutrient broth) was weigh using different foil paper, and it was removed and poured inside clean different beaker. 200ml of distilled water was added and allow dissolving and settle for some minute. After some minute the top layer of the nutrient broth was slowly removed into another beaker in which 2-3 drop of phenol red was added before the addition of each sugar, 10ml was dispense inside sterile test tubes using 10ml of syringe. The test tubes used was labeled according to the name of each sugar test .After dispensing the broth inside each test tubes, Durham's tube was introduce immediately after dispensing the broth inside test tubes, the test tubes 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

removed to phenolphthalein with 5N sodium hydroxide. Make up to the mark with distilled water.

The Lane and Eynon titration with the inverted solution was perform using 10ml of Fehling's solution and calculated as invert sugar.

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completing this process the test tubes was arranged inside a beaker and put inside the incubator for 24hrs at 37°c for result analysis.

### 3.9.3 Gelatin test

Nutrient gelatin is a differential medium that tests the ability of an organism to produce an exoenzyme, 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.

### 3.9.4 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.

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

### 3.9.5 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.

### 3.10 Vitamin Procedure

The vitamin content profile of the samples was analyzed following the methods of AOAC MTHD 992.03, 992.04 and 992.04 (2006).

Each sample was made to attain the laboratory atmospheric condition on the bench after removing the samples from the storage chamber at least than $4^{o}C$.

He sample was pressed and completely homogenized in the mortar carefully with pestle to avoid forming balls. About 0.10g of the sample was weighed into 10ml beaker capacity. The sample

was extracted in the container by the above stated modified methods. After the extraction, the extract was concentrated to 1.0ml for the chromatographic analysis.

**Gas Chromatographic Conditions**

GC= HP 6890 Powered with HP Chem Station Rev. A.0901 (1206)

Injection Temperature= Split Injection

Split Ratio= 20:1

Carrier Gas= Nitrogen

Flow Rate=1.0ml/min

Inlet Temperature= 250°C

Column Type= HP5

Oven program= initial at 50°C for 2mins.

First Ramp at 10°C/min for 20 min and maintained for 4min.

Second ramp at 15°C /min for 4 min and kept constant for 2min.

Detector = PFPD

Detector temperature = 320°C

Hydrogen pressure= 20 psi

Compressed Air= 3

# CHAPTER FOUR

## 4.0 RESULT AND DISCUSSION

**Table 1 pH Changes in co-fermented mixtures**

| | Fermentation time (h) | | | |
|---|---|---|---|---|
| Sample I.D | 0hr | 24hrs | 48hrs | 72hrs |
| A | 6.75 | 5.70 | 5.55 | 5.44 |
| B | 6.72 | 5.66 | 5.56 | 5.40 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea

**Table 2. Titratable acidity % Lactic acid sample of co-fermented mixtures**

| | Titratable acidity/ Fermentation time (h) | | | |
|---|---|---|---|---|
| Sample I.D | 0hr | 24hrs | 48hrs | 72hrs |
| A | 1.35 | 0.9 | 0.63 | 0.27 |
| B | 1.98 | 1.08 | 0.81 | 0.045 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea

**Table 3. Trypsin inhibitory activity (mg/g) of food samples**

| Sample I.D | A | B | Average |
|---|---|---|---|
| A | -0.13 | -0.13 | 0.13 |
| B | 0.68 | 0.70 | 0.69 |
| CA | 1.06 | 1.05 | 1.06 |
| CB | 1.77 | 1.75 | 1.76 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control).

**Table 4. Reducing sugar contents of food samples (mg/100g)**

| Sample I.D | A | B | Average |
|---|---|---|---|
| A | 9.23 | 9.23 | 9.23 |
| B | 8.14 | 8.15 | 8.145 |
| CA | 10.30 | 10.28 | 10.29 |
| CB | 10.25 | 10.21 | 10.23 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control)

**Table 5. Sensory evaluation of co-fermented and unfermented gruels**

| SAMPLE | COLOUR | TEXTURE | TASTE | AROMA | ACCEPTABILITY |
|---|---|---|---|---|---|
| A | $5.12^{a}$ | $5.90^{b}$ | $6.60^{c}$ | $7.10^{c}$ | $4.60^{b}$ |
| B | $5.30^{b}$ | $6.40^{c}$ | $6.70^{c}$ | $7.60^{d}$ | $4.11^{a}$ |
| CA | $5.90^{c}$ | $4.10^{a}$ | $5.80^{b}$ | $6.10^{b}$ | $5.40^{c}$ |
| CB | 5.10a | 4.11 | 5.30 | 5.90 | 6.00 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control).

Samples with the same superscripts were not significantly different.

**Table 6 Bostwick Consistency Measurement**

| Sample I.D | A | B | CA | CB |
|---|---|---|---|---|
| 2g | N.R | N.R | N.R | N.R |
| 6g | 14.5 | 15.8 | 26.0 | 11.0 |
| 9g | 10.4 | 9.5 | 8.1 | 3.5 |
| 10g | 6.2 | 4.0 | 4.5 | 1.5 |
| 12g | 4.1 | 3.4 | 2.6 | 0.7 |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control). N.R: No readings.

**Table 7 Bacterial count (CFU/ML) of co-fermented and un-fermented food samples.**

| | Fermentation Time (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample I.D** | **0 hr** | | **24hrs** | | **48hrs** | | **72hrs** | |
| **Dillution factor** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **A** | **2.8X10** | **N.G** | **2.7X10** | **1.7X10** | **7.8x10** | **2.8x10** | **7.8x10** | **2.8x10** |
| **B** | **$1.3X10^{-4}$** | **N.G** | **$4.4X10^{-4}$** | **N.G** | **$5.6x10^{-7}$** | **$1.3x10^{-5}$** | **$5.6x10^{-7}$** | **$1.3x10^{-5}$** |
| **CA** | **N.G** | **N.G** | **$8.6X10^{-4}$** | **$1.8X10^{-7}$** | **$1.8x10^{-7}$** | **$1.9x10^{-5}$** | **$1.8x10^{-7}$** | **$1.9x10^{-5}$** |
| **CB** | **N.G** | **N.G** | **$9.8X10^{-4}$** | **$1.7X10^{-7}$** | **$1.0x10^{-7}$** | **N.G** | **$1.0x10^{-7}$** | **N.G** |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control). N.G: No growth.

**Table 9 Isolation ad biochemical characteristics of Lactobacillus Spp. co-fermented and un-fermented food samples**

| Sample I.D | Gram rxn | Catalase | Utilization of gelatin | Suc | Lac | Mal | Ure | Cit | Probable organism | Isola code |
|---|---|---|---|---|---|---|---|---|---|---|
| Fermented A 1 | +ve cocci | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus. Planterum* | ATC 1491 |
| A2 | +ve short rod | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus Spp.* | |
| Fermented B1 | +ve short rod | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus Spp.* | |
| B2 | +ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *L. planterum* | |
| Control A1 | +ve short rod | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus Spp.* | |
| A2 | +ve short rod | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus Spp.* | |
| Control B2 | +ve cocci | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *L. planterum* | |
| B2 | +ve cocci | -ve | -ve | +ve | +ve | +ve | +ve | +ve | *Lactobacillus Spp.* | |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control). +Ve: Positive, -Ve: Negative. ATCC= American Type Culture Collection ( Rock Ville Mary Land). Suc= sucrose, Lac= lactose, Mal= maltose, Ure= urease , Cit= citrate

**Table. 10. Yeast Count co-fermented and un-fermented food samples**

| | Fermentation time(h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample I.D** | **0 hr** | | **24 hrs** | | **48 hrs** | | **72hrs** | |
| **Dilution factor** | $10^2$ | $10^5$ | $10^2$ | $10^5$ | $10^2$ | $10^5$ | $10^2$ | $10^5$ |
| **A** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** |
| **B** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** |
| **CA** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** |
| **CB** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** | **N.G** |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea, CA= Co-fermented millet/sweet potato/pigeon pea, CB= Unfermented millet/sweet potato/pigeon pea (control). N.G: No growth.

**Table 11. Vitamin profile (mg/100g) of co-fermented and un-fermented samples**

| Sample | VIitamins profile (mg/100g and microgram/100 and to IURE of co-fermented and unfermented Samples | | | | | | | | | | | TOTAL (mg/100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B3 µg | B4 | B6 | C | A µg | B1 | B2 | E | B9 µg | K µg | B5 | |
| A | 1345 | 0.003 | 0.119 | 20.204 | 52.6 | 0.171 | 0.067 | 0.062 | 0.000 | 2.0 | 0.353 | 22.374 |
| B | 3850 | 0.005 | 0.345 | 10.142 | 78.4 | 0.306 | 0.000 | 0.113 | 0.000 | 126.9 | 0.641 | 15.361 |
| CA | 1153 | 0.000 | 0.249 | 14.961 | 6.0 | 0.079 | 0.036 | 0.068 | 0.000 | 2.0 | 0.061 | 17.164 |
| CB | 1298 | 0.003 | 0.189 | 18.685 | 19 | 0.188 | 0.008 | 0.071 | 0.000 | 2.0 | 0.384 | 20.886 |
| RDA for Infant | 6.0 µg | | 0.5mg | 330mg | 400µg | 0.5mg | 0.5mg | 5.0mg | 150µg | 15µg | 2.0mg | |

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control),RDA = Recommended Daily Allowance from Infant Complementary food (WHO/FAO 2005)

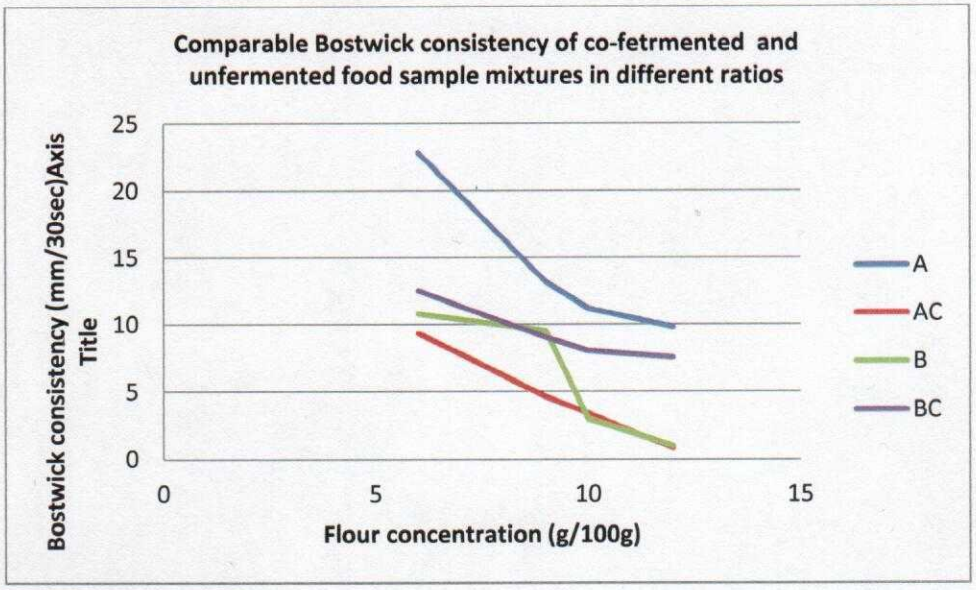


**Figure 2. Showing Bostwick Consistency Flow mm/ 30sec of co-fermented and un-fermented gruels**

Key: A= Co- fermented maize/carrot/pigeon pea, B= Co-fermented maize/ carrot/pigeon pea CA= Co-fermented millet/sweet potato/pigeon pea CB= Unfermented millet/sweet potato/pigeon pea (control)

## DISCUSSSION

### 4.1 Biochemical result discussion

The biochemical test for sucrose, lactose, maltose, urease and citrate show positive result indicating the present of these following sugars in the food sample. The catalase and gelatin test also show positive and negative result. For the gram reaction the result show positive cocci and positive rod which indicate that the bacteria present in the sample are both cocci and rod shape bacteria. There is also the presence of *Lactobacillus spp,* which indicate 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 Microbial Isolation

In Table 6, the evolution of isolated microorganisms during the co-fermentation process of food sample is presented. At the end of the fermentation process analysis for microbial contamination as safety of food must be guaranteed at the time of consumption. The tests were carried out for coliform, yeast and other pathogenic organism bacteria. As it can be observed that the total count was decreasing from 0hr to 72 hrs. this is in agreement with Golshan *et al.,* (2013) there was presence of *Lactobacillus spp* during the fermentation process mostly in 24hrs, 48hrs and 72hrs, 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 is growth in the co-fermented samples (A&B) when there e is no growth in the unfermented sample (A&B). The result shows that there are some coliform counts. 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. The presence of nitrogenous source from pigeon pea might have contributed to the survival of *Lactobacillus spp* up to 48 hours of fermentation in agreement with Wakil Onilude (2011). Fermentation is a novel preservation process where the low pH and high acid content are credited with the microbial integrity of the fermented product (panda *et al.,* 2007).

Over a study period of nine months, a group of children fed with lactic acid fermentation gruel had a mean number of 2,1 diarrhea episodes compared to 3,5 for the group fed with unfermented gruel (lorri and Svanberg, 1994). 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).

### 4.2.1 Yeast count

The plates for yeast showed no growth of colonies for the co-fermented food sample.

### 4.3 The Effects of fermentation on the Sensory profile and consumer acceptability

Sensory evaluation performs other function such as quality control, tracking of quality changes in a product on storage and investigation of general consumer perception of a product. The result data helps rate product based on the chosen attributes. Sensory analysis therefore showed the panelists' scores on the basis of their acceptability and preference for the selected attributed after taste, appearance and flavor.

The sensory evaluation results for the fermented samples are presented in Table 5. For flavor, the panelists' scores ranged from 7.6 to 4.1, on a hedonic scale of 1 to 9, indicating that they like the products moderately. The comparative study of but food sample indicates that they prefer the fermented sample to unfermented sample.

**4.4 pH and Titratable acidityS**

There was concurrence decrease in pH with increase in fermentation time the decrease was more pronounced at 72hrs to pH of 5.4 which is inhibitory to most bacterial growth. Lactic acid bacteria grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able to grow. The presence of pigeon pea must have made the fermenting medium a bit alkaline in nature thus preventing the pH from further reduction to pH 4.0 or below this is in agreement with the finding of (Oyarekua 2012.). Preservation of foods by microorganisms during fermentation process depends on pH 4.0–4.5; some are active at pH 9.6 and others at pH 3.2 (Oyarekua.2013.).

Also, the decrease in the pH of co-fermented samples might be due to availability of more nutrients probably from microbial proliferation and enhanced metabolic activities, (Table 1) and also cultivation and hybrid of maize grains and sweet potato tubers used. Decrease in pH during fermentation is usually due to accumulation of organic acid especially lactic acid (Arvanitoyannis, 2006; Kim,Min, Lee & Ji, 2000). The acidic nature of the products of both samples could be due to the production of lactic acid by microorganism associated with maize dough fermentation. In this study, co-fermented mixture increased the acid production which is desirable for souring and acceptability of product. However the population of bacteria and yeasts in this work was more reduced than the reported population by Sanni, *et al.,* (2002). Yeasts and

bacteria in this work may also be contaminants in cereals Abegaz *et al.,* (2002); and may not appear to play any significant role in the fermentation process however 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. The values of Titratable acidity decreased from 1.35 to 0.27mg/kg in sample A (fermented) and from 1.98 to 0.045mg/kg in sample B (fermented) over the period of fermentation. Titratable acidity (TA) is an important parameter in following the progress of a fermentation process. TA is either inherent or induced as a result of the conversation of sugars to lactic acid by the lactic acid cultures

**4.5 Reducing sugar**

The reducing sugar is high in control A and control B (unfermented sample) but lower in the co-fermented sample A and B (fermented sample), this can be as a result of the presence of sweet potato tuber through compared to that one that does not pass through fermented together with another food product. Also decrease in starch and sugar is as a result of their utility by *Lactobacillus planterum* for growth and metabolism (Gardner, Savard, Obermeier, Caldwell, & Champagne, 2001)

However, in the fermented sweet potato some amylolytic activity by microorganism by utilization of sugar by fermenting microbes must have been taking place which has led to the reduction of the sugar content on the co-fermented bases.

**4.6 Trypsin inhibitor activity (mg/g) of co-fermented and un-ferment food samples**

The result for the trypsin inhibitor activity shows that there is reduction in the trypsin activity of co-fermented sample A and B and increase in trypsin activity of sample A and B unfermented

(control). The trypsin inhibitor activity in the co-fermented sample ranged from 0.13 – 0.69 and 1.06 – 1.76 Tiu/mg proteins for the unfermented sample. The reduction of the trypsin in so-fermented sample A and B may be due to the fermentation process. These results are also lower to that of Gurumoorthi *et al.*, (2003), who found the trypsin inhibitor activities of both black and white colored accessions of Mucosa beans, South Indian pulses to be between 45.2 and 49.6 units mg-1 protein.

**4.7 Consistency**

From table 8, it can be deduced that fermentation increased the swelling capacity of starch component and hot paste viscosity of the co-fermented food product. The diameter and covalently bonded phosphate monoester groups of sweet potato starch granule could have given a higher paste viscosity (Oyarekua 2013). The result shows that for 9g of flour was desirable sample to prepare infant food. The viscosity is normal, not watery, not too fast to be measure and can be swallow easy in a way that when is been taken by infant it will stay a long time before another food is been given. At 12g the result shows that it will be too thick for the infant to swallow.

Reduction in viscosity of co-fermented gruel could be due to a slightly greater breakdown of the paste during cooking, protein-starch interaction and amylose-lipid formation or due to differences in starch granules, size and composition of the co-fermented raw materials with the protein part contributing to giving lower paste viscosity. This agrees with the finding that supplementation of sample flour reduced the peak viscosity of the product. The traditionally cereals-based fermented foods which are prepared as gruels in Africa especially Nigeria, are frequently fed to infants as complementary foods. These foods are characterized by low energy

and nutrient densities which are well below the recommended values by Dewey and Brown (2003).

High energy density (ED) gruels which depend on the flour concentration and nutrient contents improve infant's energy and nutrient intakes (Golden 2009).

The flour content is negatively influenced by the thickening of gruels due to starch swelling capacity during cooking resulting in low EDs. (Treche *et al.,* 2005). However to obtain a thin-based gruel with high ED, fermentation or partial starch hydrolysis can be assessed (Treche *et al.,* 2005).

Spontaneous lactic acid fermentation has been reported to have a slight viscosity reducing effect by implication, thin gruel with high ED and probably high nutrient density (Oyarekua 2012). Different processing methods like fermentation, germination, use of amylolytic starter cultures, and α-amylase. The processes of germinated seeds followed by fermentation have been employed to increase ED (Oyarekua 2012.). The dry matter content of cereal gruel given to infants in Nigeria is very low resulting into complementary foods of poor nutritional quality in terms of nutrient and energy densities; therefore modification of the substrates can be achieved by incorporation and co-fermentation with legumes, tubers, and vegetables.

**4.8 Vitamins**

The value of vit B3 (Niacin) in all the samples were significantly (P0>0.5) higher than recommended value. Vit B6 (Pyridoxine) and B1 (Thiamine) values in CA were comparable to recommended values from infant complementary food WHO/FAO, while the values in other samples were lower. Sample CA had significantly (P0>0.5) higher vitamin K (Phylloquinone)

value than recommended value of while the value were significantly (P0.>05) lower in the other samples. In comparison with its unfermented analogue (CA); Sample A had lower values B3, B4, B6, B1 E (αTocopherol), K and B5….; while CA had higher values in these vitamins. The reduction in B6 is in agreement with the finding of (Murdock & Fields, 1984). However sample A had a significantly (P0>0.5) higher of Vitamin C (Ascorbic acid) than CA. This means that co-fermentation process reduced most vitamins in maize/carrot/ pigeon mixture when compared with its unfermented analogues.

Sample B had lower value of vitamin B3, A, B4, C, B1 and B5 than CA which is the unfermented analogue. This is contrary to the finding of Hoque *et al.,* (2013) that fermented whole onion plant retained 97% of vitamin A activity, while fermented eggplant only retained 34% of the vitamin A activity However, CB had higher value of vitamin B3, CA (Retinol), B1 and B5. The values of B4, E, B9 and K were comparable in sample B and CB and these values were significantly (P0>0.5) lower than recommended values. From this study fermentation process involving sieving and filtering might have contributed to reduction of vitamins in the co-fermented mixture also drying of the co-fermented mixture at $60^0$c might have contributed to this loss.

Vit C though had higher value in all the samples when compared with other vitamins, the values were still significantly (P0.>05) lower than recommended value. The vitamins B1, B2 (Riboflavin), B5, B9 (Folate ), A and E, C were significantly (P0.>05) lower in all the samples than recommended values. FAO/WHO (2005).

Fermented milk compared to raw milk showed that B- vitamins were affected only slightly in comparison to raw milk. Hoque *et al.,* 2013. Contrary to the finding of Speek et al 1988, vitamin

A was not affected by fermentation in this study. B3 was higher in all samples than recommended value. The slight decrease in B vitamins is in agreement with Hoque *et al.,* (2013).

**4.9 Micro flora in fermented foods**

By tradition, lactic acid bacteria (LAB) are the most commonly used microorganisms for preservation of foods. 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 which makes the finished product to have long-life and natural acceptance as GRAS (Generally Recognized As Safe) for human consumption (Dullon 2004, Wakil and onilude 2011)). In very often, a mixed culture originating from the native microflora of the raw materials is in action in most of the food fermentation processes.

# CHAPTER FIVE

## CONCLUSION AND RECOMMENDATION

The co-fermentation of maize/carrot/ pigeon pea and millet/sweet potato/pigeon pea had significant influence on the acid production, reducing sugar content and trypsin inhibiting activity profile and also led to reduction in viscosity which might give higher energy and nutrient densities. The β- carotenoid level improved in co-fermented mixture. The co-fermentation process in this study is easy and cost-effective and can be applied locally especially by low-socio economic mothers to improve the nutritive quantity and quality of infant complementary food. The co-fermented product compared well with RDAs recommended for infant complementary foods and thus can contribute to reduction of malnutrition in infants.

## RECOMMENDATIONS

Investigation of microbial contamination in the fermented products needs to be carried out. Also, effective programs to promote the use of traditionally fermented processed complementary foods should be put in place in developing countries like Nigeria.

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# APPENDIX

## 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**.....

.....

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## 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. .. .....