EFFECTS OF *GARLIC* AND *GINGER* GRATES ON MICROBIAL PROFILE AND PHYSICO-CHEMICAL PROPERTIES OF FERMENTED CASSAVA PRODUCTS

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CERTIFICATION

This is to certify that this project titled "**Effects of *Garlic* and *Ginger* Grates on Microbial Profile and Physico-Chemical Properties of Fermented Cassava Products"** was carried out by **Emekoma Ezinne Abigail (20114773878)** in partial fulfillment of the requirement for the award of M.Sc. in Food/Industrial Microbiology of the Federal University of Technology, Owerri.

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DEDICATION

I dedicate this work to my father Engr. Clinton Emekoma for his love, care and advice in the course of this work.

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ABSTRACT

The effects of ginger and garlic grate on the microbial succession, sensory, cyanide and physicochemical properties of fermented cassava products prepared in different methods were investigated. The results showed that species of *Bacillus, Lactobacillus, E.coli, Rhizopus, Aspergillus, Geotrichum* and *Trichoderma* were implicated in the fermentation process. Microbial successions monitored every 48hours for 10days showed that among the species already prevalent, *Proteus spp* were isolated on the 6^{th} to 10^{th} day of fermenting unpeeled cassava with ginger and cassava only. The microbial counts increased following days of fermentation with decline from the 8^{th} and 10^{th} day. The results for the sensory evaluation, showed an overall acceptability of the products for aroma, colour and texture. The fermented cassava products were analysed for nutritional, anti-nutritional and mineral properties using standard methods. The mean cassava preparations averaged over rates and treatment types revealed that the percentage Moisture contents of 19.702, 16.987 and 16.723% has unpeeled, peeled and mashed cassava preparations, Ash; 1.022, 0.796 and 1.373%, Fat; 3.305, 2.66 and 3.059%, Protein; 4.341, 4.66 and 4.955%, Carbohydrate; 67.671, 69.219 and 70.845% and Fibre; 3.953, 5.67 and 3.042% respectively. The investigation also revealed that Calcium contents were 0.258, 0.152 and 0.102mg/100g, Magnesium; 0.2099, 0.1639 and 0.1598mg/100g, Sodium; 0.0623, 0.0517 and 0.05mg/100g, Phosphorus; 40.548, 56.994 and 44.678mg/100g and Potassium; 0.125, 0.143 and 0.086mg/100g for unpeeled, mashed and peeled cassava preparations respectively. The contents of cyanogenic glucosides were 18.017, 16.348 and 15.075mg/100g, Flavonoid; 1.78, 2.03 and 1.83mg/100g, Alkaloid; 2.47, 2.83 and 2.58mg/100g and Tannin; 0.108, 0.244 and 0.128mg/100g for unpeeled, peeled and mashed cassava preparations averaged over treatment types and rates respectively. In conclusion, the results obtained in this work, revealed that cassava prepared in different preparation of cassava in different methods with the addition of varying quantities of garlic and ginger grates significantly affected the microbial, sensory and physicochemical properties of the fermented cassava products.

Key words: Cassava, Garlic, Ginger, Microbial Succession, Physicochemical Properties

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cassava is a plant originating from South America and is known under various names: *Manihot esculenta*, manioc, yucca and tapioca (FAO, 1977). The tubers (part of the root system) and the leaves are used as food sources (Lancaster and Brooks, 1983). Cassava is one of the most important staple food crops grown in tropical Africa. It plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food systems in Africa (Hahn and Keyser 1985, Hahn *et al*. 1987).

Cassava is used in many ways: as a binding agent in paper, textiles and cosmetics, in the production of monosodium glutamate (MSG) and alcohol, added to animal feed, transformed into tapioca pellets and cooked, boiled or fried for a meal.

Cassava tubers contain toxic compounds known as cyanogenic glucosides (Narty, 1978, Brauman *et al* 1996). If consumed in sufficient quantities, these compounds can cause severe cyanide poisoning and death in humans and animals (Soto-Blanco *et al* 2002). The amount of these compounds present within the tuber varies according to growing conditions and variety (bitter or sweet cassava). The bitter variety contains higher amounts of toxic compounds than the sweet variety and must be detoxified before consumption. The consumption of large amounts of the bitter variety can cause severe toxicity resulting in Konzo, a paralytic disorder, or even death (Soto- Blanco *et al.,* 2002). Routine ingestion of low levels of cyanide leads to chronic toxicity possibly developing into goiter (enlargement of the thyroid gland) or

tropical ataxic neuropathy (a disorder involving the nervous system) (Soto- Blanco *et al.,* 2008), (Manzo *et al.,* 2007) .

Cassava tubers are processed using varying processing procedures, ranging from simple processing (peel, boil and eat) to complicated procedures for processing into garri, for example, which involve many more steps, namely peeling, grating, pressing, fermenting, sifting, and roasting (Cooke and Maduagwu, 1985). Some of these steps reduce cyanide more effectively than others. Processing techniques and procedures differ with countries and localities within a country according to food cultures, environmental factors such as availability of water and fuel wood, the cassava varieties used, and the types of processing equipment and technologies available.

Cassava tubers are rich in starch and are a major source of energy but compared to cereals has little nutritional value hence, the need to improve the nutritional value of cassava. Cassava is grown in areas where mineral and vitamin deficiencies are widespread, especially in Africa (Burns, 2006). A marginal nutrient status increases the risk of morbidity and mortality. Therefore, improving the nutritional value of cassava could alleviate some aspects of hidden hunger, that is, subclinical nutrient deficiencies without overt clinical signs of malnutrition. The relationship between hidden hunger and food insecurity has been reviewed (Tanumihardjo *et al.,* 2008). The process of adding nutritional value to a crop is called biofortification (Tanumihardjo *et al.,* 2008) and so, cassava has been targeted for biofortification using spices such as ginger and garlic because of its unique geographical distribution and its importance as a staple food.

Ginger or ginger root is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae) (Oyagbemo, *et al.,* 2010). *Allium sativum*, commonly known as garlic, is a species in the onion genus,

Allium. Its close relatives include the onion, shallot, leek, chive and rakkyo (Block, 2010). Ginger and garlic are spices that can be used for culinary, medical purposes. They have a large scale of nutritional value, which has made them unique, effective and useful. Ginger is a medicinal plant; its root portion is mainly used to consume and to cure ailments. Ginger roots are highly beneficial and effectual in dealing with the several diseases including nausea, abdominal cramps, motion ailments, heartburn and disturbed stomach (Terry *et al.,* 2011). Garlic is regarded as wonder drug. The curative properties and advantages of garlic have widely been known to the consumers. It is used as a natural herbal drug to cure many health disorders. Garlic contains numerous potent and effective constituents, including allicin, ajoene, vitamin B, diallysulfide, minerals, saponins, proteins, enzymes and flavonoids (Block, 1992). Garlic contains antiviral, antibacterial and antifungal properties. It helps to prevent atherosclerosis, high blood pressure, high cholesterol and cancer (Gardner *et al.,* 2007). It is used against the skin diseases caused by fungus.

The effects of these spices (ginger and garlic) on cassava fermentation were studied to determine their effects on cassava cyanide content, sensory/acceptability of cassava, physico chemical properties of cassava and the succession of microorganisms involved in fermentation.

1.2 STATEMENT OF PROBLEM

1. Too much consumption of synthetic food products.
2. Low quality of available food products.
3. High cost of other food supplements.
4. Over- dependence on existing food products.
5. Ignorance on the health benefits of these supplements/ additives.

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1.3 AIM OF THIS STUDY

The aim of this project was to determine the effect of Ginger and Garlic grates on Microbial Profile and Physico-Chemical Properties of Fermented Cassava Products.

1.4 OBJECTIVES OF THIS STUDY

This project was carried out to determine the effect of ginger and garlic grates on the:

1. Cyanide level of fermented cassava products by different preparation methods.
2. Sensory properties of fermented cassava products by different preparation methods.
3. Physicochemical properties of fermented cassava products by different preparation methods.
4. Microbial succession of fermented cassava products by different preparation methods.

1.5 SIGNIFICANCE OF STUDY

1. To create awareness of a new formulation in cassava processing.
2. To ensure improved cassava products by its fortification with the additives.
3. To encourage the production and consumption of cassava products thereby increasing the nations income.
4. To make available natural food and dietary nutrients that will be available to every class of people.
5. To ensure food security.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CASSAVA CONSUMPTION IN AFRICA

Cassava production in Africa is used almost exclusively for consumption as food. In fact, 95 percent of the total cassava production, after accounting for waste, was used as food in Africa in the late 1990s (Ezemenari *et al.,* 1998) In Africa, total cassava consumption more than doubled from 24 million tonnes per year in the early 1960s to 58 million tonnes per year in the late 1990s (FAOSTAT). The large increase in the total cassava consumption in Africa is due to a significant increase in per capita consumption in countries such as Ghana and Nigeria where cassava is produced as a cash crop for urban consumption. The availability of cassava in a convenient food form, such as *gari,* played a major role in the increase in the per capita cassava consumption in Ghana and Nigeria. Future increases in cassava consumption in other African countries will depend on how well cassava is prepared into food forms, which make an alternative to wheat, rice, maize and sorghum to urban consumers.

Cassava roots are the single largest source of calories in seven African countries having 40 percent of the population in the late 1990s (Ezumah & Domenico, 1995). In these seven countries, cassava contributed an average of nearly 600 calories per person per day. In another 11 countries with about 25 percent of Africa's population, cassava was the second largest source of calories (Fresco, 1986). In those countries, cassava provided more than 300 calories per person per day in the late 1990s (FAOSTAT). Thus, in Africa, cassava roots are an important source of calories for about 65 percent of the total population.

These averages underestimate the importance of cassava in specific countries. In the Congo, for example, many families eat cassava for breakfast, lunch and dinner. In the Congo, cassava

contributed over 1 000 calories per person per day or about 55 percent of the average daily calorie intake in the late 1990s (FAOSTAT). Cassava leaves are widely consumed as a vegetable in several places where cassava is grown such as in the Congo and Tanzania. Since cassava leaves are rich in protein, vitamins A and C and some minerals (iron and calcium) (Latham, 1979) they partially compensate for, the shortage of these nutrients in the roots.

Cassava was found to be the cheapest source of calories among all food crops in each of the six study countries. As family incomes increased, the consumption of cassava as dried root flour declined while consumption in convenient food forms such as *gari* increased. Dried cassava root flour is cheaper than *gari* because of the high cost of processing *gari.* Medium and high income families were found to consume *gari* because it is cheaper and more convenient to cook than grains. The future of cassava as a rural and urban food staple will depend on cassava's ability to compete with wheat, rice, maize, sorghum and other grains in terms of cost, convenience and availability in urban markets. Cassava can retain its competitive edge only through investments in labour-saving production, harvesting and processing technologies.

2.1.1 Overview of Cassava Consumption in Nigeria

Cassava was originally a crop of South America, it was introduced in to Nigeria's southern part during the period of slave trade proliferated by Portuguese explorers and colonizers in the sixteenth century (Adeniji *et al,* 2005). However, its importance to the country got a boost in the late nineteenth century when more slaves returned to their homeland and introduced processing techniques. Over the years, it has become a major economic sustenance crop and it has attained the status of largest producer in the world with recorded production of 34 million tonnes and is a cash crop of great importance to the people of Nigeria (Adeniji *et al,* 2005).

2.1.2 Cassava as a Nigerian Food

In Nigeria, cassava production is well-developed as an organized agricultural crop. It has well-established multiplication and processing techniques for food products and cattle feed. There are more than 40 cassava varieties in use. Though the crop is produced in 24 of the country's 36 states (IITA, 2009), cassava production dominates the southern part of the country, both in terms of area covered and number of farmers growing the crop. Planting occurs during four planting seasons in the various geo-ecological zones. The major states of Nigeria which produce cassava are Anambra, Benue, Cross River, Imo, Oyo, and Rivers, and to a lesser extent Kwara and Ondo (IITA, 2009).

In 1999, Nigeria produced 33 million tonnes, (IITA, 2009) while a decade later, it produced approximately 45 million tonnes, which is almost 19% of production in the world (Asante-Pok, 2013). As of 2000, the average yield per hectare was 10.6 tonnes (IITA, 2009).

Cassava is grown throughout the year, making it preferable to the seasonal crops of yam, beans or peas. It displays an exceptional ability to adapt to climate change, (CIAT, 2007) with a tolerance to low soil fertility, resistance to drought conditions, pests and diseases, and suitability to store its roots for long periods underground even after they mature. Use of fertilizers is limited, and it is also grown on fallow lands (Adeniji *et al.,* 2005). Harvesting of the roots after planting varies from 6 months to 3 years.

The land holding for farming in Nigeria is between 0.5–2.5 hectares (1.2–6.2 acres), with about 90% of producers being small-scale farms (Adeniji *et al.,* 2005). In order to increase production, several varieties of cassava have been developed which are pest resistant; production in the country is hampered with problems with green mite, the cassava mealybug,

and the variegated grasshopper. Diseases affecting cassava crop are mosaic disease, bacterial blight, anthracnose, and root rot (Adeniji *et al.,* 2005).

2.2 NUTRITIONAL VALUE OF CASSAVA

The composition of cassava depends on the specific tissue (root or leaf) and on several factors, such as geographic location, variety, age of the plant, and environmental conditions. The roots and leaves, which constitute 50% and 6% of the mature cassava plant, respectively, are the nutritionally valuable parts of cassava (Tewe and Lutaladio, 2004). The nutritional value of cassava roots is important because they are the main part of the plant consumed in developing countries. In Table 1, the proximate, mineral, and vitamin compositions of cassava roots and leaves are reported.

Table1: The Proximate, Mineral and Vitamin Compositions of Cassava Roots and Leaves

Proximate composition (100 g)	**Raw Cassava**	**Cassava Roots**	**Cassava Leaves**
Food energy (kcal)	160	110 to 149	91
Food energy (KJ)	667	526 to 611	209 to 251
Moisture (g)	59.68	45.9 to 85.3	64.8 to 88.6
Dry weight (g)	40.32	29.8 to 39.3	19 to 28.3
Protein (g)	1.36	0.3 to 3.5	1.0 to 10.0
Lipid (g)	0.28	0.03 to 0.5	0.2 to 2.9
Carbohydrate, total (g)	38.06	25.3 to 35.7	7 to 18.3
Dietary fiber (g)	1.8	0.1 to 3.7	0.5 to 10.0
Ash[e] (g)	0.62	0.4 to 1.7	0.7 to 4.5
Vitamins			
Thiamin (mg)	0.087	0.03 to 0.28	0.06 to 0.31
Riboflavin (mg)	0.048	0.03 to 0.06	0.21 to 0.74
Niacin (mg)	0.854	0.6 to 1.09	1.3 to 2.8
Ascorbic acid (mg)	20.6	14.9 to 50	60 to 370
Vitamin A (μg)	-	5.0 to 35.0	8300 to 11800

Table 1: Contd

Minerals			
Calcium (mg)	16	19 to 176	34 to 708
Phosphorus, total (mg)	27	6 to 152	27 to 211
Ca/P	0.6	1.6 to 5.48	2.5
Iron (mg)	0.27	0.3 to 14.0	0.4 to 8.3
Potassium (%)	-	0.25 (0.72)	0.35 (1.23)
Magnesium (%)	-	0.03 (0.08)	0.12 (0.42)
Copper (ppm)	-	2.00 (6.00)	3.00 (12.0)
Zinc (ppm)	-	14.00 (41.00)	71.0 (249.0)
Sodium (ppm)	-	76.00 (213.00)	51.0 (177.0)
Manganese (ppm)	-	3.00 (10.00)	72.0 (252.0)

Hidayat *et al* (2002)

2.3 CASSAVA AND CYANIDE

Manihot belongs to the same sub-family as rubber (*Hevea brasiliensis*) and like rubber contains both cyanogenic glucosides and latex (Jorgensen *et al.,* 2005)

A food safety problem with cassava is that cassava roots contain considerable quantities of cyanide which occurs in the form of cyanogenic glucosides, primarily linamarin and a small amount of lotaustralin (Uyoh *et al.,* 2007). Thses cyanogenic glycosides break down to release toxic hydrogen cyanide gas during digestion (Poulton, 1998). The consumption of cassava can therefore be harmful to human health. Despite the presence of these naturally occurring toxins, millions of people all over the world have been safely consuming cassava for hundreds of years. The on-going challenge is to ensure that the presence of these cyanogenic glycosides are minimized through proper understanding and possibly control of factors that affect cyanogenic glycoside content of cassava. Roots and leaves contain the highest amount of linamarin (Cereda and Mattos, 1996).

2.3.1 Factors Affecting Cyanide Content of Cassava

Cultivar

Thousands of cassava cultivare have been developed that are adapted to local conditions and differ in their ability to tolerate pest and diseases, yield, nutritional and cooking quantities of food products. Cassava is propagated clonally from stem cuttings so there is minimal variation between individuals of one cultivar when grown under the same environmental conditions. All cassava cultivars contain cyanogenic glucosides however a wide variation in the concentration of cyanogens exists among different cultivars. This can range from 1 to 2,000mg/kg (Cardoso *et al*., 2005, CIAT 2007). Cultivars with ≤ 100mg/kg hydrogen cyanide are called sweet while those with > 100mg/kg are called bitter (Wheatley *et al*., 1993).

Climatic Conditions

Cassava, a perennial shrub thrives in tropical and subtropical conditions. In general, the crop requires a warm humid climate. Temperature is important, as all growth stops at about 10^{o}c typically, the crop is grown in areas that are frost free the year round. The highest root production can be expected in the tropical lowlands, below 150m altitude, where temperatures average 25-27^{o}c, but some varieties grow at altitudes of up to 1500m.

The plant produces best when rainfall is fairly abundant, but it can be grown where annual rainfall is as low as 500mm or where it is as high as 5,000mm. The plant can stand prolonged periods of drought in which most other food crops would perish. This makes it valuable in regions where annual rainfall is low or where seasonal distribution is irregular. In tropical climates the dry season has about the same effect on cassava as low temperature has on deciduous perennials in other parts of the world. The period of dormancy lasts two to three months and growth resumes when the rains begin again. Cassava is drought resistant and grows well in poor soil (Java Cassava, 2007).

The probem however is that cyanide content of cassava tends to increase during periods of drought and or prolonged dry weather due to water stress on the plant (Bokanga *et al*., 1994). For example, in Mozambique, about 55% of the sweet fresh roots were extremely toxic and the remainder moderately so during drought like conditions. Similar observations were recorded in The Democratic Republic of Congo (Gitebo *et al*., 2009), and various citations in Africa (Cardoso *et al*., 2005)

Fertilizer

There is a general consensus that crop yields do increase with application of fertilizer, there is debate however on the relationship between addition of fertilizer and cyanide content of cassava.

Studies in the Philippines (Rolinda *et al*., 2008) concluded that application of fertilizer does not significantly affect cyanide content. It further suggested that the amount of nutrient in the soil does not considerably contribute to the cyanogenic character of the cultivar. In Ethiopia, Endris (1977) suggested that the cyanogenic content of cassava roots were significantly reduced by potassium application.

Harvesting

Harvesting of cassava can be done throughout the year when the roots reach matrurity. Maturity differs from one variety to another, but for food, the tubers can be harvested at almost any age below 12 months (FAO, 1977) and can remain the soil for up to three years after maturity (Lebot, 2009).

Harvesting is still generally a manual operation. Before harvest, the plants are 'topped' (stalks are cut off 40-60cm above ground) leaving an adequate length of stalks as a handle for pulling. In the light soils the roots are slowly drawn from the soil simply by pulling the stems or with the help of a kind of crowbar and the tubers are cut off the stock. In heavier soils, digging up the roots before the plant is pulled out may be required (Java Cassava, 2007). Once the plants have been topped, uprooting must not be delayed, as sprouting causes a drstic fall in the starch content of the tubers. While the effect of harvesting method on cyanide is not clear, injuring the roots increases the rate of post harvest deterioration.

Age of Cassava at Harvesting

A study by Hidayat *et al.,* (2002) on ninety variety of cassava showed that there is a significant correlation between cyanide potential of roots and leaves. The cyanide content was higher in younger leaves compared to older ones, suggesting that cyanide potential of roots drops as plant ages. This seems to agree with investigations by Chotineeranati *et al.,* (2006). Cooke and Elba, (1982) reported that the root parenchymal tissue and root cortex

were not significantly different between 6 and 14 months; both tissues displayed peak concentrations at 6 and 14 months.

Post Harvest Practises

Post harvest deterioration is the most important cause of loss in cassava production and this is mainly as a result of microbial invasion of the tuber (Okigbo *et al.,* 2009). Post harvest deterioration can render cassava unpalatable and un-marketable within 24-72hrs (Rielly *et al.,* 2004). Cassava must also be processed before being eaten.

The Amerindians, who first cultivated cassava, over the years, have devised numerous processing techniques not only to increase palatability and extend shelf life, but also to decrease its cyanogenic potential. Today, a great diversity of processing methods is found in the various parts of the world where cassava is consumed (Lancaster *et al*., 1982). These methods consist of different combinations of peeling, chopping, grating, soaking, drying frying, boiling and fermenting. In Africa where cassava flour is a major product, wetting (Bradbury 2006; Cumbana *et al.,* 2007) is an effective method of cyanide removal.

Methods which use grating and crushing are very effective in removing cyanide because of the intimate contact in the finely- divided wet parenchyma between linamarin and the hydrolyzing enzyme linamarase, which promotes rapid breakdown of linamarin to hydrogen cyanide gas that escapes into the air (Cardoso *et al*., 2005). This in combination with wetting, fermentation and drying can reduce cyanide contents up to 99%.

Cassava is generally harvested fresh and boiled in preparations where roots are eaten. Storage at ambient temperature may take up to three days. Storage of cassava roots at ambient temperatures for up to four days can result in cyanide loss of up to 7% (FAO, 1977).

2.3.2 Pathophysiology of Cyanide Intoxication

Cyanide is detoxicated in the body by conversion to thiocyanate, a sulphurcontaining compound with goitrogenic properties. The conversion is catalysed by an enzyme thiosulphate cyanide sulphur transferase (rhodanase) present in most tissues in humans, and to a lesser extent by mercaptopyruvate cyanide sulphur transferase which is present in red blood cells (Fielder and Wood, 1956). The essential substrates for conversion of cyanide to thiocyanate are thiosulphate and 3-mercaptopyruvate, derived mainly from cysteine, cystine and methionine, the sulphur-containing aminoacids. Vitamin B12 in the form of hydroxycobalamin probably influences the conversion of cyanide to thiocyanate. Hydroxycobalamin has been reported to increase the urinary excretion of thiocyanate in experimental animals given small doses of cyanide (Wokes and Picard, 1955; Smith and Duckett, 1965). About 60 to 100 percent of the injected cyanide in toxic concentration is converted to thiocyanate within 20 hours and enzymatic conversion accounts for more than 80 percent of cyanide detoxification (Wood and Cooley, 1956). Thiocyanate is widely distributed throughout body fluids including saliva, in which it can readily be detected. In normal health, a dynamic equilibrium between cyanide and thiocyanate is maintained. A low protein diet, particularly one which is deficient in sulphurcontaining amino-acids may decrease the detoxification capacity and thus make a person more vulnerable to the toxic effect of cyanide (Oke, 1973). Excessive consumption of cassava, as the sole source of dietary energy and main source of protein, could thus increase vulnerability to cyanide toxicity.

2.3.3 Diseases Related to Cassava Toxicity

Several diseases have been associated with the toxic effects of cassava. Its causative role has been confirmed in the pathological condition of acute cyanide intoxication and in goitre.

There is also some evidence linking two types of paralysis to the combined effects of a high cyanide and low sulphur intake, such as could result from a diet dominated by inefficiently processed cassava. In these two diseases, tropical atoxic neuropathy and epidemic spastic paraparesis, paralysis follows damage to the spinal cord (Sousa *et al* 2002). The role of cyanide toxicity in the causation of tropical diabetes, and in congenital malformation has not been established. Similarly it's supposed beneficial effects on sickle cell anaemia, shistosomiasis and malignancies are still hypothetical.

Acute cyanide intoxication: Symptoms appear four hours after the consumption of raw or insufficiently processed cassava and consist of vertigo, vomiting, collapse and in some cases death within one or two hours. Treatment is quite effective and cheap. The principle is to increase the detoxicating capacity of the patient by giving an intravenous injection of thiosulphate and thereby making more sulphur available for conversion of cyanide to thiocyanate (Soto-Blanco *et al.,* 2008).

Endemic goitre: Cyanide taken in the diet is detoxified in the body, resulting in the production of thiocyanate. Thiocyanate has the same molecular size as iodine and interferes with iodine uptake by the thyroid gland (Soto-Blanco *et al*., 2008). Under conditions of high ingestion of inefficiently processed cassava, there may be a chronic cyanide overload leading to a high level of serum thiocyanate of 1 to 3 mg/100 ml, compared to a normal level of about 0.2 mg/100 ml. Under such conditions there is an increased excretion of iodine and a reduced iodine uptake by the thyroid gland, resulting in a low thiocyanate/iodine (SCN/I) excretion ratio. The value of the threshold level for this ratio seems to be three (Sousa *et al*., 2002) after which endemic goitre appears. This phenomenon can occur only when the iodine intake is below about 100 mg per day. At SCN/I ratios of lower than two there is a risk of endemic cretinism, a condition characterized by severe mental retardation and severe neurologic abnormalities (Soto-Blanco *et al*., 2008).

Studies in Zaire have shown that the population of Ubangi, who consume a high amount of sun dried but unfermented cassava products, have a low SCN/I ratio of 2 to 4 and suffer from endemic goitre and cretinism. Whereas in Kim, where fermented and dried cassava paste is eaten, the SCN/I ratio goes up to three to five and there is a low incidence of goitre. In Bas Zaire, where properly processed cassava products are eaten, the SCN/I ratio is higher than seven and there is no goitre. A low ratio leads to abnormal levels of the thyroid stimulating hormone (TSH) and low thyroxine (T4). Ayangade *et al*., (1982) found that in pregnant women the thiocyanate level of the cord blood was proportional to the maternal serum thiocyanate level, indicating that thiocyanate can cross the placental barrier and affect the foetus. However, there is very little thiocyanate in breast milk indicating that the mammary gland does not concentrate thiocyanate and so breast-fed infants are not affected.

When iodine supplements are given, for example, by adding potassium iodide to local supplies of salt, goitre is reduced in spite of a continued high intake of cassava products. Where salt intake is small or variable, iodized oil, given by mouth, provides protection for one to two years. In the Amazon jungle some tribal people eat as much as one kg of cooked fresh cassava per person per day and consume up to three litres of fermented cassava beer, but there have been no reported cases of either goitre or ataxic neuropathy. These tribes also consume a considerable amount of animal and fish protein and thus have high levels of sulphur-amino acids and iodine in their diet.

Neurological disorders: Cyanide intake from a cassava-dominated diet has been proposed as a contributing factor in two forms of nutritional neuropathies, tropical ataxic neuropathy in Nigeria (Osuntokun, 1981) and epidemic spastic paraparesis (Cliff *et al.,* 1984). These disorders are also found in some cassava growing areas of Tanzania and Zaire.

Tropical ataxic neuropathy: This disease is common in a particular area in Nigeria where a lot of cassava is consumed without the addition of sufficient protein-rich supplementary foods to provide an adequate supply of sulphur amino-acids for the detoxification of ingested cyanide. The consumed cassava product, called purupuru, is processed by an insufficient fermentation of the cassava, which leaves a residual cyanide content of up to 0.10 M mole/g. As much as two kg of this foodstuff is consumed daily, leading to the ingestion of about 50 mg of cyanide. The toxic level for an adult is about 60 mg. The clinical picture is dominated by damage to one of the sensory tracts in the spinal cord resulting in an uncoordinated gait called ataxia.

When patients are brought to the hospital they have a high plasma thiocyanate level. On admission they are put on a hospital diet which is highly nutritious and includes cassava only twice a week. Within a short period the plasma thiocyanate level returns to normal, and the patients recover. However, on discharge, they go back to their original diet of cassava and so the condition reappears (Osuntokun, 1968).

All the cases reported came from the area where cassava is cultivated and eaten in large quantities, with no cases in the nearby areas where yam predominates. A change in the diet of the population at risk in Nigeria has reduced the incidence of this disease.

Epidemic spastic paraparesis: This is a situation of depending on very toxic varieties of cassava as a food security crop (Cliff *et al.*, 1984). In parts of Mozambique a bitter toxic type of cassava is often planted as a food reserve because of its high yield. As cassava constitutes about 80 percent of the basic diet, there is nominally a standard method of preparation which makes the cassava safe for consumption. Cassava, containing about 327 mg HCN/ kg, is peeled, sliced and sun dried for about three weeks after which the cyanide level is reduced to about 95 mg/kg. It is then pounded to flour which is mixed with hot water to make a paste

called chima. This paste is normally eaten with a relish of beans, fish or vegetables, to provide a well balanced meal.

During a prolonged period of drought all the food crops in this area were lost except the toxic variety of cassava. The food stores were depleted and many families had no alternative, but to resort to the toxic cassava. Normal processing time was reduced because of the emergency and so there was no proper detoxification. The people knew this but they had no other choice of action except to die of starvation. On eating the underprocessed chima without their usual protein-rich supplement they complained that it was more bitter than normal. After about four to six hours they suffered from nausea, vertigo and confusion. Sufferers showed a high serum thiocyanate level and a urinary thiocyanate excretion of about ten times that of non-cassava-eating groups in Mozambique. There followed a sudden appearance of many cases of spastic paraparesis, indicating an extensive epidemic. This disease affects mainly women and children. It damages the nerve tract in the spinal cord that transmits signals for movement, thus causing a spastic paralysis of both legs (Rolling, 1983). Outbreaks have been reported during the dry season from two areas in Zaire (Nkamany and Kayinge, 1982) and during droughts in one area in Mozambique (Cliff *et al*., 1984) and one area in Tanzania (Howlett, 1985).

During these drought periods about 500 g of dried cassava, or 1.5 kg on a fresh weight basis, is consumed daily, representing an intake of 1 500 kcal and 50 mg cyanide per day. This level approaches the toxic level of 60 mg. The body can safely detoxify about 20 mg cyanide per day but when this level increases to 30 mg symptoms of acute intoxication develop in many consumers and hence the epidemics. If there is a period during which a high cassava intake and a low protein-rich food intake, to supply sulphur amino-acids for detoxification, coincide, this combination precipitates the outbreak of this disease. The situation may be compared to

the epidemics of lathyrism that occured in drought-affected areas of India owing to the high-level intake of the drought-resistant pea, Lathyrus saliva.

2.4 PROCESSING TECHNIQUES AND REDUCTION OF CYANIDE IN CASSAVA

Cassava contains the cyanogenic glucosides, linamarin and lotaustralin which are hydrolyzed after tissue damage, by the endogenous enzyme, linamarase to the corresponding cyanohydrins and further to hydrogen cyanide [HCN](Conn 1969). The hydrogen cyanide is responsible for chronic toxicity when inadequately processed cassava products are consumed by humans and animals for prolonged periods. Therefore, traditional processing procedures must aim at reducing cyanide and improving storability, convenience and palatability.

2.4.1 Cassava Fermentation

Fermentation consists of two distinct methods: aerobic and anaerobic fermentation. For aerobic fermentation, the peeled and sliced cassava roots are first surface-dried for 1-2 hours and then heaped together, covered with straw or leaves and left to ferment in air for 3-4 days until the pieces become moldy. The fermented moldy pieces are sun-dried after the mold has been scraped off. The processed and dried pieces (called "Mokopa" in Uganda) are then milled into flour, which is prepared into a "fufu" called "kowan" in Uganda. The growth of mold on the root pieces, increases the protein content of the final products three to eight times (Amey 1987, Sauti *et al.*, 1987). This fermentation method is also very popular in other parts of East Africa such as Tanzania, Rwanda, and Zaire.

In anaerobic fermentation, grated cassava for processing into "gari" is placed in sacks and pressed with stones or a jack between wooden platforms. Whole roots or pieces of peeled roots for processing into "fufu" are placed in water for 3-5 days. During the first stage of gari production, the bacterium *Corynebacteria manihot* attacks the starch of the roots, leading to

the production of various organic acids (such as lactic and formic acids) and the lowering of substrate pH. In the second stage, the acidic condition stimulates the growth of a mold, *Geotrichum candidium*, which proliferates rapidly, causing further acidification and production of a series of aldehydes and esters that are responsible for the taste and aroma of gari (Odunfa, 1985). The optimum temperature for the fermentation for gari processing is 35°C, increasing up to 45°C.

For "lafun" production in Nigeria, peeled or unpeeled cassava tubers are immersed in a stream, in stationary water (near a stream) or in an earthenware vessel, and fermented until the roots become soft. The peel and central fibres of the fermented roots are manually removed and the recovered pulp is hand mashed or pounded. The microorganisms involved in "lafun" production include four yeasts: *Pichia onychis, Candida tropicalis, Geotrichum candida,* and *Rhodotorula* sp.; two molds: *Aspergillus niger* and *Penicillium* sp.; and two bacteria: *Leuconostoc* sp. and *Corynebacterium* sp. (Nwachukwu and Edwards, 1987). Moisture, pH and temperature conditions are critical for the growth of these microorganisms in roots and thus for fermentation.

Dewatering the Fermented Cassava

During or after fermentation of roots for gari production, the grated pulp is put in sacks (jute or polypropylene) on which stones are placed or jacked-wood platforms are set to drain or press off the excess liquid from the pulp. In Zaire, the cassava pulp is taken out and heaped up on the racks in the sun for further fermentation and draining of the excess moisture. In this way, much of the cyanide is effectively lost with the liquid.

Tissue Disintegration

Tissue disintegration in the presence of excess moisture during grating or fermenting in water permits the rapid hydrolysis of glucosides, effectively reducing both free and residual cyanide in the products. Fermentation in water appears a more efficient method for reducing the cyanide of roots. For example, this process reduced cyanide by 70-95 percent of the original level after the roots were soaked in water for 3 days (Hahn *et al*., 1987). Gari obtained through the processing procedures involving grating and/or fermentation showed 80-90 percent reduction in total cyanide content relative to freshly peeled roots (Mahungu *et al*., 1987). Oke (1968) reported HCN content of 1.9 mg/100g for gari, 2.5 mg/100g for fufu (Nigeria) and 1.0 mg/100g for fufu (Zaire) or lafun (Nigeria). HCN concentration in 202 gari samples collected across the cassava growing areas of Nigeria had 0-3.2 mg/100g with a mean of 0.6 mg/100g. Akinrele *et al*., (1962) stated that 0.3 mg HCN/ 100g was an acceptable level in gari. Therefore, adequately processed gari in Nigeria would contain acceptable levels of HCN. When gari is prepared into "eba", HCN is further reduced to even safer levels. By processing roots into "chickwangue" cyanide reduction of at least 90 percent was achieved (Mahungu *et al*., 1987).

Drying

Drying is the simplest method of processing cassava. Drying reduces moisture, volume and cyanide content of roots, thereby prolonging product shelf life. This processing is practiced primarily in areas with fewer water supplies. Total cyanide content of cassava chips could be decreased by only 10-30 percent through fast air drying. Slow sun-drying, however, produces greater loss of cyanide. Sun-dying the peeled cut pieces of roots gave a HCN concentration lower than 10 mg/100g and loss was more effective than oven drying (Mahungu *et al*., 1987).

Drying may be in the sun or over a fire. The former is more common because it is simple and does not require fuelwood.

Boiling

Boiling the peeled roots did not effectively remove HCN. Pounding the boiled roots into "pounded fufu" decreased the HCN concentration by only 10 percent. Therefore, only cultivars containing low cyanide are recommended for this method of preparation (Mahungu *et al*., 1987).

Milling

The dried root pieces and fermented/dried pulp are milled into flour by pounding in mortar or using hammer mills. Milling with hammer mills, done at village level, may also reduce cyanide. The dried cassava roots (both fermented and unfermented) are often mixed in a ratio of 2-3 parts cassava with one part of sorghum, millet and/or maize and milled into composite flour. Mixing cassava with cereals increases food protein, and enhances palatability by improving consistency.

2.5 GARLIC

2.5.1 Properties

Alliin, a sulfur-containing compound found in garlic. When crushed, Allium sativum yields allicin, an antibiotic (Shuford *et al.,* 2005) and antifungal compound (phytoncide) discovered by Chester J. Cavallito and colleagues in 1944. Fresh or crushed garlic also affords the sulfur-containing compounds alliin, ajoene, diallyl polysulfides, vinyldithiins, S-allylcysteine, and enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds. Furthermore, a phytoalexin (allixin)

was found, a nonsulfur compound with a γ-pyrone skeleton structure with antioxidant effects, antimicrobial effects, (Lawson and Wang 2001) antitumor promoting effects, (Jones and Goebel 2001) inhibition of aflatoxin B2 DNA binding,(Sovova and Sova 2004) and neurotrophic effects. Allixin showed an antitumor promoting effect in vivo, inhibiting skin tumor formation by TPA and DMBA initiated mice (Kodera *et al.,* 2002). Analogs of this compound have exhibited antitumor promoting effects in in- vitro experimental conditions. So allixin and/or its analogs may be useful compounds for cancer prevention.

The composition of the bulbs is approximately 84.09% water, 13.38% organic matter, and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic matter (Block, 1992).

The phytochemicals responsible for the sharp flavor of garlic are produced when the plant's cells are damaged. When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulfur-containing compounds stored in the cell fluids (cytosol). The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Some of the compounds are unstable and continue to react over time. Among the members of the onion family, garlic has by far the highest concentrations of initial reaction products, making garlic much more potent than onion, shallot, or leeks (Mc Gee, 2004). Although many humans enjoy the taste of garlic, these compounds are believed to have evolved as a defensive mechanism, deterring animals such as birds, insects, and worms from eating the plant (Kamenestsky *et al.,* 2004).

A large number of sulfur compounds contribute to the smell and taste of garlic. Allicin has been found to be the compound most responsible for the "hot" sensation of raw garlic. This chemical opens thermotransient receptor potential channels that are responsible for the burning sense of heat in foods. The process of cooking garlic removes allicin, thus mellowing

its spiciness (Kodera *et al.,* 2002). Allicin, along with its decomposition products diallyl disulfide and diallyl trisulfide, are major contributors to the characteristic odor of garlic, while other allicin-derived compounds, such as vinyldithiins and ajoene show beneficial in vitro biological activity (Block, 2010), Because of its strong odor, garlic is sometimes called the "stinking rose". When eaten in quantity, garlic may be strongly evident in the diner's sweat and garlic breath the following day. This is because garlic's strong-smelling sulfur compounds are metabolized, forming allyl methyl sulfide. Allyl methyl sulfide (AMS) cannot be digested and is passed into the blood. It is carried to the lungs and the skin, where it is excreted. Since digestion takes several hours and release of AMS several hours more, the effect of eating garlic may be present for a long time (Block, 2010).

Table 2: Nutritional value per 100 g (3.5 oz) of raw Garlic

Energy	333 kJ (80 kcal)
Carbohydrates	17.77 g
Sugars	1.7 g
Dietary fiber	2 g
Fat	0.75 g
Protein	1.82 g
Thiamine (vit. B1)	0.025 mg (2%)
Riboflavin (vit. B2)	0.034 mg (3%)
Niacin (vit. B3)	0.75 mg (5%)
Pantothenic acid (B5)	0.203 mg (4%)
Vitamin B6	0.16 mg (12%)
Folate (vit. B9)	11 µg (3%)
Vitamin C	5 mg (6%)
Vitamin E	0.26 mg (2%)
Calcium	16 mg (2%)
Iron	0.6 mg (5%)
Magnesium	43 mg (12%)
Manganese	0.229 mg (11%)
Phosphorus	34 mg (5%)
Potassium	415 mg (9%)
Selenium	4.2µg
Sodium	13 mg (1%)
Zinc	0.34 mg (4%)

Percentages are roughly approximated using US recommendations for adults.

Source: USDA Nutrient Database

2.5.2 Health Benefits of Garlic

Antibacterial and Antiviral: Garlic is most well-known for its antibacterial and antiviral properties. They help control bacterial, viral, fungal, yeast and worm infections. Fresh garlic is thought to play a role in preventing food poisoning by killing bacteria like E. coli, Salmonella enteritidis, etc (Groppo *et al.,* 2007).

To treat skin infections: The chemical ajoene found in garlic may help treat fungal skin infections like ringworm and athlete's foot (Shuford *et al.,* 2005).

Blood thinning: The anti-clotting properties of ajoene found in garlic help in preventing the formation of blood clots in the body (Rahman, K. 2007). Hence, it may also increase the risk of bleeding after surgery.

Reduce blood pressure: Angiotensin II is a protein that helps our blood vessels contract thereby increasing the blood pressure. Allicin in garlic blocks the activity of angiotensin II and helps in reducing blood pressure (Reid *et al.,* 2010). The polysulphides present in garlic are converted into a gas called hydrogen sulphide by the red blood cells. Hydrogen sulphide dilates our blood vessels and helps control blood pressure.

Protect heart: Garlic protects our heart against cardiovascular problems like heart attacks and atherosclerosis. This cardio-protective property can be attributed to various factors. With age, the arteries tend to lose their ability to stretch. Garlic may help reduce this and may also protect the heart from the damaging effects of free oxygen radicals (Breithaupt- Grogler *et al* 1997). The sulphur-containing compounds of garlic also prevent our blood vessels from becoming blocked and slow the development of atherosclerosis (hardening of the arteries). The anti-clotting properties of ajoene help prevent clots from forming inside the blood vessels.

Reduce cholesterol: Garlic has the ability to moderately lower our blood triglycerides and total cholesterol and reduce arterial plaque formation (Durak *et al.,* 2004).

Combat allergies: Garlic is known to have anti-inflammatory property. It can help the body fight against allergies. The anti-arthritic property of garlic is due to diallyl sulphide and thiacremonone. Garlic has been show to improve allergic airway inflammation (allergic rhinitis). Raw garlic juice may be used to immediately stop the itching due to rashes and bug bites.

Remedy for respiratory problems: Daily use of garlic might reduce the frequency and number of colds. Its antibacterial properties help in treating throat irritations. Garlic may also reduce the severity of upper respiratory tract infections (Alnaqeeb *et al.,* 1996). Its benefits in disorders of the lungs like asthma, difficulty of breathing, etc. make it a priceless medicine. Its ability to promote expectoration makes it irreplaceable in chronic bronchitis.

Diabetes: Garlic increases insulin release and regulates blood sugar levels in diabetics.

Effective against warts and corns: Applying fat dissolving garlic extracts to corns on the feet and warts on the hands is thought to improve these conditions (Gardner *et al.,* 2007).

Cancer prevention: Daily intake of garlic has been found to lower risk of most types of cancer. This anti-cancer property is due to allyl sulphides found in garlic. PhIP, a type of heterocyclic amine (HCA), has been associated with increased incidence of breast cancer among women (Fleischauer and Arab, 2001). According to studies, diallyl sulphide found in garlic inhibits the transformation of PhIP into carcinogens.

Improve iron metabolism: Ferroportin is a protein which helps in iron absorption and release. Diallyl sulphides in garlic increase production of ferroportin and help improve iron metabolism (Yeh, Y 1999).

Stir up passions: Garlic's aphrodisiac property is due to its ability to increase the circulation.

Toothaches: Simply put some crushed garlic clove directly on the affected tooth can help relieve toothaches due to its antibacterial and analgesic properties. But be aware that it can be irritating to the gum.

Reduce weight: Many researchers believe that obesity is a state of long-term low-grade inflammation. According to recent research, garlic may help to regulate the formation of fat cells in our body. Pre-adipocytes are converted into fat cells (adipocytes) through inflammatory system activity. The anti-inflammatory property of 1, 2-DT (1, 2-vinyldithiin) found in garlic may help inhibit this conversion. This may help prevent weight gain.

2.6. GINGER

2.6.1 Properties

The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols, volatile oils that compose one to three percent of the weight of fresh ginger. In laboratory animals, the gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties (O'Hara *et al.,* 1998). A study at the University of Michigan demonstrated that gingerols can inhibit growth of ovarian cancer cells in vitro (Rhode *et al.,* 2007). [6]-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) is the major pungent principle of ginger (Oyagbemi *et al.,* 2010).

Ginger contains up to three percent of a fragrant essential oil whose main constituents are sesquiterpenoids, with (-)-zingiberene as the main component. Smaller amounts of other sesquiterpenoids (β-sesquiphellandrene, bisabolene and farnesene) and a small

monoterpenoid fraction (β-phelladrene, cineol, and citral) have also been identified (Oyagbemi *et al.,* 2010).

The pungent taste of ginger is due to nonvolatile phenylpropanoid-derived compounds, particularly gingerols and shogaols, which form from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process; this compound is less pungent and has a spicy-sweet aroma (Langner *et al.,* 1998). Ginger is also a minor chemical irritant, and because of this was used as a horse suppository by pre-World War I mounted regiments for feaguing.

Ginger has a sialagogue action, stimulating the production of saliva, which makes swallowing easier (Mc Gee, H. 2004).

Table 3: Nutritional value per 100 g (3.5 oz) of Ginger root (raw)

Energy	333 kJ (80 kcal)
Carbohydrates	17.77 g
- Sugars	1.7 g
- Dietary fiber	2 g
Fat	0.75 g
Protein	1.82 g
Thiamine (vit. B_1)	0.025 mg (2%)
Riboflavin (vit. B_2)	0.034 mg (3%)
Niacin (vit. B_3)	0.75 mg (5%)
Pantothenic acid (B_5)	0.203 mg (4%)
Vitamin B_6	0.16 mg (12%)
Folate (vit. B_9)	11 μg (3%)
Vitamin C	5 mg (6%)
Vitamin E	0.26 mg (2%)
Calcium	16 mg (2%)
Iron	0.6 mg (5%)
Magnesium	43 mg (12%)
Manganese	0.229 mg (11%)
Phosphorus	34 mg (5%)
Potassium	415 mg (9%)
Sodium	13 mg (1%)
Zinc	0.34 mg (4%)

Percentages are roughly approximated using US recommendations for adults.

Source: USDA Nutrient Database

2.6.2 Health Benefits of Ginger

Digestion: Digestion has a key role in keeping you healthy. According to Organic Facts, ginger can help digestion by monitoring high sugar levels. If these sugar levels are too high, the stomach may not empty, as it should. Ginger soothes the stomach and helps return the emptying to normal state.

Reduces Blood Clotting: Motley Health states that ginger was shown to reduce production of thromboxane, a powerful blood clotting stimulant, by 60 per cent.

Diarrhoea: Diarrhoea is a difficult problem to have, but according to studies, ginger can help defeat the symptoms of diarrhoea. The ginger helps with any stomach spasms and gas that may be in effect (Cheng *et al.,* 2007).

Sexual Properties: Ginger increases blood circulation, which can directly affect male stimulation. It has also been cited as an aphrodisiac, and ginger has been scientifically proven to increase sexual desire.

Respiratory Ailments: Ginger relieves congestion, soothes aching muscles (Black and O'Connor, 2010) and can comfort a sore throat.

May Relieve Arthritis Symptoms: Ginger consumption can control or diminish swelling. Ginger can play a role in reducing inflammation and has been used for this purpose by traditional medicine (Wigler *et al.,* 2003). Ginger contains natural components that are similar to those found in FDA-approved over-the-counter anti-inflammatory compounds, according to the American Academy of Rheumatology. Ginger may help relieve your arthritis pain, also. Make certain you do not drink too much ginger tea, however. Excessive ginger intake may lead to inflammation of your intestines and/or stomach. Some studies have confirmed that ginger can produce pain relief, according to the UMMC, but one trial found

that it was no more effective than ibuprofen or a placebo. It works as well to reduce joint swellings in people who suffer from rheumatoid arthritis. A recent study found that ginger eased the symptoms in 55 per cent of people with osteoarthritis and 74 per cent of those with rheumatoid arthritis (Altman and Maraissen, 2001).

Relieves Flu and Cold Symptoms: Ginger consumption can help relieve cold symptoms. Ginger is considered the best remedy for colds in Chinese traditional and ayurvedic medicine, according to Holisticonline.com. Ginger contains antiviral properties that may help fight cold symptoms such as stomach upset and/or nausea, dizziness and overall pain (Ethelbert *et al.,* 2003).

Relieves Motion Sickness Symptoms: Ginger may help relieve some symptoms of motion sickness, most notably nausea (Wood and Pittler, 2000). Motion sickness involves symptoms such as cold sweats, excessive saliva production, headache, nausea and/or an upset stomach, vomiting, vertigo and breathing difficulties (Stewart *et al.,* 1991). Consuming ginger products, including ginger tea and/or ginger ale, can help. Ginger can be a safe alternative to prescription-based motion sickness medication.

Diminish Morning Sickness Symptoms: Ginger may help diminish morning sickness, a symptom experienced during pregnancy. Morning sickness occurs any time of the day. Common symptoms, such as vomiting and/or nausea, can be relieved with ginger, states the American College of Nurse-Midwives. Get your ginger in tea form. Symptoms of morning sickness can increase, due to hormonal fluctuations, fatigue, stress or foods. Ginger and ginger products, such as tea, are effective for treating morning sickness (Wilkinson, 2000).

Acts as a Natural Blood Thinner: Ginger can be used to nourish and support cardiovascular health. Ginger provides a natural blood thinner, since it makes blood platelets less sticky. It

prevents excessive blood clotting. In turn, this helps reduce your blood cholesterol and circulatory problems. Ginger increases blood circulation, according to Holisticonline.com.

Headaches: Its effectiveness against headaches has been documented. Taken at the first sign of migraine, ginger can reduce the symptoms and severity of headaches by blocking prostaglandins, the chemicals that cause inflammation in blood vessels in the brain. This anti-inflammatory activity in ginger can shorten the discomfort of headaches, colds and flu (Haghighi *et al.,* 2005). Ginger blocks the production of substances that cause bronchial congestion and stuffiness. Its main compounds, gingerols, are natural cough suppressants (Oyagbemi *et al.,* 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLE LOCATION AND COLLECTION

The samples for this work were obtained from Imo State in the first week of December, 2013. The cassava tubers were harvested from a farmland at Irete in Owerri West Local Government Area of Imo State, Nigeria. The ginger and garlic bulbs were obtained from Relief Market in Owerri Municipal Imo State, Nigeria. All the samples were labelled and transported to the laboratory for analysis.

3.2 PREPARATION OF SAMPLE, INOCULATION AND INCUBATION

The samples were prepared accordingly; the garlic bulbs and ginger roots were peeled, washed, and grated/mashed. The additives (garlic only, ginger only and garlic plus ginger) were weighed into 0.1kg, 0.2kg and 0.3kg. The cassava samples were prepared and processed accordingly; by the different methods that is mashed cassava, peeled and unpeeled cassava. Using a weighing scale, one kilogram (1kg) of the processed cassava (mashed, peeled and unpeeled) was steeped in warm water (50^{o}C) containing varying quantities of the garlic, ginger and garlic plus ginger mash/grates. Control experiments were also setup containing the processed cassava (mashed, peeled and unpeeled) and steeped in warm water (50^{o}C) without any additive (garlic, ginger and garlic plus ginger). The setups were left to ferment and these were monitored every 48hour for microbial succession.

Ten grams (10g) of each of the food samples were weighed using a chemical beam balance (Model No 710-T0: OHAUS) and dispensed into 90ml of sterile peptone water to obtain 10^{-1} dilution. Further dilutions were made serially until 10^{-8} was obtained.

One tenth milliliter (0.1) of the dilutions was spread inoculated in duplicate onto Nutrient Agar, MacConkey Agar, de-Man Rogosa Sharpe Agar (MRS Agar) and Potato Dextrose Agar (PDA). The inocula were spread with sterile spreader to ensure even distribution before incubating the plates.

Nutrient agar and MacConkey agar were incubated at 37^{o}C for 24-48 hours for the growth of heterotrophic bacteria and coliforms, respectively. de-Man Rogosa Sharpe Agar (MRS Agar) were incubated 35^{o}C for 24 – 72 hours in an enriched CO_2 environment for the isolation of *Lactobaccillus spp.* Potato dextrose agar plates (PDA) were incubated at room temperature (28 ± 2^{0}C for 3-5 days) for isolation of heterotrophic fungi.

3.3 ENUMERATION OF BACTERIA AND FUNGI ISOLATES

The total viable counts of the samples were analyzed by the method of Obiajuru and Ozumba (2009). The mean of replicate platings were calculated and the total number expressed as cfu/g. (colony forming units per gram). Isolates were purified by repeated subculture on nutrient agar and the pure cultures of isolates obtained were stored on nutrient agar slants in a refrigerator (at 4^{o}C) waiting to be characterized.

3.4 CHARACTERIZATION OF ISOLATES

Isolates were characterized on the bases of colonial, morphology, microscopic, and biochemical characteristics.

3.4.1 Culture/Colonial Characterization of Isolates

Isolates were characterized colonially based on features presented on the culture plates. Such features include: size, shape, colour, margin, elevation, and surface appearance (Cheesbrough, 1998). Fungal isolates on the other hand were characterized by the mycelial arrangement and pigmentation.

3.4.2 Microscopic Characterization

The microscopic morphology and Gram's reaction of bacteria were determined using the conventional Gram's staining technique (Obiajuru and Ozumba, 2009). The fungi were identified by their colonial appearance and microscopic morphology after growth for 2 days to 2 weeks, mycelial characteristics in lactophenol cotton blue mount and arrangement of asexual spores (Obiajuru and Ozumba, 2009).

3.4.3 Biochemical Characterization of Bacteria Isolates

Bacterial isolates were further subjected to biochemical tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, coagulase, urease, starch hydrolysis, cystein and gelatin liquefaction, citrate utilization, fermentation of glucose, lactose, sucrose, maltose, and manitol (MacFaddin, 2000).

Further characterization was based on the Analytical Profile Index (A.P.I.) kit and with reference to Bergey's manual of Systematic Bacteriology.

3.5 PROXIMATE ANALYSIS

The main compositional components of interest are moisture, fat, protein, ash, fibre and available and unavailable carbohydrate.

3.5.1 Moisture Content Analysis

3.5.1.1 Procedure:

The crucible used was washed and dried in the oven. The dried container was transferred to desicator and weighed. Then, 2g of each of the samples were weighed and dried in the oven at a temperature of 105^0C for 2hrs. The container and samples were reweighed, taken back to

the oven and dried, put in the desicator and weighed again. This process was continued until a consistent result was obtained for each of the samples (AOAC, 2002).

3.5.1.2 Calculation:

$$\% \text{ Moisture Content} = \{(W_1 - W_2) / (W_1 - W)\} \times 100$$

Where:

W_1 = Mass of container + sample before drying

W_2 = Mass of container + sample after drying

W = Mass of container.

3.5.2 Ash Content Analysis

3.5.2.1 Procedure:

The crucible was washed and dried in desiccator, and then weighed. Also, 2g of each of the samples were weighed into the crucible. The crucible and contents were placed in the muffle furnace. The temperature was regulated at 575 ± 25^0 C until it was carbonized and calcinated until black particles were no more. Then the furnace was switched off and allowed to cool somewhat. Finally, the crucible and content were placed in a desiccator and weighed (James, 1995).

3.5.2.2 Calculation:

$$\% \text{ Ash Content} = \{(W_3 - W_1) / (W_2 - W_1)\} \times 100$$

Where:

W_1 = Mass of crucible

W_2 = Mass of crucible + Sample before ignition

W_3 = Mass of crucible + Ash after ignition

$W_2 - W_1$ = Mass of sample taken for ignition

3.5.3 Crude Fibre Analysis

3.5.3.1 Procedure:

About 2g of each of the samples were weighed and placed in a hot 200ml of 1.25% H_2SO_4 and boiled for 30 minutes. The samples were filtered through a Buckner funnel equipped with muslin cloth and held firm with elastic band. The funnel was made hot by pouring boiling water on to it. The hot acid sample solutions were filtered and the residues washed with boiling water to remove acid from them. Then, the residues were returned to 200ml boiling 1.25% NaOH and boiled for 30mins, filtered and progressively washed with boiling water, 1% HCl and boiling water to remove acid from it. The residues were further washed twice with alcohol and three times with petroleum ether using small quantities. The residues were drained and transferred completely to a porcelain crucible and dried in oven to a constant mass, cooled and weighed. The crucible was incinerated at 600^0 C for 2hrs in muffle furnace. Finally, the crucible and the content were weighed after cooling in desiccators. The loss on incineration was obtained as the mass of the crude fibre (AOAC, 2000).

3.5.3.2 Calculation:

% Crude Fiber = $\{(M_3 - M_4) / (M_2 - M_1)\} \times 100$

Where:

M_1 = Mass of the crucible

M_2 = Mass of sample + Crucible

M_3 = Mass of crucible + Residue after drying

M_4 = Mass of crucible + ash after incineration.

3.5.4 Crude Fat (Ether Extract)

3.5.4.1 Procedure:

Here, 2g of each of the samples were weighed into a filter paper, carefully wrapped and tied with thread. The filter and contents were then placed in the soxhlet extractor column and extracted for about 6 hours. When the solvent was clear in the column, it indicated that the fat must have been extracted. The defatted samples were carefully removed and the solvent recovered. Further, the flask and oil was oven dried until all the solvent was gone. Finally, the flask and the content were reweighed (James, 1995).

3.5.4.2 Calculation:

$$\% \text{ Crude fat} = \{(M_2 - M_1) / (M_3)\} \times 100$$

Where:

M_1 = Mass of the Flask

M_2 = Mass of flask + fat

M_3 = Mass of the sample

3.5.5 Crude Protein

3.5.5.1 Procedure:

Twenty grams (20g) of the samples were weighed and carefully transferred to a kjeldahl flask containing boiling chips. Using a spatula, a mixture of copper and sodium were added, and this raised the boiling temperature. Then, 20 ml concentration of H_2SO_4 was added to the flask to assist oxidation. The mixture was heated until it becomes clear. After cooling to room

temperature, it was quantitatively transferred to 100ml volumetric flask. This procedure was carried out for a blank experiment. Further, 20ml of the digest was pipetted and transferred to the distillation flask. Then, 10ml of 2% Boric was measured out into a receiver (small beaker) and two drops of methyl red indicator added, ensuring that the tip of the delivery tube extends below the surface of the Boric acid solution. More, 35ml of 40% NaOH was added to the distillation flask and the plug replaced quickly. The mixture was distilled until 30ml of the distillate was collected. The same procedure also was carried out for the blank experiment. Titration was against standard 0.1N HCl (Chang, 2003).

3.5.5.2 Calculation:

$$\% \text{ Crude protein} = \frac{(T-B) \times N_{HCl} \times 6.25 \times \text{Vol. Made} \times 0.00014}{\text{Aliquot} \times \text{Mass of Sample used}} \times \frac{100}{1}$$

Where: T = titre value of the sample

B = Blank titre value

N_{HCl} = Normality of HCl used

Aliquot = Sample aliquot (Volume) taken

The volume it was made up to = $100cm^3$

3.5.6 Carbohydrate Analysis

The total carbohydrate content was determined by difference as described by Sarkiayaji and Agar (2010). It involved summing up the percentage moisture, ash, crude lipid, crude protein and crude fiber and subtracting this sum from 100%.

Hence, Carbohydrate = 100 - (% moisture + % ash + % protein + % lipids + % fiber) for each sample.

3.6 ANTI-NUTRITIONAL COMPONENTS ANALYSIS

This involves analysis of food samples to identify those antinutritionals present in them. In this work, the antinutritionals of interest are alkaloid, cyanide, flavonoid, oxalate, saponin, and tannin.

3.6.1 Determination of Alkaloid

The alkaline precipitation method by Harborne (1973) was used. A measured weight of each processed sample was dispersed in 100ml of 10% acetic acid in ethanol solution. The mixture was shaken vigorously and allowed to stand for 4 hours at room temperature with shaking every 30min. At the end of this period, the mixture was filtered through Whatman filter paper (No. 42).

The filtered extract was concentrated by evaporation, to a quarter of the original volume. The extract was treated with dropwise addition of concentrated ammonia solution to precipitate the alkaloids. Ammonia was continually added until it was in excess.

The precipitated alkaloid was filtered using Whatman filter paper (No. 42). After washing with 1% NH_4OH soulution, the precipitated alkaloid was dried at 60^0 C and weighed after cooling in dessicator. The alkaloid content was calculated as shown below:

% Alkaloids = $\{(W_2-W_1) / \text{Weight of sample}\} \times 100$

Where;

W_1 = weight of empty filter paper

W_2 = weight of filter paper and alkaloid precipitate.

3.6.2 Cyanide Determination

The method used was as described by Anhwange *et al.,* 2011). Ten grams of each of the samples were soaked in the mixture of 200 cm^3 of distilled water and 10 cm^3 of orthophosphoric acid. The mixture was kept for 12 hours to release all the bonded cyanide. The mixture was then distilled until 150 cm^3 of the distillate was collected. 20 cm^3 of the distillate was taken into a conical flask containing 40 cm^3 of distilled water, 8 cm^3 of ammonia solution (6 $moldm^{-3}$) and 2 cm^3 of potassium iodide (5%) solution were added. The mixture was titrated with silver nitrate (0.02 $mold \cdot m^{-3}$) to faint but permanent turbidity (1 cm3 0.02 $moldm^{-3}$ $AgNO_3 \equiv 1.08$ mg HCN). Replicates determination were done for each of the samples (Anhwange *et al*., 2011).

3.6.3 Determination of Flavonoid

The method described by Harborne (1973) was used to determine the flavonoid content of the samples. A measured weight (5g) of each of the processed sample was boiled in 100ml of 2MHCl solution for 40min. It was allowed to cool to room temperature before being filtered through Whatman filter paper (No. 42) to obtain the extract.

Flavonoid in the extract was then precipitated by dropwise addition of concentrated ethyl acetate until in excess. The flavonoid precipitate was recovered in weighed filter paper following filtration. After drying in the oven and cooling in a dessicator, the weight of flavonoid was obtained by difference and expressed as a percentage of the sample analyzed. It was calculated as shown below:

% Flavonoid = (w2 – w1) / w) x 100

3.6.4 Determination of Tannin

The method described by Sarkiayaiji and Agar (2010) was adopted. Briefly, 400 mg of each of the samples were placed into four conical flasks and 40 ml diethyl ether containing 1% acetic acid (v/v) was added, then the mixtures were properly mixed to remove the pigment materials. Each supernatant was carefully discarded after 5 min and 20 ml of 70% aqueous acetone was added and the flasks were sealed with cotton plug covered with aluminum foil, then kept in electrical shaker for 2 h for extraction. Each content in the flasks was filtered through Whatman filter paper and samples (filtrates) were used for analyzing. 50 ml of tannins extract from each sample was taken into test tubes and the volume of each was made up to 1.0 ml with distilled water. 0.5 ml Folic ciocalteu reagent was added to each and mixed properly. Then 2.5 ml of 20% sodium carbonate solution was added and mixed. The mixtures were kept for 40 min at room temperature, after which absorbance was taken using spectrophotometer and concentration was estimated from the tannic acid standard curve.

3.7 MINERAL DETERMINATION

3.7.1 Determination of Potassium and Sodium by Flame Photometry

3.7.1.1 Procedure

The crucible was washed and dried in the oven at 105^0C for 30minutes, cooled in a desiccator. 0.05g of the sample was weighed into the crucible and placed in the hearth of the furnace and incinerated for 6-8hours at 575 ± 25^0C and allowed to cool. 5mls of 1MHNO_3 was added and evaporated to dryness using hot plate. The crucible and the content is returned to the furnace and incinerated for 10-15minutes and allowed to cool. 10mls of 1MHCl is added and filtered into 50ml volumetric flask with filter paper and funnel. The residue on the

filter paper was rinsed with 0.1MHCl and made up to mark with 0.1MHCl and labelled appropriately (Buhler, 2000).

Standard solutions for Na^+ and K^+ were prepared using 2.5g NaCl (A.R) and 1.91g KCl (A.R) respectively and dissolved each in $1dm^3$ volumetric flask with distilled water. For Sodium, it was equivalent to 1g Sodium per dm^3 (of 1mg Na/ml or 1000ppm Na) For Potassium (K^+) IT was equivalent to 1g K per dm^3 (or 1mg per ml or 1000ppmk). For Na, aliquots of the stock solution, 0.40, 0.80, 1.20, 1.60 and 2ml were taken in different 100ml volumetric flasks and made up to mark with distilled water. These gave 4, 8, 12, 16, and 20ppm Na respectively. With the sodium filter in position, the flame photometer was adjusted to zero, calibrated with standard sodium solutions and readings taken. A calibration graph was plotted from which concentrations of the unknown samples were determined.

For Potassium, aliquots of the stock solutions 0.40, 0.80, 1.20, 1.60 and 2ml were taken and made up to 100ml mark which corresponded to 4, 8, 12, 16 and 20 ppm K. With K filter in position, the flame photometer was calibrated and the instrument readings for each of the standard solutions recorded as well as that of the unknown. A calibration graph of emission intensity against concentration of the unknown samples was determined.

3.7.2 Determination of Phosphorus by Spectrophotometry

3.7.2.1 Procedure

The procedures for the preparation of samples for phosphorus determination were the same as that of Na and K. A standard solution for P^{3+} using 0.4397g KH_2PO_4 (A.R) and dissolved in $1dm^3$ volumetric flask with distilled water. For Phosphorus, this was equivalent to 0.10g P per 50ml (or 2mgP per ml or 2000ppm P). Aliquots of the stock solution 0.2, 0.4, 0.8, 1.2 and 1.6mls were taken and made up to 100ml mark using volumetric flasks with distilled water,

these gave 4, 8, 16, 24 and 32 ppm P respectively. The colours were developed with Ammonium molybdate and Stannous chloride. The Absorbance of each stanadard solution and that of the unknown were measured at 660nm and the readings recorded. A graph of Absorbance against Concentration of the unknown was determined (AOAC, 1984)

3.7.3 Determination of Calcium and Magnesium by Complexiometric method using EDTA

3.7.3.1 Reagents:

0.931g of EDTA was dissolved in 1litre

CALCON: 20mg of CALCON was dissolved in 50ml methanol.

1M NaOH: 40g of NaOH was weighed out and dissolved in 1 litre.

Calcium Determination by EDTA:

Procedure: A reference end point was first obtained by mixing 5 drops of 1M NaOH with 5 drops of calcon and diluted to 100ml with water. 5ml of the aliquot was pipette into a conical flask, 100ml of water, 5 ml of NaOH and 5 drops of the indicator and 15ml of buffer were added. EDTA solution was titrated to obtain the end point which was indicated by matching the colour change to a reference end point. Blank titration was carried out in similar way and subtracted from the sample reading (AOAC, 2000).

Calculation:

If Xml of EDTA solution were reguired for titration;

$$Ca\ (g/kg) = \frac{Xml \times Vol.\ Of\ Solution}{10 \times aliquot \times sample\ mass(g)}$$

Determination of Ca + Mg by EDTA

Reagents: 0.931 g of EDTA in 1 Litre of distilled water was dissolved. 0.25 g of Eriochrome Black T was dissolved in 50ml industrial spirit. Buffer Solution: 65.70g NH_4Cl was dissolved in water, 570ml of 0.88N NH_4OH and dilute to 1litre.

Procedure: 5ml of the sample solution was pipette into a conical flask and diluted to 100ml with water. 15ml of buffer solution, 10 drops of indicator and 2ml of triethanolamine were added. EDTA was titrated from red to a clear blue colour. Blank titration was also carried out and subtracted from the sample reading.

This procedure is necessary if Mg is to be determined from the sample digest or extract. Mg is then obtained by difference between Calcium determined above and (Ca + Mg) determination.

3.8. STATISTICAL TOOLS

3.8.1 Experimental Design for the physicochemical analysis

The experimental design was a three (3) factorial experiment in Randomized Complete Block Design (RCBD) with three (3) replications.

The Factor A was: Cassava preparations (3 preparation methods; mashed, peeled and unpeeled).

The Factor B was: Additives/Spices consisting of three (3) additives/spices namely: Ginger, Garlic, Ginger and Garlic.

The Factor C was Rates of the additives/spices consisting of four (4) rates at 0.0kg, 0.1kg, 0.2kg, 0.3kg.

3.8.2 Sensory Analysis

Sensory analysis for aroma, colour and texture were performed using the discrimation/effective test method and these were performed using trained panelists.

The results were analysed using CHI-SQUARE with:

$X^2 = (O- E)/ E.$

Where, O is the Observed Value

E is the Expected Value.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 showed the isolates identified in fermented cassava using three different preparation methods (mashed, peeled and unpeeled cassava preparations). *Bacillus spp*. and *Rhizopus spp* were isolated in all the three preparation methods (ie mashed, peeled and unpeeled). *Escherichia coli* were isolated in the peeled and unpeeled cassava preparations while *Penicillium spp* were found only in mashed cassava preparations.

Table 4.2 showed the isolates identified in fermented cassava using the three different preparation methods with varying quantities of garlic plus ginger. *Bacillus spp* were isolated in the three preparation methods and at the different rates of garlic plus ginger. With increasing quantity of garlic plus ginger grates (from 0.2kg to 0.3kg) in the three preparation methods, there were decline in the presence of isolates.

Table 4.3 showed the isolates identified in fermented cassava using the three different preparation methods (mashed, peeled and unpeeled) with varying quantities of garlic only. At 0.1kg of garlic only in mashed, peeled and unpeeled cassava preparations, isolates such as: *Bacillus spp., Lactobacillus spp*. and *Geotrichium spp* were obtained. With mashed fermented cassava, *Bacillus spp* were recovered in 0.2kg and 0.3kg of garlic only. With peeled fermented cassava containing 0.2kg and 0.3kg garlic only, *Escherichia spp*. were obtained while with the unpeeled cassava preparation with 0.2kg of garlic (the following organisms were obtained *Bacillus spp., Lactobacillus spp., Geotrichium spp* and *Rhizopus spp* and 0.3kg of garlic only (*Bacillus spp*.)

Table 4.1: Microbial flora of mashed, peeled and unpeeled cassava preparations after fermentation

Sample	Microbial Isolates
Unpeeled cassava	*Bacillus spp.,* *Rhizopus spp.*
Peeled cassava	*Bacillus spp.,* *Rhizopus spp.*
Mash cassava	*Bacillus spp.,* *Penicillum spp., Rhizopus spp.*

Table 4.2: Microbial isolates from fermented cassava treated with varying quantities of garlic plus ginger grates

Sample	Garlic & Ginger grates (kg)	Isolates
Unpeeled	0.1kg	*E. coli, Lactobacillus spp., Bacillus spp., Rhizopus spp.*
	0.2kg	*Bacillus spp., Lactobacillus spp, Aspergillus spp.*
	0.3kg	*Bacillus Spp.*
Peeled	0.1kg	*Lactobacillus spp., Bacillus spp., Geotrichium, spp., Rhizopus spp.*
	0.2kg	*Lactobacillus spp., Bacillus spp.*
	0.3kg	*Lactobacillus spp., Bacillus spp.*
Mashed	0.1kg	*Bacillus spp., Lactobacillus spp., Geotrichium, spp., Rhizopus spp.*
	0.2kg	*Bacillus spp., Penicillum spp., Rhizopus spp.*
	0.3kg	*Bacillus spp., Rhizopus spp.*

Table 4.3: Microbial isolates from fermented cassava preparations treated with varying quantities of garlic grates.

Sample	Garlic grates (kg)	Isolates
Unpeeled	0.1kg	*Bacillus spp., Lactobacillus spp., Pseudomonas spp., Aspergillus spp., Rhizopus spp., Geotrichium spp.*
	0.2kg	*Bacillus spp., Lactobacillus spp., Geotrichium, spp., Rhizopus spp.*
	0.3kg	*Bacillus spp.*
Peeled	0.1kg	*Bacillus spp., Lactobacillus spp., E. coli. Aspergillus spp., Geotrichium, spp., Rhizopus spp.,*
	0.2kg	*E. coli.*
	0.3kg	*E. coli.*
Mashed	0.1kg	*Bacillus spp., Lactobacillus spp., Corynebacterium spp., Geotrichium spp., Penicillum spp.*
	0.2kg	*Bacillus spp.*
	0.3kg	*Bacillus spp.*

Table 4.4 showed the isolates identified in fermented cassava using the three different preparation methods (mashed, peeled and unpeeled) with varying concentrations of ginger only. In all the three preparation methods at the different concentration rates of ginger, *Bacillus spp* were isolated. With mashed cassava preparation at 0.1kg, 0.2kg and 0.3kg ginger, isolates such as *Bacillus spp*. and *Rhizopus spp* were obtained. With peeled cassava preparation at 0.1kg, 0.2kg and 0.3kg ginger only isolates such as *Escherichia coli, Bacillus spp, Rhizopus spp* and *Trichoderma spp* were found while with the unpeeled cassava preparations, *Bacillus spp., Escherichia coli* and *Geotrichium spp* were obtained in the three different concentrations of ginger only.

Table 4.4: Microbial isolates from fermented cassava treated with varying quantities of ginger grates

Sample	Ginger Concentration (kg)	Isolates
Unpeeled	0.1kg	*Bacillus spp., Lactobacillus spp., E. coli., Geotrichium spp.*
	0.2kg	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Penicillum spp.*
	0.3kg	*E. coli, Bacillus spp., Geotrichium spp.,*
Peeled	0.1kg	*E. coli, Bacillus spp., Trichoderma spp., Geotrichium spp., Rhizopus spp.*
	0.2kg	*E. coli, Bacillus spp., Trichoderma spp., Geotrichium spp., Rhizopus spp.*
	0.3kg	*E. coli, Bacillus spp., Trichoderma spp., Rhizopus spp.*
Mashed	0.1kg	*Bacillus spp., Lactobacillus spp., Geotrichium spp., Rhizopus spp.*
	0.2kg	*Bacillus spp., Penicillum spp., Rhizopus spp.*
	0.3kg	*Bacillus spp., Rhizopus spp.*

Table 4.5 showed the microbial succession of fermenting cassava alone prepared in three different preparation methods. In Day 1 of fermentation, the following organisms were isolated in the different preparation methods: *Bacillus spp., Lactobacillus spp., Escherichia coli* and *Rhizopus spp*. *Trichoderma spp* and *Aspergillus spp* were isolated in the unpeeled fermenting cassava. In Day 2 of fermentation, isolates identified included: *Bacillus spp., Lactobacillus spp., Escherichia coli, Geotrichium spp., Rhizopus spp.* and *Penicillium spp*, these were present in mashed, peeled and unpeeled cassava preparations. *Trichoderma spp* and *Aspergillus spp* were present only in the unpeeled fermenting cassava preparation. By the 4^{th} day of fermentation, the organisms isolated were the same as that of day 2. In Day 6 of fermentation, the following isolates were common/present in the three preparation methods: *Bacillus spp., Lactobacillus spp., Escherichia coli, Geotrichium spp., Rhizopus spp*. and *Penicillium spp. Trichoderma spp* were present only in the unpeeled and peeled cassava preparation methods. *Proteus spp* were identified on the sixth (6^{th}) day of fermentation and were present in the unpeeled and mashed cassava preparations. On the 8^{th} day of fermenting cassava alone, Proteus spp were only significant in the unpeeled cassava preparation. Other isolates such as *Bacillus spp., Lactobacillus spp., Escherichia coli, Geotrichium spp., Rhizopus spp*. and *Penicillium spp* were found in the three preparation methods. *Aspergillus spp* were found only in the unpeeled cassava preparation. *Trichoderma spp* were present in the unpeeled and peeled cassava preparations. By the 10^{th} day of fermenting cassava alone, isolates namely: *Bacillus spp., Lactobacillus spp., Escherichia coli, Geotrichium spp., Rhizopus spp*. and *Penicillium spp* were found in the mashed and unpeeled cassava preparations. In addition, *Proteus spp* and *Aspergillus spp* were present in the unpeeled cassava preparation. The peeled cassava preparation had *Bacillus spp., Lactobacillus spp., Escherichia coli* and *Geotrichium spp. Trichoderma spp* were present in the unpeeled and peeled cassava preparations just as was the case on the 6^{th} and 8^{th} day of fermentation.

Table 4.5: Microbial succession of mashed, peeled and unpeeled cassava during fermentation

Day 1

Sample	Microbial Isolates
Unpeeled only	*Bacillus spp., Lactobacillus spp., E. coli., Trichoderma spp., Aspergillus spp., Rhizopus spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Rhizopus spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E.coli, Rhizopus spp.*

Day 2

Sample	Microbial Isolates
Unpeeled only	*Bacillus spp., Lactobacillus spp., E. coli., Trichoderma spp., Aspergillus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp., Penicillum spp.*

Day 4

Sample	Isolates
Unpeeled only	*Bacillus spp., Lactobacillus spp., E. coli., Trichoderma spp., Aspergillus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp., Penicillum spp.*

Day 6

Sample	Isolates
Unpeeled Only	*Bacillus spp., Lactobacillus spp., E. coli, Proteus spp., Trichoderma spp., Aspergillus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma, Geotrichium spp., Rhizopus spp., Penicillum spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E. coli, Proteus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*

Table 4.5 Cont'd

Day 8

Sample	Isolates
Unpeeled only	*Bacillus spp., Lactobacillus spp., E. coli, Proteus spp., Trichoderma spp., Aspergillus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp., Penicillum spp.*

Day 10

Sample	Isolates
Unpeeled only	*Bacillus spp., Lactobacillus spp., E. coli, Proteus spp., Trichoderma spp., Aspergillus spp., Geotrichium spp., Rhizopus spp., Penicillum spp.*
Peeled only	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma spp., Geotrichium spp.*
Mashed only	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium spp., Rhizopus spp., Penicillum spp.*

Table 4.6 showed the microbial succession of fermenting cassava with garlic only prepared in three different preparation methods. In Day 1 of fermentation, the following organisms were isolated in the different preparation methods: *Bacillus spp., Lactobacillus spp., Escherichia coli* and *Rhizopus spp. Trichoderma spp* and *Aspergillus spp* were isolated in the unpeeled fermenting cassava. In Day 2 of fermentation, isolates identified in the peeled cassava preparation with garlic only included: *Bacillus spp., Lactobacillus spp., Escherichia coli, Geotrichium spp.* and *Rhizopus spp. Bacillus spp., Lactobacillus spp., Rhizopus spp* and *Penicillium spp*, were present in mashed cassava preparation with garlic only. *Bacillus spp., Lactobacillus spp* and *Aspergillus spp* were present only in the unpeeled fermenting cassava preparation with garlic only. By the 4th day of fermentation, the organisms isolated were the same as that of day 2 only that *Geotrichium spp* were introduced/ present in the mashed, peeled and unpeeled cassava preparations with garlic only. *Aspergillus spp* were also found/ isolated in the unpeeled and peeled cassava preparations with garlic only. In Day 6 of fermentation, the following isolates were common/present in the three preparation methods: *Bacillus spp.* and *Trichoderma spp. Lactobacillus spp.* and *Aspergillus spp.* were present in the unpeeled and peeled preparations of cassava with garlic only. *Rhizopus spp*. was present in the peeled and mashed cassava with garlic only. *Penicillium spp.* was present only in the mashed cassava preparation methods with garlic only. On the 8th day of fermenting cassava prepared in different methods with garlic only, *Bacillus spp* were present in the three methods. *Trichoderma spp* and *Aspergillus spp* were isolated in the unpeeled and peeled cassava preparation methods with garlic. *Lactobacillus spp* were isolated in the unpeeled and mashed cassava with garlic only. *Rhizopus spp* was isolated in the mashed cassava preparation with garlic only. By the 10th day of fermenting cassava with garlic, isolates namely: *Bacillus spp.,* and *Trichoderma* spp were isolated in the mashed, peeled and unpeeled cassava preparations with garlic only. *Aspergillus spp* were present in the unpeeled and peeled cassava preparation.

Table 4.6: Microbial succession of fermenting cassava treated with garlic grates.

Day 1

Sample	Isolates
Unpeeled + garlic	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma spp., Aspergillus spp., Rhizopus spp.*
Peeled + garlic	*Bacillus spp., Lactobacillus spp., E. coli, Rhizopus spp.*
Mashed + garlic	*Bacillus spp., Lactobacillus spp., E. coli, Rhizopus spp.*

Day 2

Sample	Isolates
Unpeeled + Garlic	*Bacillus spp., Lactobacillus spp., Aspergillus spp.*
Peeled + Garlic	*Bacillus spp., Lactobacillus spp., E. coli, Geotrichium, spp., Rhizopus spp.*
Mashed + Garlic	*Bacillus spp., Lactobacillus spp., Rhizopus spp penicillum spp.*

Day 4

Sample	Isolates
Unpeeled + Garlic	*Bacillus spp., Lactobacillus spp., Aspergillus spp., Geotrichium spp., Rhizopus spp.*
Peeled + Garlic	*Bacillus spp., Lactobacillus spp. Aspergillus spp., Rhizopus spp., Geotrichium spp.*
Mashed + Garlic	*Bacillus spp., Lactobacillus spp. Penicillum spp., Geotrichium spp.*

Day 6

Sample	Isolates
Unpeeled + Garlic	*Bacillus spp., Lactobacillus spp., Trichoderma spp., Aspergillus spp.,*
Peeled + Garlic	*Bacillus spp., Lactobacillus spp., Trichoderma spp., Rhizopus spp.*
Mashed + Garlic	*Bacillus spp., Trichoderma spp., Rhizopus spp. Penicillum spp.*

Table 4.6 Cont'd

Day 8

Sample	Isolates
Unpeeled + Garlic	*Bacillus spp., Lactobacillus spp., Trichoderma spp., Aspergillus spp.*
Peeled + Garlic	*Bacillus spp., Lactobacillus spp., Trichoderma spp., Aspergillus spp.*
Mashed + Garlic	*Bacillus spp., Lactobacillus spp., Trichoderma spp., Rhizopus spp.*

Day 10

Sample	Isolates
Unpeeled + Garlic	*Bacillus spp., Trichoderma spp., Aspergillus spp.*
Peeled + Garlic	*Bacillus spp., Trichoderma spp., Aspergillus spp.*
Mashed + Garlic	*Bacillus spp., Trichoderma spp.*

Table 4.7 showed the microbial succession of fermenting cassava prepared in three different preparation methods with ginger grates. In Day 1 of fermentation, the following organisms were isolated in all the different preparation methods: *Bacillus spp., Lactobacillus spp., Escherichia coli* and *Rhizopus spp. Trichoderma spp* and *Aspergillus spp* were isolated in the unpeeled fermenting cassava with ginger only. In Day 2 of fermentation, isolates identified in the peeled cassava preparation with ginger only included: *Bacillus spp., Geotrichium spp.* and *Rhizopus spp. Bacillus spp., Lactobacillus spp., Rhizopus spp* and *Penicillium spp*, were present in mashed cassava preparation with ginger only. *Bacillus spp., Lactobacillus spp., Escherichia coli, Trichoderma spp., Geotrichium spp., Penicillium spp., Rhizopus spp* and *Aspergillus spp* were present only in the unpeeled fermenting cassava preparation with ginger only. By the 4th day of fermentation, the organisms isolated were the same as that of day 2 only that *Penicillium spp., Aspergillus spp* and *Trichoderma spp* were absent in the unpeeled cassava preparations with ginger only on the 4th day of fermentation. *Escherichia coli* were isolated in the peeled and mashed cassava preparations which were not present on the 2nd day of fermentation. In Day 6 of fermentation, the following isolates were common/present in the three preparation methods: *Bacillus spp.*, Rhizopus *spp., Penicillium spp., Lactobacillus spp.* and *Proteus spp.* were present in the unpeeled and mashed preparations of cassava with ginger only. *Bacillus spp., Penicillium spp* and *Rhizopus spp*. was present in the peeled cassava with ginger only. On the 8th day of fermenting cassava prepared in different methods with ginger only, *Bacillus spp., Rhizopus spp* and *Penicillium spp* were present in the three methods. *Aspergillus sp., Escherichia coli* and *Proteus spp* were isolated only in the unpeeled cassava preparation methods with ginger. *Lactobacillus spp* were isolated in the unpeeled and mashed cassava with ginger only. By the 10th day of fermenting cassava with ginger, *Bacillus spp* were present in the three preparation methods with ginger only. *Penicillium spp., Aspergillus spp and Proteus spp* were isolated in the mashed cassava preparations with ginger only. *Rhizopus spp* were present in the peeled and mashed cassava preparations. *Lactobacillus spp* were present in unpeeled and mashed cassava preparations with ginger.

Table 4.7: Microbial succession of fermenting cassava treated with Ginger grates.

Day 1

Sample	Isolates
Unpeeled + Ginger	*Bacillus spp., Lactobacillus spp. E. coli, Trichoderma spp., Aspergillus spp., Rhizopus spp.*
Peeled + Ginger	*Bacillus Spp., Lactobacillus spp. E. coli, Rhizopus spp.*
Mashed + Ginger	*Bacillus Spp., Lactobacillus spp. E. coli, Rhizopus spp.*

Day 2

Sample	Isolates
Unpeeled + Ginger	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma spp., Aspergillus spp., Geotricum spp, Rhizopus spp., Penicillum spp.*
Peeled + Ginger	*Bacillus spp., Geotricum spp., Rhizopus spp.*
Mashed + Ginger	*Bacillus spp., Lactobacillus spp., Rhizopus spp. Penicillum spp.*

Day 4

Sample	Isolates
Unpeeled + Ginger	*Bacillus spp., Lactobacillus spp., E. coli, Geotricum spp., Rhizopus spp.*
Peeled + Ginger	*Bacillus spp., E .coli, Trichoderma spp., Geotricum spp., Rhizopus spp.*
Mashed + Ginger	*Bacillus spp., Lactobacillus spp., E. coli, Rhizopus spp., Geotricum spp.*

Day 6

Sample	Isolates
Unpeeled + Ginger	*Bacillus spp., Lactobacillus spp., E. coli, Proteus spp., Aspergillus spp., Geotricum spp., Rhizopus spp., Penicillum spp.*
Peeled + Ginger	*Bacillus Spp., Rhizopus spp., Penicillum spp.*
Mashed + Ginger	*Bacillus Spp., Lactobacillus Spp., Proteus spp., Rhizopus spp., Penicillum spp.*

Table 4.7 Cont'd

Day 8

Sample	Isolates
Unpeeled + Ginger	*Bacillus Spp., Lactobacillus spp., E. coli, Proteus spp., Aspergillus spp., Geotricum spp., Penicillum spp.*
Peeled + Ginger	*Bacillus Spp., Geotricum spp., Rhizopus spp., Penicillum spp.*
Mashed + Ginger	*Bacillus Spp., Lactobacillus Spp., Rhizopus spp., Penicillum spp.*

Day 10

Sample	Isolates
Unpeeled + Ginger	*Bacillus spp., Lactobacillus spp., Proteus spp., Aspergillus spp., Geotricum spp., Penicillum spp.*
Peeled + Ginger	*Bacillus Spp., Geotricum spp., Rhizopus spp.*
Mashed + Ginger	*Bacillus Spp. Lactobacillus Spp., Rhizopus spp.*

Table 4.8 showed the microbial succession of fermenting cassava prepared in three different preparation methods with garlic plus ginger grates. In Day 1 of fermentation, the following organisms were isolated in all the different preparation methods: *Bacillus spp., Lactobacillus spp., Escherichia coli* and *Rhizopus spp. Trichoderma spp* and *Aspergillus spp* were isolated in the unpeeled fermenting cassava with garlic plus ginger. In Day 2 of fermentation, isolates identified in the three preparation methods with garlic plus ginger grates included: *Bacillus spp.* and *Lactobacillus spp. Rhizopus spp* were present in peeled and mashed cassava preparations with garlic plus ginger grates. *Aspergillus spp* were present only in the unpeeled fermenting cassava preparation with garlic plus ginger grates. By the 4th day of fermentation, the organisms isolated in mashed, peeled and unpeeled cassava preparations with garlic plus ginger grates were *Bacillus spp., Rhizopus spp* and *Lactobacillus spp. Geotrichium spp* were present in the peeled and mashed cassava preparations with garlic plus ginger grates. In Day 6 of fermentation, the following isolates were common/present in the three preparation methods: *Bacillus spp* and *Geotrichium spp. Lactobacillus spp.* and *Aspergillus spp.* were present in the unpeeled preparation of cassava with garlic plus ginger grates. *Rhizopus spp*. was present in the peeled and mashed cassava with garlic plus ginger grates/mash. On the 8th day of fermenting cassava prepared in different methods with garlic plus ginger grates, *Bacillus spp* and *Geotrichium spp* were present in the three methods. *Aspergillus spp* were isolated only in the unpeeled cassava preparation methods with garlic plus ginger. *Lactobacillus spp* were isolated in the unpeeled and mashed cassava with garlic plus ginger grates. By the 10th day of fermenting cassava with garlic plus ginger, *Bacillus spp* were present in the three preparation methods with garlic plus ginger. *Aspergillus spp* were isolated in the mashed cassava preparations with garlic plus ginger mash. *Geotrichium spp* were present in the peeled and mashed cassava preparations with garlic and ginger.

Table 4.8: Microbial succession of fermenting cassava treated with Garlic plus Ginger grates

Day 1

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., E. coli, Trichoderma spp., Aspegillus spp., Rhizopus spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., E. coli, Rhizopus spp.*
Mashed + Ginger. and Garlic	*Bacillus Spp., Lactobacillus Spp., E. coli, Rhizopus spp.*

Day 2

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Aspergillus spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Rhizopus spp.*
Mashed + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Rhizopus spp.*

Day 4

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Rhizopus spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Geotricum spp., Rhizopus spp.*
Mashed + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Geotricum spp., Rhizopus spp.*

Day 6

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Aspergillus spp., Geotricum spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Geotricum spp., Rhizopus spp.*
Mashed + Ginger. and Garlic	*Bacillus spp., Geotricum spp., Rhizopus spp.*

Table 4.8 Cont'd

Day 8

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Aspergillus spp., Geotricum spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Geotricum spp.*
Mashed + Ginger. and Garlic	*Bacillus spp., Lactobacillus spp., Geotricum spp., Rhizopus spp.*

Day 10

Sample	Isolates
Unpeeled + Ginger. and Garlic	*Bacillus spp., Aspergillus spp.*
Peeled + Ginger. and Garlic	*Bacillus spp., Geotricum spp.*
Mashed + Ginger. and Garlic	*Bacillus spp., Geotricum spp.*

Table 4.9 showed the microbial counts of fermenting cassava with garlic, ginger, garlic plus ginger grates prepared in different methods. In Day 1 of fermentation, the total heterotrophic counts for the unpeeled cassava preparations were 9.3×10^4, 8.9×10^4, 9.5×10^4 and 9.7×10^4 for unpeeled only, unpeeled with garlic, unpeeled with ginger and unpeeled with garlic plus ginger respectively. For the peeled cassava preparations, the total heterotrophic counts were 8.6×10^4, 7.4×10^4, 8.5×10^4 and 8.1×10^4 which were for, peeled only, peeled with garlic, peeled with ginger, peeled with garlic plus ginger respectively. For the mashed preparation, the total heterotrophic counts were 8.4×10^4, 9.0×10^4, 8.1×10^4 and 8.9×10^4 for mashed only, mashed with garlic, mashed with ginger, mashed with garlic plus ginger respectively. The table revealed that the highest total heterotrophic count was $9.7\times10^4 cfug^{-1}$ for unpeeled cassava preparation with garlic plus ginger grates while the lowest value was with peeled cassava preparation method with garlic only $7.4\times10^4 cfug^{-1}$. In Day 2 of fermentation, the results showed that the highest total heterotrophic bacterial counts was with unpeeled cassava preparation method only $2.06\times10^5 cfug^{-1}$ while the least value was with peeled cassava preparation method with garlic plus ginger grates $8.4\times10^4 cfug^{-1}$. In Day 4 of fermentation, the results showed that unpeeled cassava only (without any additive) had the highest total heterotrophic bacterial count value of $1.76\times10^6 cfug^{-1}$ while the lowest value was $9.0\times10^5 cfug^{-1}$ for peeled fermenting cassava with garlic plus ginger grates. In Day 6 of fermentation, the results showed that unpeeled cassava only (without any additive) had the highest total heterotrophic bacterial count value of $2.14\times10^6 cfug^{-1}$ while the lowest value was $1.01\times10^5 cfug^{-1}$ for peeled fermenting cassava with garlic plus ginger grates. By the 8^{th} day of fermenting cassava prepared in three different methods, the table showed that the highest value of total heterotrophic bacterial count was $1.62\times10^6 cfug^{-1}$ for unpeeled cassava only while the lowest value was $7.2\times10^5 cfug^{-1}$ (peeled cassava with garlic plus ginger). By the 10^{th} day of fermentation, the results showed that unpeeled cassava only (without any additive) had the highest total heterotrophic bacterial count value of $1.05\times10^5 cfug^{-1}$ while the lowest value was $6.0\times10^4 cfug^{-1}$ for peeled fermenting cassava with garlic plus ginger grates.

Table 4.9: Total Heterotrophic Bacterial Counts of Fermenting Cassava.

SAMPLE	TOTAL HETEROTROPHIC BACTERIAL COUNT					
	DAY 1	DAY 2	DAY4	DAY6	DAY 8	DAY10
Unpeeled Only	9.3×10^4	2.06×10^5	1.76×10^6	2.14×10^6	1.62×10^6	1.05×10^5
Unpeeled + Garlic	8.9×10^4	1.08×10^5	1.04×10^6	1.30×10^6	1.09×10^6	8.7×10^4
Unpeeled + Ginger	9.5×10^4	1.34×10^5	1.27×10^6	1.46×10^6	1.17×10^6	9.5×10^4
Unpeeled+ Ginger & Garlic	9.7×10^4	9.2×10^4	1.12×10^6	1.39×10^6	1.06×10^6	9.0×10^4
Peeled Only	8.6×10^4	1.41×10^5	1.21×10^6	1.59×10^6	8.4×10^5	6.5×10^4
Peeled + Garlic	7.4×10^4	1.18×10^5	1.06×10^6	1.30×10^6	8.8×10^5	7.4×10^4
Peeled + Ginger	8.5×10^4	1.07×10^5	1.14×10^6	1.24×10^6	8.0×10^5	6.6×10^4
Peeled + Ginger & Garlic	8.1×10^4	8.4×10^4	9.0×10^5	1.01×10^5	7.2×10^5	6.0×10^4
Mashed Only	8.4×10^4	1.65×10^5	1.32×10^6	1.76×10^6	1.21×10^6	1.02×10^5
Mashed + Garlic	9.0×10^4	1.20×10^5	1.13×10^6	1.64×10^6	1.05×10^5	8.5×10^4
Mashed + Ginger	8.1×10^4	1.36×10^5	1.42×10^6	1.81×10^6	9.2×10^5	6.9×10^4
Mashed + Ginger & Garlic	8.9×10^4	1.05×10^5	1.28×10^6	1.69×10^6	9.6×10^5	6.5×10^4

Table 4.10 showed the total heterotrophic fungal counts of fermenting cassava.

In Day 1 of fermentation, the results showed that peeled cassava only (without any additive) had the highest total heterotrophic fungal count value of 5.1×10^{4}cfug^{-1} while the lowest value was 3.6×10^{4}cfug^{-1} for peeled fermenting cassava with garlic plus ginger grates and also for unpeeled fermenting cassava with garlic.

In Day 2 of fermentation, the results showed that unpeeled cassava only (without any additive) had the highest total heterotrophic fungal count value of 1.7×10^{5}cfug^{-1} while the lowest value was 8.4×10^{4}cfug^{-1} for mashed fermenting cassava with garlic plus ginger grates.

In Day 4 of fermentation, the results showed that unpeeled cassava with garlic plus ginger grates had the highest total heterotrophic fungal count value of 1.54×10^{6}cfug^{-1} while the lowest value was 7.9×10^{5}cfug^{-1} for peeled fermenting cassava with garlic plus ginger grates.

By the 6th day of fermentation, the results showed that unpeeled cassava only (without any additive) had the highest total heterotrophic fungal count value of 1.94×10^{6}cfug^{-1} while the lowest value was 1.09×10^{5}cfug^{-1} for mashed fermenting cassava with ginger grates.

By the 8th day of fermenting cassava prepared in three different methods, the table showed that the highest value of total heterotrophic fungal count was 1.86×10^{6}cfug^{-1} for unpeeled cassava only while the lowest value was 9.2×10^{5}cfug^{-1} (peeled cassava only).

By the 10th day of fermenting cassava prepared in three different methods, the table showed that the highest value of total heterotrophic fungal count was 1.20×10^{5}cfug^{-1} for unpeeled cassava with garlic while the lowest value was 6.2×10^{4}cfug^{-1} (peeled cassava only).

Table 4.10: Total Heterotrophic Fungal Counts of Fermenting Cassava

SAMPLE	TOTAL HETEROTROPHIC FUNGAL COUNT					
	DAY 1	DAY 2	DAY4	DAY6	DAY 8	DAY10
Unpeeled Only	4.3×10^4	1.70×10^5	1.45×10^6	1.94×10^6	1.86×10^6	1.18×10^5
Unpeeled + Garlic	3.6×10^4	1.65×10^5	1.33×10^6	1.72×10^6	1.65×10^6	1.20×10^5
Unpeeled + Ginger	4.5×10^4	1.63×10^5	1.24×10^6	1.86×10^6	1.81×10^6	1.03×10^5
Unpeeled+ Ginger & Garlic	4.7×10^4	1.51×10^5	1.54×10^6	1.70×10^5	1.74×10^6	1.08×10^5
Peeled Only	5.1×10^4	9.6×10^4	9.3×10^5	1.10×10^6	9.2×10^5	6.2×10^4
Peeled + Garlic	4.0×10^4	9.0×10^4	8.6×10^5	1.15×10^6	9.6×10^5	7.0×10^4
Peeled + Ginger	4.4×10^4	9.2×10^4	9.4×10^5	1.08×10^6	1.06×10^5	8.0×10^4
Peeled + Ginger & Garlic	3.6×10^4	9.1×10^4	7.9×10^5	1.26×10^6	1.24×10^5	8.5×10^4
Mashed Only	3.9×10^4	1.04×10^5	1.32×10^6	1.52×10^6	1.20×10^6	8.9×10^4
Mashed + Garlic	4.1×10^4	9.3×10^4	1.01×10^6	1.26×10^5	1.15×10^5	8.0×10^4
Mashed + Ginger	3.2×10^4	8.1×10^4	9.7×10^5	1.09×10^5	1.05×10^5	7.1×10^4
Mashed + Ginger & Garlic	4.5×10^4	8.4×10^4	8.8×10^5	1.12×10^5	1.06×10^5	7.4×10^4

Rates of ginger, garlic and garlic plus ginger on alkaloid content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.11

Mean concentrations averaged over rates and types of treatments were 2.47%, 2.58% and 2.83% for unpeeled, mashed and peeled cassava preparations respectively. This showed an increasing order from unpeeled, mashed and peeled cassava preparations.

Addition of mashed and garlic gave 2.64% while that with ginger and garlic plus ginger were 2.56%. Also, combinations of peeled cassava preparations with ginger gave 3.11% while that with garlic plus ginger and garlic alone were 2.88% and 2.50% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 2.88% while that with garlic and ginger and ginger alone gave 2.40% and 2.13% respectively. This showed that combinations of peeled cassava preparations with ginger gave the highest alkaloid concentration while the least was with unpeeled cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that alkaloid content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 2.70%, 2.33%, 2.80% and 2.52% respectively indicating that addition of 0.2kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates exception being the control (zero) rate. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that alkaloid contents were 2.90%, 2.53%, 3.03% and 2.85% respectively indicating that addition of 0.2kg of treatments of peeled cassava was significantly higher (LSD 0.05) exception being the control (zero) rates and 0.3kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that alkaloid contents were 2.65%, 2.20%, 2.48% and 2.53% respectively indicating that 0(zero) rates of treatments for unpeeled cassava preparations were only significantly higher (LSD 0.05) than 0.1kg

Addition of various cassava preparations and treatments in their various rates showed that the alkaloid content was significantly higher (LSD 0.05) in 0.2kg garlic of unpeeled cassava preparations (3.25%) than in most others.

Table 4.11: Effect of Ginger and Garlic on Alkaloid content Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean%
Mashed	Gaig	2.70	2.20	2.90	2.90	2.56
	Ga	2.70	2.50	3.10	3.10	2.64
	Gi	2.70	2.30	2.40	2.40	2.56
	Mean	**2.70**	**2.33**	**2.80**	**2.52**	**2.58**
Peeled	Gaig	2.90	2.75	2.80	3.05	2.88
	Ga	2.90	1.90	3.10	2.10	2.50
	Gi	2.90	2.95	3.20	3.40	3.11
	Mean	**2.90**	**2.53**	**3.03**	**2.85**	**2.83**
Unpeeeled	Gaig	2.65	2.10	2.20	2.65	2.40
	Ga	2.65	2.60	3.25	3.00	2.88
	Gi	2.65	1.90	2.00	1.95	2.13
	Mean	**2.65**	**2.20**	**2.48**	**2.53**	**2.47**

LSD 0.05	Fact A (Cassava preparations) = 0.10 Fact B (Treatments) = 0.20 Fact C (Treatment rates) = 0.13 Fact A x B = 0.28 Fact A x C = 0.20 Fact B x C = 0.26 Fact A x B x C = 0.41
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on ash content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.12

Mean concentrations averaged over rates and types of treatments were 0.796%, 1.022% and 1.373% for peeled, unpeeled and mashed cassava preparations respectively. This showed an increasing order from peeled, unpeeled and mashed cassava preparations.

Addition of mashed and garlic gave 1.425% while that with ginger and garlic plus ginger were 1.371% and 1.323% respectively. Also, combinations of peeled cassava preparations with ginger gave 0.847% while that with garlic plus ginger and garlic alone were 0.783% and 0.759% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic plus ginger gave 1.140% while that with garlic and ginger alone gave 0.725% and 1.200% respectively. This showed that combinations of mashed cassava preparations with garlic gave the highest ash concentration while the least was with unpeeled cassava preparations with garlic alone.

Addition of various cassava preparations and rates of different treatments showed that ash contents of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.391%, 1.654%, 1.420% and 2.026% respectively indicating that addition of 0.3kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that ash contents were 0.676%, 0.919%, 0.634% and 0.955% respectively indicating that addition of 0.3kg of treatments of peeled cassava were significantly higher (LSD 0.05) exception being 0.1kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that ash contents were 0.934%, 1.049%, 1.106% and 0.997% respectively indicating that addition of 0.2kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than others

Addition of various cassava preparations and treatments in their various rates showed that the ash content was significantly higher (LSD 0.05) in 0.3kg ginger of mashed cassava preparations (2.091%) than in most others.

Table 4.12: Effect of Ginger and Garlic on Ash content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean%
Mashed	Gaig	0.391	1.497	1.368	2.037	1.323
	Ga	0.391	1.647	1.711	1.952	1.425
	Gi	0.391	1.819	1.181	2.091	1.371
	Mean	**0.391**	**1.654**	**1.420**	**2.026**	**1.373**
Peeled	Gaig	0.676	0.828	0.690	0.937	0.783
	Ga	0.676	1.277	0.486	0.596	0.759
	Gi	0.676	0.652	0.726	1.332	0.847
	Mean	**0.676**	**0.919**	**0.634**	**0.955**	**0.796**
Unpeeeled	Gaig	0.934	0.908	1.782	0.937	1.140
	Ga	0.934	0.487	0.671	0.807	0.725
	Gi	0.934	1.754	0.864	1.248	1.200
	Mean	**0.934**	**1.049**	**1.106**	**0.997**	**1.022**

L.S.D 0.05	Fact A (Cassava Preparation) = 0.0024 Fact B (Treatments) = 0.0031 Fact C(Treatment Rates) = 0.0024 Fact A x B = 0.0045 Fact A x C = 0.0038 Fact B x C = 0.0044 Fact A x B x C = 0.0073
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the carbohydrate content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.13

Mean concentrations averaged over rates and types of treatments were 67.671%, 69.219% and 70.845% for unpeeled, peeled and mashed cassava preparations respectively. This showed an increasing order of unpeeled, peeled, mashed cassava preparations.

Addition of mashed cassava preparations and ginger gave 71.27% while that with garlic and garlic plus ginger were 71.243% and 70.021% respectively. Also, combinations of peeled cassava preparations with garlic plus ginger gave 70.568% while that with ginger and garlic alone were 69.678% and 67.411% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic plus ginger gave 70.253% while that with garlic and ginger alone gave 66.581% and 66.178% respectively. This showed that combinations of mashed cassava preparations with ginger alone gave the highest carbohydrate concentration while the least was with unpeeled cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that the carbohydrate contents of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 66.27%, 75.245%, 73.823% and 68.04% respectively indicating that addition of 0.1kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that carbohydrate contents were 63.31%, 68.55%, 72.473% and 72.542% respectively indicating that addition of 0.3kg of treatments of peeled cassava were significantly higher (LSD 0.05) exception being 0.2kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that ash contents were 75.82%, 61.61%, 65.183% and 68.068% respectively indicating that

addition of 0(zero) rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than others

Addition of various cassava preparations and treatments in their various rates showed that the carbohydrate content was significantly higher (LSD 0.05) in 0.2kg garlic of peeled cassava preparations (75.839%) than in most others.

Table 4.13: Effect of Ginger and Garlic on Carbohydrate content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	66.27	74.825	74.15	64.84	70.021
	Ga	66.27	76.1	72.41	70.19	71.243
	Gi	66.27	74.81	74.91	69.09	71.27
	Mean	**66.27**	**75.245**	**73.823**	**68.04**	**70.845**
Peeled	Gaig	63.31	71.91	72.44	74.61	70.568
	Ga	63.31	60.84	75.839	69.655	67.411
	Gi	63.31	72.9	69.14	73.36	69.678
	Mean	**63.31**	**68.55**	**72.473**	**72.542**	**69.219**
Unpeeeled	Gaig	75.82	68.05	68.52	68.62	70.253
	Ga	75.82	65.82	58.68	66.005	66.581
	Gi	75.82	50.96	68.35	69.58	66.178
	Mean	**75.82**	**61.61**	**65.183**	**68.068**	**67.671**

L.S.D 0.05	Fact A(Cassava Preparation) = 0.33 Fact B (Treatments) = 0.17 Fact C (Treatment Rates) = 0.17 Fact A x B = 0.29 Fact A x C = 0.30 Fact B x C = 0.29 Fact A x B x C = 0.50

Rates of ginger, garlic and garlic plus ginger on calcium content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.14

Mean concentrations averaged over rates and types of treatments were 0.102%, 0.152% and 0.258% for peeled, mashed and unpeeled cassava preparations respectively. This showed an increasing order of peeled, mashed and unpeeled cassava preparations.

Addition of mashed and garlic gave 0.183% while that with garlic plus ginger and ginger were 0.169% and 0.104% respectively. Also, combinations of peeled cassava preparations with garlic plus ginger gave 0.117% while that with ginger and garlic alone were 0.101% and 0.089% respectively. Furthermore, concentrations of unpeeled cassava preparations with ginger gave 0.333% while that with garlic and ginger and garlic alone gave 0.226% and 0.214% respectively. This showed that combinations of unpeeled cassava preparations with ginger gave the highest calcium concentration while the least was with peeled cassava preparations with garlic alone.

Addition of various cassava preparations and rates of different treatments showed that calcium content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.097%, 0.164%, 0.121% and 0.226% respectively indicating that addition of 0.3kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that calcium contents were 0.093%, 0.124%, 0.079% and 0.113% respectively indicating that addition of 0.1kg of treatments of peeled cassava was significantly higher (LSD 0.05) exception being 0.3kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that calcium contents were 0.319%, 0.224%, 0.227% and 0.239% respectively indicating that addition of

0(zero) rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than all the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the calcium content was significantly higher (LSD 0.05) in 0.2kg ginger of unpeeled cassava preparations (0.363%) than in most others.

Table 4.14: Effect of Ginger and Garlic on Calcium content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	0.097	0.168	0.128	0.282	0.169
	Ga	0.097	0.188	0.157	0.289	0.183
	Gi	0.097	0.135	0.079	0.106	0.104
	Mean	**0.097**	**0.164**	**0.121**	**0.226**	**0.152**
Peeled	Gaig	0.093	0.103	0.148	0.124	0.117
	Ga	0.093	0.171	0.017	0.075	0.089
	Gi	0.093	0.098	0.073	0.14	0.101
	Mean	**0.093**	**0.124**	**0.079**	**0.113**	**0.102**
Unpeeeled	Gaig	0.319	0.272	0.223	0.088	0.226
	Ga	0.319	0.142	0.095	0.3	0.214
	Gi	0.319	0.319	0.363	0.33	0.333
	Mean	**0.319**	**0.244**	**0.227**	**0.239**	**0.258**

L.S.D 0.05	Fact A(Cassava Preparation) = 0.030 Fact B(Treatments) = 0.016 Fact C(Treatment Rates) = 0.016 Fact A x B = 0.028 Fact A x C = 0.029 Fact B x C = 0.028 Fact A x B x C = 0.048
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the fat content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.15

Mean concentrations averaged over rates and types of treatments were 2.66%, 3.059% and 3.305% for peeled, mashed and unpeeled cassava preparations respectively. This showed an increasing order of peeled, mashed, unpeeled cassava preparations.

Addition of mashed and garlic plus ginger gave 3.803% while that with ginger and garlic were 3.108% and 2.265%. Also, combinations of peeled cassava preparations with ginger gave 2.86% while that with garlic plus ginger and garlic alone were 2.8% and 2.33% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 4.015% while that with ginger and garlic plus ginger gave 3.088% and 2.813% respectively. This showed that combinations of unpeeled cassava preparations with garlic gave the highest fat concentration while the least was with mashed cassava preparations with garlic alone.

Addition of various cassava preparations and rates of different treatments showed that the fat content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 2.9%, 2.913%, 3.067% and 3.42% respectively indicating that addition of 0.3kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates exception being the 0.2kg rate. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that fat contents were 3.05%, 2.33%, 2.58% and 2.69% respectively indicating that addition of 0(zero) rates of treatment to peeled cassava was significantly higher (LSD 0.05) exception being 0.2kg rates and 0.3kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the fat contents were 2.65%, 2.977%, 3.56% and 4.03% respectively indicating that addition of 0.3kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than all exception being the 0.2kg rates.

Addition of various cassava preparations and treatments in their various rates showed that the fat content was significantly higher (LSD 0.05) in 0.2kg garlic of unpeeled cassava preparations (4.90%) than in most others.

Table 4.15: Effect of Ginger and Garlic on Fat content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	2.9	3.9	4.13	4.28	3.803
	Ga	2.9	1.84	2.07	2.25	2.265
	Gi	2.9	3	2.8	3.73	3.108
	Mean	**2.9**	**2.913**	**3.067**	**3.42**	**3.059**
Peeled	Gaig	3.05	2.57	2.8	2.78	2.8
	Ga	3.05	1.73	2.03	2.5	2.33
	Gi	3.05	2.7	2.9	2.8	2.86
	Mean	**3.05**	**2.33**	**2.58**	**2.69**	**2.66**
Unpeeeled	Gaig	2.65	2.33	2.68	3.59	2.813
	Ga	2.65	3.7	4.9	4.81	4.015
	Gi	2.65	2.9	3.1	3.7	3.088
	Mean	**2.65**	**2.977**	**3.56**	**4.03**	**3.305**

L.S.D 0.05	Fact A (Cassava preparation) = 0.033 Fact B (Treatment) = 0.118 Fact C (Treatment Rates) = 0.113 Fact A x B = 0.153 Fact A x C = 0.170 Fact B x C = 0.191 Fact A x B x C = 0.318
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the fibre content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.16

Mean concentrations averaged over rates and types of treatments were 3.042%, 3.953% and 5.67% for mashed, unpeeled and peeled cassava preparations respectively. This showed an increasing order of mashed, unpeeled, peeled cassava preparations.

Addition of mashed and garlic gave 3.863% while that with ginger and garlic plus ginger were 2.5% and 2.763%. Also, combinations of peeled cassava preparations with garlic plus ginger gave 6.6% while that with ginger and garlic alone were 5.23% and 5.19% respectively. Furthermore, concentrations of unpeeled cassava preparations with ginger gave 5.3% while that with garlic plus ginger and garlic alone gave 3.35% and 3.21% respectively. This showed that combinations of unpeeled cassava preparations with ginger gave the highest fibre concentration while the least was with mashed cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that fibre content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 2.80%, 2.87%, 2.15% and 4.35% respectively indicating that addition of 0.3kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the fibre contents were 11.45%, 5%, 3.42% and 2.82% respectively indicating that addition of 0(zero) rate of treatments of peeled cassava was significantly higher (LSD 0.05) than all the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the fibre contents were 2.0%, 5.85%, 3.933% and 4.033% respectively indicating that addition of 0.1kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than all the other rates

Addition of various cassava preparations and treatments in their various rates showed that the fibre content was significantly higher (LSD 0.05) in the 0(zero/ control) rates of peeled cassava preparations than in most others.

Table 4.16: Effect of Ginger and Garlic on Fibre content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	2.8	3	1.8	3.45	2.763
	Ga	2.8	2.6	3.55	6.5	3.863
	Gi	2.8	3	1.1	3.1	2.5
	Mean	**2.8**	**2.87**	**2.15**	**4.35**	**3.042**
Peeled	Gaig	11.45	6.45	4.9	3.6	6.6
	Ga	11.45	4.4	2.35	2.55	5.19
	Gi	11.45	4.15	3	2.3	5.23
	Mean	**11.45**	**5**	**3.42**	**2.82**	**5.67**
Unpeeeled	Gaig	2	4.55	3.5	3.35	3.35
	Ga	2	4	3.45	3.4	3.21
	Gi	2	9	4.85	5.35	5.3
	Mean	**2**	**5.85**	**3.933**	**4.033**	**3.953**

L.S.D 0.05	Fact A (Cassava Preparation) = 0.511 Fact B (Treatment) = 0.319 Fact C (Treatment Rates) =0.271 Fact A x B = 0.507 Fact A x C = 0.486 Fact B x C = 0.485 Fact A x B x C = 0.829
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the flavonoid content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.17

Mean concentrations averaged over rates and types of treatments were 1.78%, 1.83% and 2.03% for unpeeled, mashed and peeled cassava preparations respectively. This showed an increasing order of unpeeled, mashed, peeled cassava preparations.

Addition of mashed and garlic plus ginger gave 1.86% while that with garlic and ginger were 1.8% and 1.83% respectively. Also, combinations of peeled cassava preparations with ginger gave 2.19% while that with garlic plus ginger and garlic alone were 2% and 1.91% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 1.93% while that with garlic and ginger and ginger alone gave 1.75% and 1.65% respectively. This showed that combinations of peeled cassava preparations with ginger gave the highest flavonoid concentration while the least was with unpeeled cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that flavonoid content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 2.1%, 1.58%, 2.0% and 1.63% respectively indicating that the addition of 0(zero) rates of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates exception being 0.2kg rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that flavonooid contents were 1.95%, 1.83%, 2.22% and 2.13% respectively indicating that addition of 0.2kg of treatments of peeled cassava were significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that flavonoid contents were 1.9%, 1.67%, 1.72% and 1.82% respectively

indicating that addition of 0(zero) rates of treatments to unpeeled cassava preparations were only significantly higher (LSD 0.05) than 0.1kg.

Addition of various cassava preparations and treatments in their various rates showed that the flavonoid content was significantly higher (LSD 0.05) in 0.2kg garlic of peeled cassava preparations (2.40%) than in most others.

Table 4.17: Effect of Ginger and Garlic on Flavonoid content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	2.1	1.45	2.1	1.8	1.86
	Ga	2.1	1.7	2.2	1.2	1.8
	Gi	2.1	1.6	1.7	1.9	1.83
	Mean	**2.1**	**1.58**	**2**	**1.63**	**1.83**
Peeled	Gaig	1.95	2	1.95	2.1	2
	Ga	1.95	1.5	2.4	1.8	1.91
	Gi	1.95	2	2.3	2.5	2.19
	Mean	**1.95**	**1.83**	**2.22**	**2.13**	**2.03**
Unpeeeled	Gaig	1.9	1.7	1.6	1.8	1.75
	Ga	1.9	1.7	1.9	2.2	1.93
	Gi	1.9	1.6	1.65	1.45	1.65
	Mean	**1.9**	**1.67**	**1.72**	**1.82**	**1.78**

L.S.D 0.05	Fact A Cassava preparation = 0.028 Fact B (Treatment) = 0.15 Fact C (Treatment Rates) = 0.14 Fact A x B = 0.212 Fact A x C = 0.210 Fact B x C = 0.242 Fact A x B x C = 0.400
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the cyanide contents of mashed, peeled and unpeeled cassava preparations are presented in Table 4.18

Mean concentrations averaged over rates and types of treatments were 15.075%, 16.348% and 18.017% for mashed, peeled and unpeeled cassava preparations respectively. This showed an increasing order of mashed, peeled, unpeeled cassava preparations.

Addition of mashed and ginger gave 18.8% while that with garlic and garlic plus ginger were 15.4% and 11.025% respectively. Also, combinations of peeled cassava preparations with ginger gave 16.925% while that with garlic plus ginger and garlic alone were 16.56% and 15.55% respectively. Furthermore, concentrations of unpeeled cassava preparations with ginger gave 19.25% while that with garlic and garlic plus ginger gave 18.675% and 16.125% respectively. This showed that combinations of unpeeled cassava preparations with ginger gave the highest cyanide concentration while the least was with mashed cassava preparations with garlic alone.

Addition of various cassava preparations and rates of different treatments showed that cyanide content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 19.3%, 18.77%, 10.17% and 12.067% respectively indicating that addition of 0(zero/control) rates of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates exception being the 0.1kg rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the cyanide contents were 28.2%, 18.8%, 11.4% and 6.983% respectively indicating that addition of 0(zero/ control) rates of treatments to peeled cassava was significantly higher (LSD 0.05) than all the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that alkaloid contents were 17.4%, 25.933%, 17.1%

and 11.633% respectively indicating that addition of 0.1kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than all the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the cyanide content was significantly higher (LSD 0.05) in 0.1kg ginger of unpeeled cassava (27.2%) preparations than in most others.

Table 4.18: Effect of Ginger and Garlic on Cyanide content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	19.3	13	9.9	19.4	15.4
	Ga	19.3	14.2	6	4.6	11.025
	Gi	19.3	29.1	14.6	12.2	18.8
	Mean	**19.3**	**18.77**	**10.17**	**12.067**	**15.075**
Peeled	Gaig	28.2	17.3	9.3	7.4	15.55
	Ga	28.2	16.2	13	8.85	16.56
	Gi	28.2	22.9	11.9	4.7	16.925
	Mean	**28.2**	**18.8**	**11.4**	**6.983**	**16.348**
Unpeeeled	Gaig	17.4	27.2	12.9	7	16.125
	Ga	17.4	27	16.3	14	18.675
	Gi	17.4	23.6	22.1	13.9	19.25
	Mean	**17.4**	**25.933**	**17.1**	**11.633**	**18.017**

L.S.D 0.05	Fact A Cassava preparation = 0.82 Fact B (Treatment) = 0.5 Fact C (Treatment Rates) = 0.35 Fact A x B = 0.80 Fact A x C = 0.69 Fact B x C = 0.67 Fact A x B x C = 1.15
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the potassium content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.19

Mean concentrations averaged over rates and types of treatments were 0.086%, 0.125% and 0.143% for peeled, unpeeled and mashed cassava preparations respectively. This showed an increasing order of peeled, unpeeled, mashed cassava preparations.

Addition of mashed and ginger gave 0.17% while that with garlic and garlic plus ginger were 0.134% and 0.126% respectively. Also, combinations of peeled cassava preparations with garlic plus ginger gave 0.089% while that with garlic and ginger alone were 0.0869% and 0.0835% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic plus ginger gave 0.149% while that with garlic and ginger alone gave 0.1195% and 0.1072% respectively. This showed that combinations of mashed cassava preparations with ginger gave the highest potassium concentration while the least was with peeled cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that potassium content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.109%, 0.154%, 0.139% and 0.172% respectively indicating that addition of 0.3kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the potassium contents were 0.064%, 0.105%, 0.077% and 0.099% respectively indicating that addition of 0.1kg of treatments to peeled cassava was significantly higher (LSD 0.05) exception being the 0.3kg rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the potassium contents were 0.079%, 0.131%, 0.139% and 0.151% respectively indicating that

the addition of 0.3kg of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than all the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the potassium content was significantly higher (LSD 0.05) in 0.3kg ginger of mashed cassava (0.199%) preparations than in most others.

Table 4.19: Effect of Ginger and Garlic on Potassium content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	0.109	0.133	0.114	0.149	0.126
	Ga	0.109	0.134	0.125	0.169	0.134
	Gi	0.109	0.195	0.177	0.199	0.17
	Mean	**0.109**	**0.154**	**0.139**	**0.172**	**0.143**
Peeled	Gaig	0.064	0.098	0.086	0.108	0.089
	Ga	0.064	0.146	0.0667	0.071	0.0869
	Gi	0.064	0.072	0.079	0.119	0.0835
	Mean	**0.064**	**0.105**	**0.077**	**0.099**	**0.086**
Unpeeled	Gaig	0.079	0.152	0.17	0.195	0.149
	Ga	0.079	0.099	0.144	0.156	0.1195
	Gi	0.079	0.143	0.104	0.1027	0.1072
	Mean	**0.079**	**0.131**	**0.139**	**0.151**	**0.125**

L.S.D 0.05	Fact A Cassava preparation = 0.0027 Fact B (Treatment) = 0.0051 Fact C (Treatment Rates) = 0.0049 Fact A x B = 0.0072 Fact A x C = 0.0074 Fact B x C = 0.0084 Fact A x B x C = 0.014
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the moisture content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.20

Mean concentrations averaged over rates and types of treatments were 16.723%, 16.987% and 19.702% for mashed, peeled and unpeeled cassava preparations respectively. This showed an increasing order of mashed, peeled, unpeeled cassava preparations.

Addition of mashed and garlic plus ginger gave 17.37% while that with ginger and garlic were 16.89% and 15.91%. Also, combinations of peeled cassava preparations with garlic gave 19.565% while that with ginger and garlic plus ginger were 16.323% and 15.072% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 20.311% while that with ginger alone and garlic plus ginger gave 20.045% and 18.75% respectively. This showed that combinations of unpeeled cassava preparations with garlic gave the highest moisture concentration while the least was with peeled cassava preparations with garlic plus ginger.

Addition of various cassava preparations and rates of different treatments showed that the moisture content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 22.722%, 13.381%, 14.661% and 16.129% respectively indicating that addition of 0(zero) rates of treatments to mashed cassava was significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the moisture contents were 15.542%, 19.161%, 16.228% and 17.015% respectively indicating that addition of 0.1kg of treatments of peeled cassava was significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the moisture contents were 14.651%, 24.90%, 21.359% and 17.902% respectively indicating that

the addition of 0.1kg rates of treatments to unpeeled cassava preparations only significantly higher (LSD 0.05) than all the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the moisture content was significantly higher (LSD 0.05) in 0.1kg ginger of unpeeled cassava preparations (31.186%) than in most others.

Table 4.20: Effect of Ginger and Garlic on Moisture content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	22.722	13.232	14.236	19.295	17.37
	Ga	22.722	13.63	13.659	13.642	15.91
	Gi	22.722	13.281	16.089	15.449	16.89
	Mean	**22.722**	**13.381**	**14.661**	**16.129**	**16.723**
Peeled	Gaig	15.542	15.152	15.062	14.532	15.072
	Ga	15.542	27.331	14.655	20.73	19.565
	Gi	15.542	15	18.966	15.782	16.323
	Mean	**15.542**	**19.161**	**16.228**	**17.015**	**16.987**
Unpeeled	Gaig	14.651	21.16	20.123	19.077	18.75
	Ga	14.651	22.355	24.805	19.433	20.311
	Gi	14.651	31.186	19.149	15.195	20.045
	Mean	**14.651**	**24.9**	**21.359**	**17.902**	**19.702**

L.S.D 0.05	Fact A Cassava preparation = 0.74 Fact B (Treatment) = 0.38 Fact C (Treatment Rates) = 0.25 Fact A x B = 0.65 Fact A x C = 0.57 Fact B x C = 0.50 Fact A x B x C = 0.87
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the magnesium content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.21

Mean concentrations averaged over rates and types of treatments were 0.1598%, 0.1639% and 0.2099% for peeled, mashed and unpeeled cassava preparations respectively. This showed an increasing order of peeled, mashed, unpeeled cassava preparations.

Addition of mashed and ginger gave 0.253% while that with garlic and garlic plus ginger were 0.1553% and 0.0833%. Also, combinations of peeled cassava preparations with ginger gave 0.2088% while that with garlic and garlic plus ginger were 0.1703% and 0.1003% respectively. Furthermore, concentrations of unpeeled cassava preparations with ginger gave 0.2193% while that with garlic and garlic and ginger gave 0.2075% and 0.203% respectively. This showed that combinations of mashed cassava preparations with ginger gave the highest magnesium concentration while the least was with mashed cassava preparations with garlic plus ginger.

Addition of various cassava preparations and rates of different treatments showed that the magnesium contents of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.138%, 0.1177%, 0.222% and 0.1777% respectively indicating that addition of 0.2kg of treatments to mashed cassava was significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the magnesium contents were 0.153%, 0.207%, 0.133% and 0.145% respectively indicating that addition of 0.1kg rates of treatments to peeled cassava was significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that magnesium contents were 0.11%, 0.231%, 0.258% and 0.241% respectively indicating that

addition of 0.2kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the magnesium content was significantly higher (LSD 0.05) in 0.2kg ginger of mashed cassava preparations (0.351%) than in most others.

Table 4.21: Effect of Ginger and Garlic on Magnesium content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	0.138	0.065	0.115	0.015	0.0833
	Ga	0.138	0.103	0.2	0.18	0.1553
	Gi	0.138	0.185	0.351	0.338	0.253
	Mean	**0.138**	**0.1177**	**0.222**	**0.1777**	**0.1639**
Peeled	Gaig	0.153	0.11	0.05	0.088	0.1003
	Ga	0.153	0.285	0.14	0.103	0.1703
	Gi	0.153	0.227	0.21	0.245	0.2088
	Mean	**0.153**	**0.207**	**0.133**	**0.145**	**0.1598**
Unpeeled	Gaig	0.11	0.204	0.309	0.207	0.2075
	Ga	0.11	0.243	0.239	0.22	0.203
	Gi	0.11	0.245	0.227	0.295	0.2193
	Mean	**0.11**	**0.231**	**0.258**	**0.241**	**0.2099**

L.S.D 0.05	Fact A (Cassava preparation) = 0.011 Fact B (Treatment) = 0.005 Fact C (Treatment Rates) = 0.005 Fact A x B = 0.009 Fact A x C = 0.010 Fact B x C = 0.009 Fact A x B x C = 0.016
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the sodium content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.22

Mean concentrations averaged over rates and types of treatments were 0.050%, 0.0517% and 0.0623% for peeled, mashed and unpeeled cassava preparations respectively. This showed an increasing order of peeled, mashed, unpeeled cassava preparations.

Addition of mashed and ginger gave 0.0585% while that with garlic plus ginger and garlic were 0.0498% and 0.0468% respectively. Also, combinations of peeled cassava preparations with ginger gave 0.056% while that with garlic and garlic plus ginger were 0.052% and 0.042% respectively. Furthermore, concentrations of unpeeled cassava preparations with ginger gave 0.075% while that with garlic plus ginger and garlic alone gave 0.0615% and 0.0503% respectively. This showed that combinations of unpeeled cassava preparations with ginger gave the highest sodium concentration while the least was with peeled cassava preparations with garlic plus ginger.

Addition of various cassava preparations and rates of different treatments showed that sodium contents of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.021%, 0.055%, 0.0653% and 0.0653% respectively indicating that the addition of 0.2kg and 0.3kg rates of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that sodium contents were 0.034%, 0.052%, 0.047% and 0.066% respectively indicating that the addition of 0.3kg rates of treatments to peeled cassava was significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that sodium contents were 0.026%, 0.0683%, 0.0807% and 0.074% respectively indicating that

the addition of 0.2kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than the other rates

Addition of various cassava preparations and treatments in their various rates showed that the sodium content was significantly higher (LSD 0.05) in 0.2kg ginger of unpeeled cassava preparations (0.118%) than in most others.

Table 4.22: Effect of Ginger and Garlic on Sodium content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	0.021	0.061	0.047	0.07	0.0498
	Ga	0.021	0.031	0.067	0.068	0.0468
	Gi	0.021	0.073	0.082	0.058	0.0585
	Mean	**0.021**	**0.055**	**0.0653**	**0.0653**	**0.0517**
Peeled	Gaig	0.034	0.027	0.035	0.073	0.042
	Ga	0.034	0.084	0.046	0.043	0.052
	Gi	0.034	0.044	0.061	0.083	0.056
	Mean	**0.034**	**0.052**	**0.047**	**0.066**	**0.05**
Unpeeled	Gaig	0.026	0.07	0.077	0.073	0.0615
	Ga	0.026	0.041	0.047	0.087	0.0503
	Gi	0.026	0.094	0.118	0.062	0.075
	Mean	**0.026**	**0.0683**	**0.0807**	**0.074**	**0.0623**

L.S.D 0.05	Fact A (Cassava preparation) = 0.0046 Fact B (Treatment) = 0.0022 Fact C (Treatment Rates) = 0.0029 Fact A x B = 0.0039 Fact A x C = 0.0045 Fact B x C = 0.0042 Fact A x B x C = 0.0074
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the phosphorus content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.23

Mean concentrations averaged over rates and types of treatments were 40.548%, 44.678% and 56.994% for unpeeled, peeled and mashed cassava preparations respectively. This showed an increasing order of unpeeled, peeled, mashed cassava preparations.

Addition of mashed and garlic gave 61.89% while that with ginger and garlic plus ginger were 55.738% and 53.355%. Also, combinations of peeled cassava preparations with garlic gave 53.613% while that with ginger and garlic plus ginger were 41.843% and 38.578% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 55.49% while that with ginger and garlic and ginger gave 33.3% and 32.85% respectively. This showed that combinations of mashed cassava preparations with garlic gave the highest phosphorus concentration while the least was with unpeeled cassava preparations with garlic plus ginger.

Addition of various cassava preparations and rates of different treatments showed that phosphorus content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 45.91%, 56.337%, 59.373% and 66.357% respectively indicating that addition of 0.3kg of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the phosphorus contents were 36.62%, 49.25%, 39.897% and 52.95% respectively indicating that addition of 0.3kg of treatments to peeled cassava were significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the phosphorus contents were 30.39%, 31.99%, 53.763% and 46.047% respectively

indicating that addition of 0.2kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than the others ie the 0(zero/ control), 0.1kg and 0.3kg rates

Addition of various cassava preparations and treatments in their various rates showed that the phosphorus content was significantly higher (LSD 0.05) in 0.2kg garlic of unpeeled cassava preparations (80.92%) than in most others.

Table 4.23: Effect of Ginger and Garlic on Phosphorus content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	45.91	50.26	52.74	64.51	53.355
	Ga	45.91	56.96	72.02	72.67	61.89
	Gi	45.91	61.79	53.36	61.89	55.738
	Mean	**45.91**	**56.337**	**59.373**	**66.357**	**56.994**
Peeled	Gaig	36.62	42.02	30.33	45.34	38.578
	Ga	36.62	78.83	52.02	46.98	53.613
	Gi	36.62	26.89	37.34	66.52	41.843
	Mean	**36.62**	**49.25**	**39.897**	**52.95**	**44.678**
Unpeeled	Gaig	30.39	25.61	40.74	34.66	32.85
	Ga	30.39	39.26	80.92	71.39	55.49
	Gi	30.39	31.1	39.63	32.09	33.3
	Mean	**30.39**	**31.99**	**53.763**	**46.047**	**40.548**

L.S.D 0.05	Fact A (Cassava preparation) =2.08 Fact B (Treatment) = 1.27 Fact C (Treatment Rates) = 1.26 Fact A x B = 2.03 Fact A x C = 2.16 Fact B x C = 2.16 Fact A x B x C = 3.71
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the protein content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.24

Mean concentrations averaged over rates and types of treatments were 4.341%, 4.660% and 4.955% for unpeeled, peeled and mashed cassava preparations respectively. This showed an increasing order of unpeeled, peeled, mashed cassava preparations.

Addition of mashed and garlic gave 5.286% while that with ginger and garlic plus ginger were 4.864% and 4.714%. Also, combinations of peeled cassava preparations with ginger gave 5.06% while that with garlic and garlic plus ginger were 4.749% and 4.172% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 5.149% while that with ginger and garlic and ginger gave 4.188% and 3.685% respectively. This showed that combinations of mashed cassava preparations with garlic gave the highest protein concentration while the least was with unpeeled cassava preparations with garlic plus ginger.

Addition of various cassava preparations and rates of different treatments showed that protein content of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 4.917%, 3.933%, 4.939% and 6.03% respectively indicating that addition of 0.3kg rates of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that protein contents were 5.966%, 4.033%, 4.669% and 3.974% respectively indicating that the addition of 0(zero/ control) rates of treatments to peeled cassava were significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that protein contents were 3.945%, 3.611%, 4.851% and 4.955% respectively indicating that

addition of 0.3kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than the others.

Addition of various cassava preparations and treatments in their various rates showed that the protein content was significantly higher (LSD 0.05) in 0.2kg garlic of unpeeled cassava preparations (7.487%) than in most others.

Table 4.24: Effect of Ginger and Garlic on Protein content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	4.917	3.539	4.308	6.091	4.714
	Ga	4.917	4.176	6.592	5.459	5.286
	Gi	4.917	4.083	3.917	6.54	4.864
	Mean	**4.917**	**3.933**	**4.939**	**6.03**	**4.955**
Peeled	Gaig	5.966	3.084	4.103	3.535	4.172
	Ga	5.966	4.422	4.64	3.97	4.749
	Gi	5.966	4.594	5.264	4.417	5.06
	Mean	**5.966**	**4.033**	**4.669**	**3.974**	**4.66**
Unpeeled	Gaig	3.945	2.998	3.387	4.409	3.685
	Ga	3.945	3.636	7.487	5.531	5.149
	Gi	3.945	4.2	3.68	4.925	4.188
	Mean	**3.945**	**3.611**	**4.851**	**4.955**	**4.341**

L.S.D 0.05	Fact A (Cassava preparation) = 0.056 Fact B (Treatment) = 0.026 Fact C (Treatment Rates) = 0.047 Fact A x B = 0.047 Fact A x C = 0.050 Fact B x C = 0.046 Fact A x B x C = 0.081
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Rates of ginger, garlic and garlic plus ginger on the tannin content of mashed, peeled and unpeeled cassava preparations are presented in Table 4.25

Mean concentrations averaged over rates and types of treatments were 0.108%, 0.128% and 0.244% for unpeeled, mashed and peeled cassava preparations respectively. This showed an increasing order of unpeeled, mashed and peeled cassava preparations.

Addition of mashed and garlic gave 0.161% while that with garlic plus ginger and ginger alone were 0.116% and 0.106% respectively. Also, combinations of peeled cassava preparations with ginger gave 0.439% while that with garlic plus ginger and garlic alone were 0.148% and 0.143% respectively. Furthermore, concentrations of unpeeled cassava preparations with garlic gave 0.154% while that with garlic and ginger and ginger alone gave 0.089% and 0.08% respectively. This showed that combinations of peeled cassava preparations with ginger gave the highest tannin concentration while the least was with unpeeled cassava preparations with ginger alone.

Addition of various cassava preparations and rates of different treatments showed that the tannin contents of combinations of mashed cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates were 0.121%, 0.086%, 0.193% and 0.11% respectively indicating that the addition of 0.2kg of treatments to mashed cassava were significantly higher (LSD 0.05) than all the other rates. Combinations of peeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the tannin contents were 0.129%, 0.124%, 0.381% and 0.341% respectively indicating that the addition of 0.2kg of treatments to peeled cassava were significantly higher (LSD 0.05) than the other rates. Combinations of unpeeled cassava preparations at 0(zero), 0.1kg, 0.2kg and 0.3kg treatment rates showed that the tannin contents were 0.114%, 0.078%, 0.093% and 0.145% respectively indicating that the addition

of 0.3kg rates of treatments to unpeeled cassava preparations were significantly higher (LSD 0.05) than the other rates.

Addition of various cassava preparations and treatments in their various rates showed that the tannin content was significantly higher (LSD 0.05) in 0.3kg ginger of peeled cassava preparations (0.729%) than in most others.

Table 4.25: Effect of Ginger and Garlic on Tannin content of Cassava Preparations

Cassava preparations	Treatments	0kg	0.1kg	0.2kg	0.3kg	Mean %
Mashed	Gaig	0.121	0.064	0.179	0.1	0.116
	Ga	0.121	0.114	0.321	0.086	0.161
	Gi	0.121	0.079	0.079	0.143	0.106
	Mean	**0.121**	**0.086**	**0.193**	**0.11**	**0.128**
Peeled	Gaig	0.129	0.121	0.136	0.207	0.148
	Ga	0.129	0.064	0.293	0.086	0.143
	Gi	0.129	0.186	0.714	0.729	0.439
	Mean	**0.129**	**0.124**	**0.381**	**0.341**	**0.244**
Unpeeled	Gaig	0.114	0.064	0.064	0.114	0.089
	Ga	0.114	0.107	0.15	0.243	0.154
	Gi	0.114	0.064	0.064	0.079	0.08
	Mean	**0.114**	**0.078**	**0.093**	**0.145**	**0.108**

L.S.D 0.05	Fact A (Cassava preparation) = 0.00076 Fact B(Treatment) = 0.00333 Fact C(Treatment Rates) = 0.00252 Fact A x B = 0.00471 Fact A x C = 0.00379 Fact B x C = 0.00469 Fact A x B x C = 0.00759
Gaig = Ginger and Garlic, Ga = Garlic and Gi = Ginger.	

Influence of Additives on the Sensory Properties of Cassava Preparations

Table 4.26 shows the effects of rates of garlic, ginger and garlic plus ginger on the aroma properties of mashed, peeled and unpeeled cassava preparations.

Addition of additives (garlic, ginger and garlic plus ginger) significantly affected the aroma of mashed, peeled and unpeeled cassava preparations.

The degree of variation for mashed cassava with garlic indicated that 1.54% of the respondents accepted that influence of additives (garlic) on aroma was good at 0.3kg rates of garlic, 1.24% at 0.2kg rates of garlic and 0.31% for 0.1 rates of garlic. Those for fair indicated that the influence was best at 0.2kg rates of garlic (2.45%) while the poorest influence was at 0.2kg rates of garlic.

The degree of variation for mashed cassava with ginger indicated that 2.45% of the respondents accepted that influence of additives (ginger) on aroma was good at 0.1kg rates of ginger, and at 0.2kg and 0.3kg rates of ginger, 0% respondents. Those for fair indicated that the influence was best at 0.2kg rates of ginger (2.45%) while the poorest influence was at 0.1kg and 0.2kg rates of ginger.

The degree of variation for mashed cassava with garlic plus ginger indicated that 2.45% of the respondents accepted that influence of additives (garlic plus ginger) on aroma was good at 0.1kg rates of garlic plus ginger, 0.93% at 0.2kg rates of garlic and 1.54% for 0.1kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.3kg rates of garlic plus ginger (2.45%) while the poorest influence was at 0.3kg rates of garlic plus ginger.

The degree of variation for peeled cassava with garlic indicated that 1.24% of the respondents accepted that influence of additives (garlic) on aroma was good at 0.3kg and 0.1kg rates of garlic, 0% at 0.2kg rates of garlic. Those for fair indicated that the influence of the garlic

grates were the same at the three rates; 0.1kg, 0.2kg and 0.3kg rates of garlic (2.45%) while the poorest were at 0.1kg and 0.3kg.

The degree of variation for peeled cassava with ginger indicated that 2.45% of the respondents accepted that influence of additives (ginger) on aroma was good at 0.1kg rates of ginger, and at 0.2kg and 0.3kg rates of ginger, 0% respondents. Those for fair indicated that the influence was best at 0.2kg rates of ginger (3.09%) while the poorest influence was at 0.2kg rates of ginger.

The degree of variation for peeled cassava with garlic plus ginger indicated that 1.85% of the respondents accepted that influence of additives (garlic plus ginger) on aroma was good at 0.1kg rates of garlic plus ginger, 1.24% at 0.2kg and 0.3kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.1kg rates of garlic plus ginger (1.85%) while the poorest influence was at 0.1kg rates of garlic plus ginger.

The degree of variation for unpeeled cassava with garlic indicated that 1.24% of the respondents accepted that influence of additives (garlic) on aroma was good at all the three (3) rates. Those for fair indicated that the influence of the garlic grates were the same at the three rates; 0.1kg, 0.2kg and 0.3kg rates of garlic (1.24%) while the poorest were at 0.1kg, 0.2kg and 0.3kg.

The degree of variation for unpeeled cassava with ginger indicated that 2.45% of the respondents accepted that influence of additives (ginger) on aroma was good at 0.1kg rates of ginger, 1.24% respondents at 0.2kg and 0% respondent at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.3kg rates of ginger (2.16%) while the poorest influence was at 0.1kg rates of ginger.

The degree of variation for unpeeled cassava with garlic plus ginger indicated that 2.45% of the respondents accepted that influence of additives (garlic plus ginger) on aroma was good at

0.1kg rates of garlic plus ginger, 1.24% at 0.2kg and 0% at 0.3kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (2.45%) while the poorest influence was at 0.2kg rates of garlic plus ginger.

Table 4.26 Effects of Rates of Additives on Aroma of Cassava Preparations

Samples	Rating of Aroma for Different Concentrations of Additives								
	$G_{0.1}$	$G_{0.2}$	$G_{0.3}$	$F_{0.1}$	$F_{0.2}$	$F_{0.3}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$
MA	1(6.11)0.31%	4(2.56)1.24%	5(2.44)1.54%	4(3.11)1.24%	8(6.0)2.45%	2(5.22)0.62%	8(2.89)2.45%	0(3.11)0%	4(4.56)1.24%
MB	8(6.11)2.45%	0(2.56)0%	0(2.44)0%	0(3.11)0%	8(6.0)2.45%	4(5.22)1.24%	4(2.89)1.24%	4(3.11)1.24%	8(4.56)2.45%
MC	8(6.11)2.45%	3(2.56)0.93%	5(2.44)1.54%	0(3.11)0%	4(6.0)1.24%	8(5.22)2.45%	4(2.89)1.24%	4(3.11)1.24%	0(4.56)0%
PA	4(6.11)1.24%	0(2.56)0%	4(2.44)1.24%	8(3.11)2.45%	8(6.0)2.45%	8(5.22)2.45%	0(2.89)0%	4(3.11)1.24%	0(4.56)0%
PB	8(6.11)2.45%	0(2.56)0%	0(2.44)0%	0(3.11)0%	10(6.0)3.09%	6(5.22)1.85%	4(2.89)1.24%	1(3.11)0.31%	7(4.56)2.16%
PC	6(6.11)1.85%	4(2.56)1.24%	4(2.44)1.24%	6(3.11)1.85%	0(6.0)0%	4(5.22)1.24%	0(2.89)0%	7(3.11)2.16%	5(4.56)1.54%
UPA	4(6.11)1.24%	4(2.56)1.24%	4(2.44)1.24%	4(3.11)1.24%	4(6.0)1.24%	4(5.22)1.24%	4(2.89)1.24%	4(3.11)1.24%	4(4.56)1.24%
UPB	8(6.11)2.45%	4(2.56)1.24%	0(2.44)0%	4(3.11)1.24%	4(6.0)1.24%	7(5.22)2.16%	0(2.89)0%	4(3.11)1.24%	5(4.56)1.54%
UPC	8(6.11)2.45%	4(2.56)1.24%	0(2.44)0%	2(3.11)0.62%	8(6.0)2.45%	4(5.22)1.24%	2(2.89)0.62%	0(3.11)0%	8(4.56)2.45%
Values in Parentesis ( ) = Expected; M= Mashed; P= Peeled; UP= Unpeeled; A= Garlic; B= Ginger; C= Ginger + Garlic.									

G = Good at 0.1, 0.2 and 0.3kg concentration of additives
F = Fair at 0.1, 0.2 and 0.3kg concentration of additives
P = Poor at 0.1, 0.2 and 0.3kg concentration of additives

Table 4.27 shows the effects of rates of garlic, ginger and garlic plus ginger on the colour properties of mashed, peeled and unpeeled cassava preparations.

Addition of additives (garlic, ginger and garlic plus ginger) significantly affected the colour of mashed, peeled and unpeeled cassava preparations.

The degree of variation for mashed cassava with garlic indicated that 2.47% of the respondents accepted that influence of additives (garlic) on colour was good at 0.1kg and 0.3kg rates of garlic, 0% at 0.2kg rates of garlic. Those for fair indicated that the influence was best at 0.2kg rates of garlic (2.47%) while the poorest influence was at 0.1kg rates of garlic.

The degree of variation for mashed cassava with ginger indicated that 1.54% of the respondents accepted that influence of additives (ginger) on colour was good at 0.1kg rates of ginger, 0.31% at 0.2kg and 0.93% at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg rates of ginger (3.40%) while the poorest influence was at 0.2kg rates of ginger.

The degree of variation for mashed cassava with garlic plus ginger indicated that 3.70% of the respondents accepted that influence of additives (garlic plus ginger) on colour was good at 0.1kg rates of garlic plus ginger, 0.62% at 0.2kg rates of garlic and 0% for 0.3kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (1.85%) while the poorest influence was at 0.1kg rates of garlic plus ginger.

The degree of variation for peeled cassava with garlic indicated that 3.40% of the respondents accepted that influence of additives (garlic) on colour was good at 0.3kg rates of garlic, 1.23% at 0.2kg rates of garlic and 1.54% at 0.1kg rates of garlic. Those for fair indicated that

the influence of the garlic grates was best at 0.1kg rates of garlic (2.47%) while the poorest were at 0.1kg and 0.3kg.

The degree of variation for peeled cassava with ginger indicated that 2.47% of the respondents accepted that influence of additives (ginger) on colour was good at 0.1kg rates of ginger, 0.93% at 0.2kg and 0.31% at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg and 0.3kg rates of ginger (1.54%) while the poorest influence was at 0.1kg rates of ginger.

The degree of variation for peeled cassava with garlic plus ginger indicated that 2.47% of the respondents accepted that influence of additives (garlic plus ginger) on colour was good at 0.1kg rates of garlic plus ginger, 0% at 0.2kg and 0.3kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (2.16%) while the poorest influence was at 0.1kg rates of garlic plus ginger.

The degree of variation for unpeeled cassava with garlic indicated that 2.47% of the respondents accepted that influence of additives (garlic) on colour was good at 0.1kg rates of garlic, 1.23% at 0.2kg and 0% at 0.3kg rates of garlic. Those for fair indicated that the influence of the garlic grates was best at 0.2kg and 0.3kg rates of additive (garlic) while the poorest was at 0.2kg.

The degree of variation for unpeeled cassava with ginger indicated that 2.47% of the respondents accepted that influence of additives (ginger) on colour was good at 0.1kg rates of ginger, 1.23% respondents at 0.2kg and 0% respondent at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg and 0.3kg rates of ginger (2.47%) while the poorest influence was at 0.2kg rates of ginger.

The degree of variation for unpeeled cassava with garlic plus ginger indicated that 3.70% of the respondents accepted that influence of additives (garlic plus ginger) on colour was good at 0.1kg rates of garlic plus ginger, 0% at 0.2kg and 0.3kg rates of garlic plus ginger respectively. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (2.47%) while the poorest influence was at 0.1kg rates of garlic plus ginger.

Table 4.27: Effects of Rates of Additives on Colour of Cassava Preparations

Samples	Rating of Colour for different concentrations of additives								
	$G_{0.1}$	$G_{0.2}$	$G_{0.3}$	$F_{0.1}$	$F_{0.2}$	$F_{0.3}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$
MA	8(8.22)2.47%	0(2.0)0%	8(2.56)2.47%	4(2.89)1.23%	8(7.22)2.47%	0(3.89)0%	0(0.89)0%	4(2.67)1.23%	4(5.67)1.23%
MB	5(8.22)1.54%	1(2.0)0.31%	3(2.56)0.93%	4(2.89)1.23%	11(7.22)3.40%	6(3.89)1.85%	2(0.89)0.62%	0(2.67)0%	4(5.67)1.23%
MC	12(8.22)3.70%	2(2.0)0.62%	0(2.56)0%	0(2.89)0%	6(7.22)1.85%	4(3.89)1.23%	0(0.89)0%	4(2.67)1.23%	8(5.67)2.47%
PA	5(8.22)1.54%	4(2.0)1.23%	11(2.56)3.40%	8(2.89)2.47%	4(7.22)1.23%	0(3.89)0%	0(0.89)0%	4(2.67)1.23%	0(5.67)0%
PB	8(8.22)2.47%	3(2.0)0.93%	1(2.56)0.31%	4(2.89)1.23%	5(7.22)1.54%	5(3.89)1.54%	0(0.89)0%	3(2.67)0.93%	7(5.67)2.16%
PC	8(8.22)2.47%	0(2.0)0%	0(2.56)0%	4(2.89)1.23%	7(7.22)2.16	4(3.89)1.23%	0(0.89)0%	5(2.67)1.54%	8(5.67)2.47%
UPA	8(8.22)2.47%	4(2.0)1.23%	0(2.56)0%	2(2.89)0.62%	8(7.22)2.47%	8(3.89)2.47%	2(0.89)0.62%	0(2.67)0%	4(5.67)1.23%
UPB	8(8.22)2.47%	4(2.0)1.23%	0(2.56)0%	0(2.89)0%	8(7.22)2.47%	8(3.89)2.47%	4(0.89)1.23%	0(2.67)0%	4(5.67)1.23%
UPC	12(8.22)3.70%	0(2.0)0%	0(2.56)0%	0(2.89)0%	8(7.22)2.47%	0(3.89)0%	0(0.89)0%	4(2.67)1.23%	12(5.67)3.70%
Value in Parenthesis = Expected values; M= Mashed; P= Peeled; UP= Unpeeled; A= Garlic; B= Ginger; C= Ginger + Garlic.									

G = Good at 0.1, 0.2 and 0.3kg concentration of additives
F = Fair at 0.1, 0.2 and 0.3kg concentration of additives
P = Poor at 0.1, 0.2 and 0.3kg concentration of additives

Table 4.28 shows the effects of rates of garlic, ginger and garlic plus ginger on the texture properties of mashed, peeled and unpeeled cassava preparations.

Addition of additives (garlic, ginger and garlic plus ginger) significantly affected the texture of mashed, peeled and unpeeled cassava preparations.

The degree of variation for mashed cassava with garlic indicated that 3.70% of the respondents accepted that influence of additives (garlic) on texture was good at 0.2kg rates of garlic, 0.93% at 0.1kg and 1.23% at 0.3kg rates of garlic. Those for fair indicated that the influence was best at 0.1kg rates of garlic (2.78%) while the poorest influence was at 0.1kg and 0.2kg rates of garlic.

The degree of variation for mashed cassava with ginger indicated that 2.16% of the respondents accepted that influence of additives (ginger) on colour was good at 0.1kg rates of ginger, 0% at 0.2kg and 0.31% at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg rates of ginger (2.47%) while the poorest influence was at 0.1kg rates of ginger.

The degree of variation for mashed cassava with garlic plus ginger indicated that 1.85% of the respondents accepted that influence of additives (garlic plus ginger) on texture was good at 0.1kg rates of garlic plus ginger, 0.62% at 0.2kg rates of garlic and 0% for 0.3kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (3.09%) while the poorest influence was at 0.1kg and 0.2kg rates of garlic plus ginger.

The degree of variation for peeled cassava with garlic indicated that 2.47% of the respondents accepted that influence of additives (garlic) on texture was good at 0.1kg rates of garlic, 1.85% at 0.2kg rates of garlic and 0% at 0.3kg rates of garlic. Those for fair indicated that the

influence of the garlic grates was best at 0.3kg rates of garlic (2.16%) while the poorest was at 0.1kg.

The degree of variation for peeled cassava with ginger indicated that 3.09% of the respondents accepted that influence of additives (ginger) on texture was good at 0.1kg rates of ginger, 1.23% at 0.2kg and 0.62% at 0.3kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg rates of ginger (0.93%) while the poorest influence was at 0.1kg rates of ginger.

The degree of variation for peeled cassava with garlic plus ginger indicated that 1.85% of the respondents accepted that influence of additives (garlic plus ginger) on texture was good at 0.1kg and 0.3kg rates of garlic plus ginger, 0% at 0.2kg rates of garlic plus ginger. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (3.70%) while the poorest influence was at 0.2kg rates of garlic plus ginger.

The degree of variation for unpeeled cassava with garlic indicated that 1.54% of the respondents accepted that influence of additives (garlic) on texture was good at 0.1kg and 0.3kg rates of garlic, 1.23% at 0.2kg rates of garlic. Those for fair indicated that the influence of the garlic grates was best at 0.2kg rates of additive (garlic) while the poorest was at 0.2kg. The degree of variation for unpeeled cassava with ginger indicated that 2.16% of the respondents accepted that influence of additives (ginger) on texture was good at 0.3kg rates of ginger, 1.23% respondents at 0.2kg and 0.31% respondent at 0.1kg rates of ginger. Those for fair indicated that the influence was best at 0.2kg rates of ginger (2.47%) while the poorest influence was at 0.2kg and 0.3kg rates of ginger.

The degree of variation for unpeeled cassava with garlic plus ginger indicated that 2.47% of the respondents accepted that influence of additives (garlic plus ginger) on texture was good

at 0.3kg rates of garlic plus ginger, 1.23% at 0.2kg and 0.31% at 0.1kg rates of garlic plus ginger respectively. Those for fair indicated that the influence was best at 0.2kg rates of garlic plus ginger (2.47%) while the poorest influence was at 0.2kg rates of garlic plus ginger.

Table 4.28: Effects of Rates of Additives on Texture of Cassava Preparations

Samples	Rating of Texture for different concentrations of additives								
	$G_{0.1}$	$G_{0.2}$	$G_{0.3}$	$F_{0.1}$	$F_{0.2}$	$F_{0.3}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$
MA	3(5.56)0.93%	12(4.0)3.70%	4(3.67)1.23%	9(4.22)2.78%	0(6.33)0%	3(2.33)0.93%	0(2.89)0%	0(1.33)0%	5(5.67)1.54%
MB	7(5.56)2.16%	0(4.0)0%	1(3.67)0.31%	7(4.22)2.16%	8(6.33)2.47%	0(2.33)0%	0(2.89)0%	1(1.33)0.31%	12(5.67)3.70%
MC	6(5.56)1.85%	2(4.0)0.62%	0(3.67)0%	6(4.22)1.85%	10(6.33)3.09%	0(2.33)0%	0(2.89)0%	0(1.33)0%	12(5.67)3.70%
PA	8(5.56)2.47%	6(4.0)1.85%	0(3.67)0%	4(4.22)1.23%	0(6.33)0%	7(2.33)2.16%	0(2.89)0%	6(1.33)1.85%	5(5.67)3.70%
PB	10(5.56)3.09%	4(4.0)1.23%	2(3.67)0.62%	2(4.22)0.62%	3(6.33)0.93%	0(2.33)0%	1(2.89)0.31%	5(1.33)1.54%	9(5.67)2.78%
PC	6(5.56)1.85%	0(4.0)0%	6(3.67)1.85%	6(4.22)1.85%	12(6.33)3.70%	3(2.33)0.93%	1(2.89)0.31%	0(1.33)0%	2(5.67)0.62%
UPA	5(5.56)1.54%	4(4.0)1.23%	5(3.67)1.54%	0(4.22)0%	8(6.33)2.47%	4(2.33)1.23%	7(2.89)2.16%	0(1.33)0%	3(5.67)0.93%
UPB	1(5.56)0.31%	4(4.0)1.23%	7(3.67)2.16%	0(4.22)0%	8(6.33)2.47%	4(2.33)1.23%	12(2.89)3.70%	0(1.33)0%	0(5.67)0%
UPC	4(5.56)0.31%	4(4.0)1.23%	8(3.67)2.47%	4(4.22)0%	8(6.33)2.47%	0(2.33)0%	5(2.89)1.54%	0(1.33)0%	3(5.67)0.93%
Values in Parenthesis = Expected values; M= Mashed; P= Peeled; UP= Unpeeled; A= Garlic; B= Ginger; C= Ginger + Garlic.									

G = Good at 0.1, 0.2 and 0.3kg concentration of additives
F = Fair at 0.1, 0.2 and 0.3kg concentration of additives
P = Poor at 0.1, 0.2 and 0.3kg concentration of additives

4.1 DISCUSSION

The microbial strains isolated from fermented cassava prepared in three methods (using varying quantities of ginger, garlic and ginger plus garlic grates) and the control setup were: *Bacillus spp., Echerichia coli., Lactobacillus spp., Corynebacterium spp., Aspergillus spp., Geotrichum spp., Rhizopus spp., Trichoderma spp.,* and *Penicillum spp*. The results agreed with the findings by Abriba *et al.,* (2012). These isolates were predominant in the preparations with the lowest/least quantity of the grates (that is at 0.1kg rates of treatment). At 0.3kg rates of treatment (spices) *Bacillus spp* were dominant and this could be attributed to the formation of spores which tolerate and survive in any adverse environment for days (Brook *et al.,* 1998).

Isolates succession results showed that with the fermenting cassava alone, the following organisms; *Bacillus spp, Lactobacillus spp, E.coli, Rhizopus spp, Trichoderma spp., Geotrichum spp* and *Penicillum spp*., were predominant from day 1 - 10 of fermentation with the introduction of *Proteus spp* from day 6 - 10 of fermentation in the unpeeled cassava preparation only. The succession results for the fermenting cassava with ginger plus garlic grates and with garlic grates alone showed that: *Bacillus spp., Lactobacillus spp., E.coli, Aspergillus spp., Geotrichum spp., Rhizopus spp* and *Trichoderma spp* were predominant. Isolates succession result for fermenting cassava with ginger grates alone showed that *Bacillus spp* and *Rhizopus spp* were most predominant in the unpeeled, peeled and mashed fermenting cassava with ginger grates. *Proteus spp* were isolated on day 6 – 10 of fermenting cassava with ginger grates alongside other isolates.

The presence of diverse species of bacteria and fungi proved that diverse organisms are implicated in the fermentation of food (Chukwu *et al*., 2011). *Bacillus* species are frequently inhabitants of places such as dust, natural water, vegetation, sediments and many foods (Jay, 1986; Bergdol, 1987). The *Bacillus subtilis* species has a long history of safe use. It has been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA), EFSA (2010) and is part of the authoritative list of microorganisms with a documented history of safe use in food established by the International Dairy Federation (IDF) in collaboration with the European Food and Feed Cultures Association (EFFCA) in 2002 and updated in 2012. On the other hand, some strains of *Bacillus cereus* are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals (Ryan and Ray, 2004).

Escherichia coli and *Staphylococcus aureus* are among the four organisms reported to be mostly encountered pathogens in many African fermented foods. Other two are *Pseudomonas aeruginosae* and *Klebsiella* spp. (Gadaga *et al*., 2004). *E. coli* normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract. (Facultative anaerobes are organisms that can grow in either the presence or absence of oxygen.)

Corynebacterium is a genus of Gram-positive, rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous – harmless (Collins *et al*., 2004). Some are useful in industrial settings such as *C. glutamicum*. Others can cause human disease. *C. diphtheriae*, for example, is the pathogen responsible for diphtheria. The most notable human infection is diphtheria, caused by *Corynebacterium diphtheriae.* Nonpathogenic species of

Corynebacterium are used for very important industrial applications, such as the production of amino acids,(Hongo and Ogata, 1972; Yamada *et al.,* 1972) nucleotides, and other nutritional factors (Martín, 1989); bioconversion of steroids (Constantinides, 1980); degradation of hydrocarbons (Cooper *et al.*, 1979); cheese aging (Lee *et al*., 1985); and production of enzymes. Some species produce metabolites similar to antibiotics: bacteriocins of the corynecin-linocin type (Suzuki *et al*., 1972; Kerry-Williams and Noble, 1984; Kerry-Williams and Noble, 1986) antitumor agents, (Milas and Scott, 1978). One of the most studied species is *C. glutamicum*, whose name refers to its capacity to produce glutamic acid in aerobic conditions (Abe *et al.,* 1967). It is used in the foods industry as monosodium glutamate (MSG) in the production of soy sauce and yogurt. Species of *Corynebacterium* have been used in the mass production of various amino acids including glutamic acid, a food additive that is made at a rate of 1.5 million tons/ year.

Lactic Acid Bacteria are a group of Gram positive, non- respiring, non-spore forming, cocci or rods which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important desirable bacteria in food fermentation. Bacteria from the genera Lactobacillus, Leuconostoc, Pediococcus and Streptococcus are the main species involved. The majority of Nigerian fermented food products are through lactic acid fermentation (Oyewole, 1992). This is desirable as it improves consumer's safety of the fermented foods. Lactic acid fermentation is generally inexpensive and has been employed in the production and preservation of wholesome fermented foods (Steinkraus, 1997). Naturally occurring lactic acid bacteria are the organisms mainly responsible for such fermentations. Lactic acid bacteria have been associated with the fermentation of foods and feeds for many centuries (Magnusson and Schnurer, 2001). They have the advantage of health and nutritional benefits and contribute to the flavour, aroma and increased shelf life of fermented products

(Aderiye and Adebayo, 1999). Lactic acid bacteria are commonly found in fermented foods and feeds and consequently are regularly consumed. Their role is to promote sugar fermentation and other modifications of the raw materials which results in changes in their rheological and organoleptic properties and in the increase of the period of consumption. They equally play a fundamental role in microbial ecology, synthesising a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins (Lindgren and Dobroqosz, 1990). The antimicrobial compounds produced by the bacteria play an essential role in ensuring safety and extending the shelf life of food. Lactic acid bacteria produce many antimicrobial metabolites like hydrogen peroxide, diacetyl, organic acids and bacteriocins during fermentation. Their ability to produce antimicrobial substances against other competing microflora ensures their predominance and food safety. The use of antimicrobials produced by bacteria traditionally used in the manufacture of food has been studied intensively as a means of improving microbial barriers in formulated or minimally processed foods (Hugas, 1998).

Rhizopus is a genus of common saprobic fungi on plants and specialized parasites on animals. They are found on a wide variety of organic substrates, including "mature fruits and vegetables" (Kirk *et al*., 2008), faeces, jellies, syrups, leather, bread, peanuts and tobacco. Some Rhizopus species are opportunistic agents of human zygomycosis (fungal infection) and can be fatal. Rhizopus infections may also be a complication of diabetic ketoacidosis (Chinn and Diamond, 1982). This widespread genus includes ten species (Zheng *et al*., 2007). *Rhizopus arrhizus, Rhizopus azygosporus, Rhizopus circinans, Rhizopus microspores, Rhizopus oligosporus, Rhizopus oryzae, Rhizopus schipperae, Rhizopus sexualis, Rhizopus stolonifer* and *R. artocarpi. R. arrhizus* causes fruit rot on apples; *R. artocarpi* causes fruit drop of jack fruit; *R. oligosporus* is used to make tempeh, a fermented food derived from soybeans; *R. oryzae* is used in the production of alcoholic beverages in parts of Asia and

Africa; *R. stolonifer* (black bread mold) causes fruit rot on strawberry, tomato, and sweet potato and used in commercial production of fumaric acid and cortisone.

Geotrichum is a genus of fungi found worldwide in soil, water, air, and sewage, as well as in plants, cereals, and dairy products; it is also commonly found in normal human flora and is isolated from sputum and feces. The genus *Geotrichum* includes several species: The most common species is *Geotrichum candidum.* Among other species are *Geotrichum clavatum* and *Geotrichum fici*. *Geotrichum fici* has an intense smell resembling that of pineapple. Yeast-like and mold-like strains have been identified (Gents *et al*., 2002).

Penicillium is a genus of ascomycetous fungi of major importance in the natural environment as well as food and drug production. Members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria inside the body. Some species of *Penicillium* include *Penicillium aurantiogriseum*, *Penicillium bilaiae*, which is agricultural inoculants, *Penicillium camemberti*, which is used in the production of Camembert and Brie cheeses, *Penicillium candidum*, which is used in making Brie and Camembert. It has been reduced to synonymy with *Penicillium camemberti, Penicillium chrysogenum* (previously known as *Penicillium notatum*), which produces the antibiotic penicillin (Marianski and Marianski, 2009).

The total heterotrophic bacterial count result showed a progressive increase as the fermentation duration/time increased (from Day1-Day 6 of fermentation). From the 8^{th} day down to the 10^{th} Day, the bacterial counts recorded declined. The results of the total heterotrophic fungal count showed an increase in fungal counts with increase in fermentation time/exposure. Declines in fungal counts were recorded on the 8^{th} day of fermentation and this decline continued down to the 10^{th} day of fermenting cassava. This agrees with the report by Willey *et al.,* 2011 that micro organisms after attaining the stationary phase of growth

over time will experience a decline in the number of viable cells which was attributed to environmental changes such as nutrient deprivation and the build up of toxic wastes.

Anti-nutritional factors are natural or synthetic substances found in the human diet or animal feed that have the potential to adversely affect health and growth by preventing the absorption of nutrients from food. They exert effect contrary to optimum nutrition (Kumar, 1990).

From the results obtained, the mean concentration of tannin averaged over rates and treatment types showed that the peeled cassava preparation had the highest value of 0.244%, while the least value of 0.108 was with the unpeeled cassava preparation method. The values obtained were lower than that reported by Sarkiyayi and Agar, (2010), this can possibly be as a result of the additives/spices that were included in the fermentation process.

Tannins are constituents of several drugs because of their astringent property. They are used in the treatment of haemorrhoids, diarrhoea, dysentery, leucorrhoea and as a useful medicine for the throat (Allport, 1970). There are quite a number of anti-nutritional effects of the tannins present in plants. These effects occur via a common mechanism. The mechanism of dietary effects of tannins may be understood by their ability to form complex with proteins. Tannins may form a less digestible complex with dietary proteins. They may as well bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). The precipitation of protein-tannin complex depends on pH, ionic strength and molecular size of tannins. Tannins may be used as a component of biological rodenticide, in the control of rodents which invade and destroy food plants, thus reducing the economic and nutritional value of human foods and animal feeds (Joslyn and Glick, 1969; Freeland *et al.*, 1985; Soetan, 2008). Some chemical agents used as insecticides include tannins, flavonoids and alkaloids (Sharma and Norris, 1991). Tannins are also able to affect microbial activity in

the soil (Lewis and Starkey, 1968). The effects of tannins on soil microbes are used to play a role in succession in plant communities (Schimel *et al*., 1996). Molan *et al*., (1999, 2000, 2000a, b, 2002) reported the inhibitory effects of tannins against gastrointestinal nematodes and deer lungworms.

From the results obtained, the mean concentration of alkaloid averaged over rates and treatment types showed that the peeled cassava preparation had the highest value of 2.83%, while the least value was with the unpeeled cassava preparation method (2.47%). The values obtained were higher than that reported by Igbabul *et al.,* (2014) in their studies on fermented cocoyam flour. The additives & the preparation methods employed may have contributed to the high values of alkaloid. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Rhoads, 1979). Alkaloids have some benefits. They as well act as insecticides in some plants. Moreso, alkaloid extracts from *Murraya koenigii* have been noted to have ovicidal and oviposition activities against two very important mosquito vectors, *Culex tritae* and *Culex quinquefasciatus* (Rajkumar and Jebanesan, 2003). Alkaloids have been used in the treatment of skin infections (Sofowora, 1982; Finar, 1987).

From the results obtained, the mean concentrations of flavonoid averaged over rates and treatment types ranged from 1.58% - 2.22%. The results obtained were lower/ lesser when compared to that of Ezemenari *et al.,* 1998 and this can be attributed to the impact of the additives on the cassava products. Studies revealed that flavonoids were found to be strong topoisomerase inhibitors and induce DNA mutations in the MLL gene, which are

common findings in neonatal acute leukemia (Thirman *et al*., 1993; Strick *et al*., 2000). The DNA changes were increased by treatment with flavonoids in cultured blood stem cells (Barjesten *et al*., 2007). Some flavonoids have been shown to prevent liver cancer (hepatoma) and to prevent the liver from lipid peroxidative effects in experimental hyperlipidaemia (Blazovics *et al*., 1993; Soetan, 2008). Natural phenols (flavonoids in one set of experiments and delphinidin in another) were found to be strong topoisomerase inhibitors, similar to some chemotherapeutic anticancer drugs including etoposide and doxorubicin (Bandele *et al*., 2008). This property may be responsible for both an anticarcinogenic-proapoptotic effect and a carcinogenic, DNA damaging potential of the substances. Further, these compounds chelate metals such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins (Beecher, 2003). An extensive study of the antiarthritic and anti-inflammatory activity of flavonoids has been documented by Hanada *et al*., (1992). Tkayama *et al*., (1984) reported that flavonoids are potent inhibitors of molecular oxygen (O_2), thus acting as free radical scavengers (anti-oxidant). Flavonoids also scavenge other free radicals as OH and N_3 (Bors *et al*., 1990). Flavonoids suppress the effects of active oxygen species (H_2O_2 and O_2) in many other vulnerable biological systems (Nakayama *et al*., 1993). Flavonoids are used as natural anti-oxidants in food, medicinal and nonnutritive plant materials due to their ability to inhibit and scavenge reactive oxygen species (Kim *et al*., 1990; Larson, 1988). Isoflavones are also known to act as antioxidants in the test tube (Ruiz-Larrea *et al*., 1997). Flavonoid drugs have been widely used in medical practice for many years in the management of circulatory disorders involving capillary dysfunction. They were also effective in preventing or alleviating capillary fragility and permeability (Fahey and Jung, 1989). Flavonoids are currently used to potentiate the *in vivo* and *in vitro* activity of other drugs and vice-versa (Hoffman *et al*., 1988). Their synergistic use with vitamin E as anti-oxidants is more potent than when either of them is used singly

(Ferriola *et al*., 1989). DeEds (1968), Robbins (1973) and Srinivasan *et al.,* (1973) reported the beneficial physiological and pharmacological effects of flavonoids on blood capillaries. The action of Flavonoids have also been shown to be capable of modifying low density lipoproteins (LDL) in order to greatly increase its uptake by macrophages, thereby reducing the level of low density lipoproteins (LDL) in the body (Rankin *et al.,* 1993). As such, flavonoids can be applied in the management of atherosclerosis (Soetan, 2008).

From the results obtained, the mean concentrations of cyanide averaged over rates and treatment types showed that the unpeeled cassava preparation had the highest value of 18.017mg/g, followed by the peeled cassava preparations (16.348mg/g) and then mashed cassava preparations with 15.075mg/g. Unpeeled cassava preparation gave the highest cyanide value of 18.017mg/g which can be attributed to the presence of the cassava peel/ skin which also contains residual cyanide (Cumbana *et al.,* 2007). This result supported the findings of Anhwange (2011) who reported the hydrogen cyanide content of *Manihort utilissima, Colocasia esculenta, Dioscorea bulbifera and Dioscorea domentorum tubers.* Nwaichi *et al.,* (2013) reported the comparative effects of processing on the cyanide content of *Manihort esculenta, Glycin max and Zea mays.* According to their report, heat treatments reduced the cyanide content (approximately 100%) in the tested food crops thereby making them suitable and safer for consumption. Mashed cassava preparations had the least cyanide content which was primarily due to the method of preparation and the effect of the additives and so is the safest among the three, for consumption. Cyanide poisoning occurs when a living organism is exposed to a compound that produces cyanide ions (CN^-) when dissolved in water. Most cyanides are highly toxic, and as an antinutritional, studies have revealed that cyanide ion halts cellular respiration by inhibiting an enzyme in the mitochondria called cytochrome c oxidase. This stops ATP formation, tissues suffer energy deprivation and death

follows rapidly (Kumar, 1990). Common poisonous cyanide compounds include hydrogen cyanide gas and the crystalline solids potassium cyanide and sodium cyanide. According to Conn (1979) the lethal dose for cyanogens would be 10-20 times greater because the HCN comprises 5-10% of their molecular weight. Further, cyanide can cause goitrogenic effects due to thiocyanate produced during detoxification. More, poor animal performance due to *Acacia sieberiana* pod feeding has been attributed to cyanogens (Tanner *et al*., 1990). Keeler (1984) revealed that cyanogens have teratogenic (anti-embryonic development agent) effects. Animals suffering from cyanide must be immediately treated by injecting a suitable dose of sodium nitrate and sodium thiosulphate (Kumar, 1990).

Proximate or nutritional analysis of the mean cassava preparations averaged over rates and treatment types showed that the moisture content was highest with the unpeeled cassava preparation method followed by the peeled cassava preparation and the least being the mashed cassava preparation method (19.702% >16.987%> 16.723%). The reduction in moisture content may probably be due to the softer and porous texture of the corms resulting in maximum moisture loss. Also the microorganisms must have utilized some moisture for metabolic activities (Igbabul *et al.,* 2014). From the results obtained, the moisture contents were higher than the results obtained by Ojo and Akande (2013) and Igbabul *et al.,* 2014. Generally, the relative high moisture content of the samples may imply that they are liable to be spoiled by microorganisms (Amadi *et al*., 2011). This is because high moisture content has been described to favor bacterial growth (Temple *et al*., 1996).

From the results obtained, the mean cassava preparations averaged over rates and treatment types showed that the fat content was highest with the unpeeled cassava preparations (3.305%) while the least was with the peeled cassava preparations (2.66%). The high fat values agrees with that of Onoja and Obizoba (2009), these could be as a result of

extensive breakdown of large fat molecules to simpler fatty acid units due to the high activities of lipolyic enzymes. Also, the high fat values might be from dead microflora or that the fermenting microflora did not use fat from these foods as source of energy (Reebe *et al.*, 2000).

The mean cassava preparations averaged over rates and treatment types showed that the carbohydrate content was highest with the mashed cassava preparations (70.845%) , followed by peeled cassava preparations (69.219%) while the least was with the unpeeled cassava preparations (67.671%). These values agree with that reported by Igbadul *et al.,* (2014) in their study on the effect of fermentation on cocoyam flour. Whereas, the values reported by Sarkiyayi and Agar (2010) in their comparative analysis on the nutritional contents of sweet and bitter cassava varieties are higher (ranging from 85.45% - 86.21%) compared to that obtained from the various cassava preparations. The high carbohydrate value recorded with the mashed cassava preparation method could have been as a result of the preparation method which accelerated the intake/conversion of the product as energy source by the fermenting microflora.

Also, the protein content obtained from the mashed, peeled and unpeeled cassava preparations averaged over rates and treatment types were higher compared to the findings by Ojo and Akande (2013) and Sarkiyayi and Agar (2010). The high rate of protein content could be attributed to the presence/effects of essential oils from ginger (*Zingiber officinale*).

The ash content from the cassava preparation methods averaged over rates and treatment types showed that the mashed cassava preparation method had the highest ash content value of (1.373%) followed by the unpeeled (1.022%) and then the peeled cassava preparation method (0.796%). These values were lower than that reported by Sarkiyayi and

Agar (2010) and can be attributed to the treatment types and rates of the additives (spices) used.

Finally, the fibre content from the various cassava preparation methods averaged over rates and treatment types showed the peeled cassava preparation method had the highest fibre content of 5.67% while the least was the mashed cassava preparation method (3.042%).The lower values obtained in the case of the unpeeled and mashed cassava preparations could have been due to the activities of microorganisms which are known for the bio- conversion of carbohydrates and lignocelluloses into protein. This agrees with the findings of Hwei- Ming *et al.,* (1994).

The results for the mineral analysis showed that the mean concentration of magnesium averaged over rates and treatment types had the highest value of 0.2099mg/100g (unpeeled cassava preparation and the least value of 0.1598mg/100g (peeled cassava preparation method). The values obtained are higher than that reported by Kadashi, (2005) these can be attributed to the high content of magnesium mineral present in the spices/ additives (garlic & ginger).

From the results obtained, the mean cassava preparations averaged over rates and treatment types showed that the sodium content was highest with the unpeeled cassava preparations (0.0623mg/100g) while the least was with the peeled cassava preparations (0.050mg/100g). The values obtained were lower than that reported by Fredrick, (2008).

The results for the potassium content showed that the mashed cassava preparation had the highest value of 0.143mg/100g, followed by the unpeeled preparation (0.125mg/100g) and then the peeled cassava preparation method (0.086mg/100g). The values are lower than that reported by the USDA Database on raw cassava roots.

From the results obtained, the mean cassava preparations averaged over rates and treatment types showed that the calcium content was highest with the unpeeled cassava preparation (0.258%) while the least value was 0.102% with the peeled cassava preparation methods. The major role of calcium also increases the permeability of the cell membrane and is also involved in the transmission of nerve impulses, 800g of calcium is recommended per day for an adult person.

The results for phosphorus were highest with the mashed preparation method (56.994mg/100g) and the least value was with the unpeeled preparation (40.548mg/100g). Phosphorus combines with calcium in bones and teeth (Davidson and Stanley 1975). Daily require of phosphorus is 800mg for adults (0.03%).

The organoleptic/ sensory assessment of the various cassava preparations averaged over rates and treatment types showed that the values obtained were significantly high at 5% level for aroma, texture and colour. These showed overall acceptability by the respondents.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

Different cassava preparations were fermented using varying quantities of garlic, ginger, garlic plus ginger grates. These brought about a significant improvement on the nutritional composition, mineral composition, anti-nutritional composition and overall acceptability of the fermented cassava products.

The need for an alternative method of improving the quality of fermented cassava has been emphasized. The use of garlic, ginger, garlic plus ginger grates in different cassava preparation methods could serve as good alternative methods for improving the quality of fermented cassava.

5.1 CONTRIBUTION TO KNOWLEDGE

1. The introduction of a new formulation in cassava processing, hence, improvement in the shelf life of processed cassava products.
2. An improved quality of processed cassava thereby encouraging the cultivation and processing and marketing of cassava products which can be felt in areas such as rural employment and vibrant cassava industrial sector.

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APPENDIX

1.0 PREPARATION OF PEPTONE WATER AND MEDIA

Buffered Peptone Water with NaCl - Diluents (HIMEDIA-Technical Data: M1275)

Buffered Peptone Water with NaCl is recommended as a diluent for carrying microbial limit test from clinical and non clinical specimens.

Composition**

Ingredients	**Gms / Litre**
Peptone	1.000
Potassium dihydrogen phosphate	3.560
Disodium hydrogen phosphate	7.230
Sodium chloride	4.300
Final pH (at 25°C)	7.0 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Preparation

Suspend 16.09 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Add 0.1 to 1% w/v polysorbate 20 or 80 if desired. Dispense in tube or flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

1.2 Preparation of Media

Three different media were used. They are nutrient agar, MacConkey agar, and potato dextrose agar. Each is known to have a unique composition.

1.3 Description of Nutrient Agar

Nutrient agar is a general culture medium for less fastidious microorganism as well as for permanent cultures. Blood, serum or other biological fluids can be added into this agar if the need arises.

1.3.1 Composition of Nutrient Agar

Nutrient agar contains 1g/l of meat extract, 2g/l of yeast extract, 5g/l of peptone, 5g/l of sodium chloride 15g/l of agar and pH of 7.4 $\pm$ 0.2 at 37^0C.

1.3.2 Procedure for Preparation of Nutrient Agar

Twenty eight gram (28g) of the powdered agar was dissolved in 1 litre of distilled water. The mixture was swirled to dissolve completely and sterilized by autoclaving at 121^0C for 15 minutes.

1.4 Preparation of MacConkey Agar

MacConkey agar is a differential plating medium recommended for the detection and isolation of coliforms and intestinal pathogens from stool, urine, water and other samples.

1.4.1 Composition of MacConkey Agar

Composition	Quantity
Peptone	20g/l
Lactose	10g/l
Bile salt	5g/l
Neutral red	0.075g/l
Agar	12g/l
Final pH	7.4±0.2 (at 37^0C)

1.4.2 Procedure for Preparation of MacConkey Agar

Fifty two grams (52g) of the powder was dissolved in 1 litre of distilled water. The mixture was shaken gently to achieve dissolution of the medium. This was followed by sterilization in autoclave at 121^0C for 15 minutes. After allowing the autoclaved medium to cool to 45^0C, it was poured asceptically into sterile petri dishes and allowed to solidify.

1.5 Preparation of Potato Dextrose Agar

1.5.1 Composition of Potato Dextrose Agar

Potatoe dextrose agar contains 200.0g of potatoe infusion, 20.0g of dextrose, 15.0g of agar and 5.6 ± 0.2 (pH) at 25^0C.

1.5.2 Procedure for Preparation of PDA

Thirty nine grams (39g) of the PDA powder was dissolved in 1000ml of distilled water. The mixture was heated to dissolve the medium completely. This was followed by sterilization in autoclave at 121^0C for 15 minutes.

1.5.3 Biochemical Characterization of Bacteria Isolates

Bacterial isolates were further subjected to biochemical tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, coagulase, urease, starch hydrolysis, cystein and gelatin liquefaction, citrate utilization, fermentation of glucose, lactose, sucrose, maltose, and manitol (Patel, 1994; James, 1995).

1.5.3.1 Oxidase Test:

1.5.3.1.1 Aim:

The oxidase test is used to assist in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella,* and *Pasteurella* species, all of which produce the enzyme cytochrome C oxidase that is able to reduce O_2 (Lansing *et al*., 1996; Monica, 2006).

1.5.3.1.2 Principle:

The principle is based on soaking a piece of filter paper with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively, an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour (Monica, 2006).

1.5.3.1.3 Procedure (Fresh Reagent):

- A piece of filter paper was placed in a clean petri dish and 3 drops of freshly prepared oxidase reagent added,
- Using a piece of sterile stick or glass rod (not an oxidized wire loop), a colony of test organism was removed and smeared on the filter paper,
- Development of a blue-purple colour within 5-10 seconds was looked for.

1.5.3.1.4 Results:

- Positive oxidase test = Blue-purple colour within 10 seconds,
- Negative oxidase test = No blue-purple colour within 10 seconds.

1.5.3.1.5 Controls

- Positive oxidase control: *Pseudomonas aeruginosae*
- Negative oxidase control: *Escherichia coli*.

1.5.3.2 Citrate Utilization Test:

1.5.3.2.1 Aim:

In the words of Monica, 'this technique is one of several techniques used occasionally to assist in the identification of enterobacteria'.

1.5.3.2.2 Principle:

The test is based on the ability of an organism to use citrate as its only source of carbon.

1.5.3.2.3 Procedure:

There are two major ways of performing a citrate test. They are:

- Method involving the use of Simmon's citrate agar. This method is adopted in this research, and,
- Method involving the use of Rosco citrate identification tablet.

CITRATE METHOD USING SIMMON'S CITRATE AGAR:

- Slopes of Simmon's citrate agar were prepared in bijou bottles as recommended by the manufacturer
- The prepared medium was tested for purity or sterility by storing it at refrigeration temperatures of 2-8^0 C for 24hrs,
- After the purity test, and using a sterile straight wire, the slope was streaked with a saline suspension of the test organism, then stabbed the butt and incubated,
- After incubation at 35^0 C for 48 hrs, a bright blue colour was looked for in the medium

1.5.3.2.4 Results:

- **Positive citrate test =** Bright blue colour ocurred in the medium,
- **Negative citrate test =** No change in colour of medium.

1.5.3.2.5 Controls:

- **Positive citrate test control:** *Klebsiella pneumonia*
- **Negative citrate test control:** *Escherichia coli.*

1.5.3.3 Catalase Test

1.5.3.3.1 Aim:

The test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

1.5.3.3.2 Principle:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

1.5.3.3.3 Procedure:

- Two-to-three mililitres of three percent hydrogen peroxide (3% H_2O_2, ten volume solution), were poured into a sterile test tube,
- Using a sterile wooden stick or glass rod (not a nichrome wire loop), several colonies of the test organism were removed and immersed in the hydrogen peroxide solution,
- Immediate bubbling was looked for.

1.5.3.3.4 Results:

- **Positive catalase test** = Active bubbling,
- **Negative catalase test** = No bubbles.

1.5.3.3.5 Controls:

- **Positive catalase control:** *Staphylococcus* spp.
- **Negative catalase control:** *Streptococcus* spp.

1.5.3.4 Carbohydrate/sugar Fermentation Tests:

***BRIEF HISTORY*:**

In 1837, Cagniard-Latour, Schwann, and Kŭtzing independently proposed that yeast, detected during alcoholic fermentation, was a microscopic plant and the responsible etiology for the conversion of sugars to ethyl alcohol and carbon dioxide (Stanier *et al*., 1963). This theory was strongly opposed by the leading contemporary chemist who subscribed to the popular belief that fermentation and putrefaction were both strictly chemical processes (Stanier *et al*., 1963). The controversy over the matter continued for years, until Pasteur, a chemist by training, was able to convince the scientific community that ‘‘all fermentation processes are the results of microbial activity’’ (Stanier *et al*., 1963; Mŭller, 2008).

Pasteur's work on fermentation was aided providentially by his involvement with ''the distillers of Lille,'' where alcohol was produced from beet sugar. This local industry had encountered difficulties with their production and being aware of Pasteur's interest in the matter, solicited his assistance. Pasteur's investigations showed that the alcoholic fermentation had been replaced, at least in part, by another kind of fermentative process, which resulted in the conversion of the sugar to lactic acid (Stanier *et al*., 1963). Pasteur found that in the vats where lactic acid had been formed, a different or new yeast was present. Successive inoculation of these small rods and spheres on the same medium always resulted in the production of lactic acid and more rods and spheres. He concluded that these new microorganisms must specifically convert sugar to lactic acid as they grew (Stanier *et al*., 1963; Muller, 2008).

For the next 20 yrs. (1856-1876), Pasteur continued to test many fermentative processes. He was able to show that fermentation was always accompanied by the growth of microorganisms and that each particular type of chemical fermentation, defined by its primary chemical end-product, was the result of a particular microorganism's involvement. These ''specific types of microorganisms'' could be characterized by their sizes and shapes and further distinguished by the specific environmental condition in which they thrive (Stanier *et al*., 1963).

Pasteur was also the first to recognize that some microorganisms were able to obtain energy by breaking down organic compound, and he was able to show that the amount of growth was directly related to the amount of energy that could be produced by the breakdown of organic compound (Stanier *et al*., 1963). He also noted that fermentation was less efficient than other biochemical pathways at producing energy (Muller, 2008; Stanier *et al*., 1963).

Th term ‘‘fermentation’’ is often used to describe the breaking down or catabolism of a carbohydrate under anaerobic conditions. Therefore, bacteria capable of fermenting a carbohydrate are usually facultative anaerobes (Hugh and Leifson, 1953; Forbes *et al.*, 2007). It should also be noted that while the terms ‘‘carbohydrate’’ and ‘‘sugar’’ are often used interchangeably, the term sugar might not indicate the true chemical composition of certain substrates such as in the case of dulcitol and mannitol (Cowan and Steel, 1965; Forbes *et al.*, 2007).

PURPOSE:

Carbohydrate fermentation tests detect the ability of microorganisms to ferment a specific carbohydrate. Fermentation patterns can be used to differentiate among bacterial groups or species (Bartelt, 2000; MacFaddin, 2000; Forbes *et al.*, 2007; Mahon *et al.*, 2011).

For example, all members of the *Enterobacteriaceae* family are classified as glucose fermenters because they can metabolize glucose anaerobically (MacFaddin, 2000; Mahon *et al.*, 2011). Within this family however, maltose fermentation differentiates *Proteus vulgaris* (positive) from *Proteeus mirabilis* (negative) (MacFaddin, 2000; Mahon *et al.*, 2011). Meanwhile, fermentation tests can be performed on microorganisms other than bacteria (Karen, 2013).

THEORY:

During the fermentation process, an organic substrate serves as the final electron acceptor (MacFaddin, 2000; Stanier *et al.*, 1963). The end product of carbohydrate fermentation is an acid or acid with gas production (Forbes *et al.*, 2007; Mahon *et al.*, 2011). Various end-products of carbohydrate fermentation can be produced. The end-product depends on the organisms involved in the fermentation reaction, the substrate being fermented, the enzymes

involved, and environmental factors such as pH and temperature (Stanier *et al*., 1963). Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbon dioxide and hydrogen (Forbes *et al*., 2007; MacFaddin, 2000; Mahon *et al*., 2011).

Fermentation reactions are detected by the color change of a pH indicator when acid products are formed. This is accomplished by adding a single carbohydrate to a basal medium containing a pH indicator. Because bacteria can also utilize peptones in the medium resulting in alkaline by-products, the pH changes only when excess acid is produced as a result of carbohydrate fermentation (Cowan and Steel, 1965).

Phenol red is commonly used as a pH indicator in carbohydrate fermentation test because most of the end-products of carbohydrate utilization are organic acids (MacFaddin, 2000). However, other pH indicators such as bromocresol/bromcresol purple, bromothymol/ bromthymol blue, and Andrade's can be used (Karen, 2013).

Fermentation tubes or Durham tubes are used to detect gas production (MacFaddin, 2000; Forbes *et al*., 2007; Mahon *et al*., 2011). These small, slender test tubes (6 by 50mm) are inserted upside down inside larger (13 by 100mm) test tubes. After sterilization, Durhan tubes become filled with the media. If gas is produced, it will be trapped inside the Durham tube and is evident by the presence of visible air bubble (Karen, 2013).

Three characteristic reactions can be observed from the fermentation of a specific carbohydrate (MacFaddin, 2000). Based on these reactions, bacteria are classified as:

- Fermenter with acid production only,
- Fermentation with acid and gas production,
- Nonfermenter.

The following sugar tests were adopted in this research. They are;

- Lactose test,
- Sucrose test,
- Maltose test, and
- Mannitol test.

1.5.3.4.1 Lactose fermentation test:

1.5.3.4.2 Aim:

The aim is to see if the test organism can ferment the carbohydrate (sugar) lactose as a carbon source.

1.5.3.4.3 Principle:

The principle is based on the fact that when lactose is fermented to produce acid end products, the pH of the medium will drop, while a pH indicator in the medium changes color to indicate acid production.

1.5.3.4.4 Procedure:

- An inoculum from a pure culture of the test organism was transferred asceptically to a sterile tube of phenol red lactose broth,
- The inoculated tube was incubated at 35-37^{0}C for 24 hours and the results were determined.

1.5.3.4.5 Results:

- **Positive lactose fermentation test =** change in colour of the broth to yellow because of acid production,

- **Negative lactose fermentation test =** change in colour of the broth to magenta or hot pink in the presence of bases/alkali.

1.5.3.4.6 Controls:

- **Positive lactose fermentation test control:** *E. coli*
- **Negative lactose fermentation test control:** *Pseudomonas aeruginosa*.

1.5.3.5 Sucrose fermentation test:

1.5.3.5.1 Aim:

The aim of this test is to see if the test organism can ferment the carbohydrate sucrose as a source of carbon.

1.5.3.5.2 Principle:

The principle is based on the fact that the fermentation of sucrose produces acid end products, which leads to the drop in pH of the medium. A pH indicator in the medium changes color to indicate acid production.

1.5.3.5.3 Procedure:

- An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red sucrose broth,
- The inoculated tube was incubated at 35-37^0 C for 24 hours and the results were determined.

1.5.3.5.4 Results:

- **A positive sucrose fermentation test** = a color change from red to yellow, indicating a p*H* change to acidic,

- **A negative sucrose fermentation test** = a colour change of the broth to magenta or hot pink in the presence of bases/alkali.

1.5.3.6 Maltose fermentation test:

1.5.3.6.1 Aim:

The aim of this test is to see if the test organism can ferment the carbohydrate (sugar) maltose as a carbon source.

1.5.3.6.2 Principle:

The principle is based on the fact that the fermentation of maltose produces acid end products, which leads to the drop in pH of the medium. A pH indicator in the medium changes color to indicate acid production.

1.5.3.6.3 Procedure:

- An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red maltose broth,
- The inoculated tube was incubated at 35-37^0 C for 24 hours and the results were determined.

1.5.3.6.4 Results:

- **A positive maltose fermentation test** = a color change from red to yellow, indicating a p*H* change to acidic,
- **A negative maltose fermentation test** = a colour change of the broth to magenta or hot pink in the presence of bases/alkali.

1.5.3.7 Mannitol salt agar (MSA) test:

1.5.3.7.1 Aim:

MSA is both a selective and differential media used in the isolation of *Staphylococci*.

- As a selective medium, MSA contains 7.5% sodium chloride and thus selects for those bacteria which can tolerate high salt concentrations, while,
- As differential medium, MSA also distinguishes bacteria based on the ability to ferment sugar mannitol, the only carbohydrate in the medium.

1.5.3.7.2 Principle:

Staphylococci can withstand the osmotic pressure created by 7.5% NaCl, while this concentration will inhibit the growth of most other Gram-positive and Gram-negative bacteria (Koch, 1942). MSA contains mannitol and uses phenol red as a pH indicator in the medium. At pH levels below 6.9, the medium is a yellow colour. In the neutral pH ranges (6.9 to 8.4) the color is red, while above pH 8.4, the colour of phenol red is pink (Gerhardt *et al*., 1981). When mannitol is fermented by a bacterium, acid was produced, which lowered the pH and results in the formation of a yellow area surrounding an isolated colony on MSA. A nonfermenting bacterium that withstands the high salt concentration would display a red to pink area due to peptone breakdown (Mahon and Manusekis, 1995).

1.5.3.7.3 Procedure:

- A sterile plate of mannitol salt agar was streaked with the test organism using quadrant streak plate method to obtain isolated colonies,
- The inoculated plates were incubated aerobically at 32-37^0 C and examined after 18-72 hrs, (APHA, 1966).

1.5.3.7.4 Results:

- **Positive mannitol salt agar fermentation test =** the yellowing of the area surrounding an isolated colony on MSA indicated a positive result. This was because when mannitol was fermented by a bacterium, acid was produced, which lowered the pH and resulted in the formation of a yellow area surrounding an isolated colony on MSA. A nonfermenting bacterium that withstood the high salt concentration displayed a red to pink area due to peptone breakdown (Mahon and Manusekis, 1995).
- **Negative mannitol salt agar test =** medium retains its colour.

1.5.3.7.5 Controls:

- **Positive control** = *Staphylococcus areus*
- **Negative control** = *Escherichia coli.*

1.5.3.8 Indole Test:

1.5.3.8.1 Aim:

This test aims at identifying those bacteria that have the ability to produce indole such as enterobacteria. Most strains of *E. coli*, *P. vulgaris, P. rettgeri, M. morganii, and Providencia* species are able to break down tryptophan, an amino acid, with the release of indole.

1.5.3.8.2 Principle:

The principle relies on the culture of test organism in a medium that contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylaminobenzaldehyde. This is believed to react with the indole to produce a red

coloured compound. Kovac's reagent is recommended in preference to Erlich's reagent for the detection of indole from enterobacteria.

1.5.3.8.3 Procedure:

Indole test can be performed in three different ways, namely:

- As a single test using tryptophan water and Kovac's reagent,
- As a combined *beta*-glucuronidase-indole test using a Rosco PGUA/Indole identification tablet and Kovac's reagent. This is useful when identifying *E. coli,*
- As a combined lysine decarboxylase-indole test using a Rosco LDC/Indole identification tablet. This is useful in helping to identify salmonellae and shigellae.

In this research, the method of detecting indole usng tryptophan water is adopted. This involves the following;

- The test organism was inoculated into a bijou bottle containing 3 ml of sterile tryptone water,
- The inoculated medium was incubated at 35-37^{0}C for up to 48 hrs,
- After incubation, indole was tested for by adding 0.5 ml of Kovac's reagent, shaked gently and examined for a red or pink colour in the surface layer within 10 minutes. (Monica, 2006).

1.5.3.8.4 Results:

- **Positive indole test =** red or pink surface layer
- **Negative indole test =** no red or pink surface.

1.5.3.9 Methyl red (2-N, N-Dimethyl-4-aminophenyl) azobenzenecarboxylic acid) Test:

1.5.3.9.1 Aim and Principle:

Methyl red also known as C.I. Acid red 2 is used to determine whether the test organism performed mixed acid fermentation when supplied glucose. Mixed acid fermentation results in accumulation of a variety of acids and a significant drop in the pH of the medium.

1.5.3.9.2 Procedure:

- The test organism was inoculated into phosphate-buffered glucose-peptone medium,
- The inoculated medium was incubated for 48 hrs at 37^0C,
- Then, a few drops of 0.04% methyl red were added, and exmined.

1.5.3.9.3 Results:

- **Positive methyl red test =** colour change to red
- **Negative methyl red test** = no colour change.

1.5.3.10 Voges-Proskauer (VP) Test:

1.5.3.10.1 Aim:

VP is a test used to detect acetoin in a bacterial broth culture.

1.5.3.10.2 Principle:

The principle involves detecting acetoin in a bacterial culture by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth which has been inoculated with bacteria.

1.5.3.10.3 Procedure:

- A phosphate - buffered glucose-peptone medium was inoculated with the test organisms and incubated at 37^0 C for 2 days,

- Also, 0.6 ml of an ethanolic solution of 5% alpha-naphthol, and 0.2ml of 40% potassium hydroxide solution were added sequentially to 1ml of culture and stoppered,
- The stoppered tube or bottle was shaken vigorously, placed in a sloping position and examined after 30 to 60 minutes.

1.5.3.10.4 Results:

- A pinkish – red colouration indicated a positive test
- A yellow-brown color indicated a negative result (MacFaddin, 1980).

1.5.3.10.5 Controls:

Positive control = *Klebsiella*

Negative control = *Citrobacter*

1.5.3.11 Coagulase Test:

1.5.3.11.1 Aim:

This test is used in the identification of *Staphylococcus aureus* which produces the enzyme coagulase.

1.5.3.11.2 Principle:

Coagulase is known to cause plasma to clot by converting fibrinogen to fibrin. Basicall, there two types of coagulase produced by most strains of *S. aureus:*

- Bound coagulase (clumping factor) converts fibrinogen directly to fibrin without requiring a coagulase-reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test,
- Free or unbound coagulase: this converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is usually detected by clotting in the tube test.

1.5.3.11.3 Procedure:

Slide test method:

- A drop of distilled water was placed on each end of a slide,
- A colony of the test organism was emulsified in each of the drops to make two thick suspension,
- A loopful of plasma was added to one of the suspensions, mixed gently and examined, no plasma was added to the second suspension. It was used to differentiate any granular appearance of the organism from true coagulase clumping.

Tube test method:

- Three small sterile test tubes were labelled thus;

  A = test organism (18 – 24 hrs broth culture)

  B = positive control (18 – 24 hrs *S. aureus* broth culture)

  C = Negative control (sterile broth),

- And 0.2 ml of plasma was pipetted into each tube,
- Then, 0.8 ml of the test broth culture was added to tube 'A',

  0.8 ml of the *S. aureus* culture was added to the tube labelled 'B', and

  0.8 ml of sterile broth was added to the tube labelled 'C', and all the tubes mixed gently,

- After mixing gently, the three tubes were incubated at 35 - 37^0C,
- After one hour of incubation, the tubes were examined for clotting. If no clotting had occurred, the tube was further examined after three hours. If the test was still negative, the tube was left at room temperature overnight and examined again while tilting each tube gently.

1.5.3.11.4 Results:

Slide Test:

- Clumping within ten seconds = *S. aureus.*
- No clumping within ten seconds = no bound coagulase

Tube Test:

- Clotting of tube contents or fibrin clot in tube = *S. aureus,*
- No clotting or fibrin clot = negative test.

1.5.3.11.5 Controls:

- **Positive coagulase control** = *Staphylococcus aureus,*
- **Negative coagulase control =** *E. coli.*

1.5.3.12 Urease Test:

1.5.3.12.1 Aim:

Testing for urease enzyme activity is important in differentiating enterobacter. *Proteus* strains are strong urease producers. *Y. enterocolitica* also shows urease activity (weakly at 35 - 37^0C). *Salmonellae* and *Shigellae* do not produce urease (Monica, 2006).

1.5.3.12.2 Principle:

The principle involves culturing the test organism in a medium which contains urea and the indicator phenol red. When the strain is urease – producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red. (Monica, 2006).

1.5.3.12.3 Procedure:

- The test organism was heavily inoculated in a bijou bottle containing 3 ml sterile Christensen's modified urea broth,
- The inoculated broth was incubated at 35 - 37^0 C for 3 – 12 hrs, and then examined for a pink colour.

1.5.3.12.4 Results:

- **Positive urease test =** Pink colour
- **Negative urease test** = No pink colour.

1.5.3.13 Starch Hydrolysis:

In this test, starch agar was inoculated with the test species. After inoculation, the plates were incubated for 24 hours at 37°C. Iodine, which changes color from a yellow-brown to blue-black in the presence of starch, was applied to the agar surface and allowed to stand for 10 minutes. Iodine turned blue-black in the presence of starch. This was a negative reaction for the starch hydrolysis test. Absence of the blue-black color indicated that starch was no longer present in the medium. This indicated a positive result. Bacteria which showed a clear zone around the growth produced the exoenzyme amylase which cleaves the starch into di- and monosaccharides (Bird and Hopkins, 1954).

APPENDIX A

Cultural and Biochemical Identification of the Bacterial Isolates

S/N	Cultural Characteristics	Gram Reaction	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Generation of Isolates
1.	Large, flat, irregular creamy colonies about 4mm in diameter	+ve spore Formin rods	+	-	-	+	+	-	-	+	A	-	A	A	-	-	*Bacillus spp.*
2.	Milk colonies on blood agar alone	-ve short rods	+	-	-	-	+	-	+	A	-	-	A	-	-	+	*Proteus spp.*
3.	Glossy, smooth and translucent rose pink coloured colonies on MacConkey about 1.5mm in diameter	-ve rods	-	-	-	-	+	+	-	-	AG	AG	AG	A	A	-	*E.coli*
4.	Large clear colonies on MRS Agar	+ve rods	-	-	-	-	-	-	-	-	-	A	-	-	A	A	*Lactobacilli spp.*

KEY: - = Negative result 1. Catalase 4. Citrate 7. Hydrogen sulphide 12. Maltose

\+ = Positive result 2. Coagulase 5. Motility 9. Glucose 13. Manitol

A = Acid Production 3. Oxidase 6. Indole 10. Lactose 14. Urease

APPENDIX B

CULTURAL AND MICROSCOPIC CHARACTERISTICS OF THE FUNGAL ISOLATES

S/N	Cultural Characteristics	Microscopy	Fungi
1.	Dark-brown/ black colony spreading on the surface of the medium attaining a diameter of 4-5cm within 7 days. Reverse is yellowish and zoned.	Radiate conidia heads. Conidiophores stipes smooth walled, hyaline often in brown colour conidia globse orb subglobse (3.5-5 Nm) brown, ornamented with irregular warth spines and ridges	*Aspergillus spp.*
2.	Slowly developing chocolate brown colonies consisting of a dense conidiophores arising from agar or from the scanty aerial mycelium	Conidiophores stalked hyaline and smooth-walled. Conidia head radiate, becoming losely columnar with age.	*Penicillium spp.*
3.	Colony whitish becoming whitish-gray with age about 10mm high.	Sporagiospores arising from stolons without rhizoids in groups of up 5, forked or smooth walled. Sporamgiospores, globose, oviod	*Rhizopus spp.*