# DEODORIZATION OF FERMENTED FOOD CONDIMENT FROM *PROSOPIS AFRICANA* (AFRICAN MESQUITE) SEEDS AND ITS EFFECT ON NUTRIENTS AND ANTINUTRIENTS COMPOSITION

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**DECEMBER, 2021**

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**A THESIS SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN NUTRITION**

**DEPARTMENT OF BIOCHEMISTRY,**
**FACULTY OF LIFE SCIENCES,**
**AHMADU BELLO UNIVERSITY,**
**ZARIA, NIGERIA**

**DECEMBER, 2021**

**DECLARATION**

I declare that the thesis entitled "**Deodorization of Fermented Food Condiment *(Okpehe)* from *Prosopis africana* (African mesquite) Seeds and its Effect on Nutrients and Antinutrients Composition**" has been carried out by me in the Department of Biochemistry under the supervision of Prof. D.A. Ameh, Dr. M. A. Musa and Prof. C. M. Z. Whong. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this of any other institution.

| UBANGIDA Suleiman | ………………… | ……............. |
|---|---|---|
| (Name of Student) | Signature | Date |

## CERTIFICATION

This thesis entitled "**DEODORIZATION OF FERMENTED FOOD CONDIMENT *(OKPEHE)* FROM *PROSOPIS AFRICANA (*AFRICAN MESQUITE) SEEDS AND ITS EFFECT ON NUTRIENTS AND ANTINUTRIENTS COMPOSITION**" by SULEIMAN UBANGIDA meets the regulation governing the award of the degree of Master of Science in Nutrition of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. D. A. Ameh Sign ___________________ Date______________
Chairman Supervisory Committee

Dr. M. A. Musa Sign ___________________ Date______________
Member Supervisory Committee

Prof. C. M. Z. Whong Sign ___________________ Date______________
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Prof. A. B. Sallau Sign ___________________ Date______________
Head of Department, Biochemistry

Prof. Sani A. Abdullahi Sign ___________________ Date______________
Dean, Postgraduate School

## DEDICATION

This work is wholeheartedly dedicated to Almighty Allah (Subhanahu Wa Ta'ala), my beloved Parents in person of Late Alhaji M. S. UBANGIDA may his soul rest in Aljannatul-Firdausy and Hajiya Gambo UBANGIDA, family, friends, Nigeria and Africa at large.

# ACKNOWLEGEMENTS

All thanks and gratitude are due to Almighty Allah (SWT) Who provided me with the life, health, strength and wisdom to carry out this research. I am highly indebted to my supervisors Prof. D. A. Ameh, Dr. M. A. Musa and Prof. C. M. Z. Whong for their unending support in supervising this work. I am grateful for the time given from the beginning of this work to a successful and useful end. May Allah (Subhanahu Wa Ta'ala) reward them abundantly. I sincerely acknowledge the role of the Head of Department Prof. A. B. Sallau and all academic and non-academic staff of Biochemistry Department for their useful suggestions.

I would also like to express my sincere gratitude to my Parents Late Alhaji M. S. UBANGIDA (may his soul rest in Aljannatul-Firdausy) and Hajiya Gambo UBANGIDA for their resilience, financial support, encouragement, patience and love. I extend my gratitude to staff of Herbarium Laboratory, Department of Botany, Ahmadu Bello University Zaria, for helping me in identifying the plant. I would also like to thank my colleagues particularly Mr. Auwal Muhammad Saliu, Mr. Halliru Zailani, and Zeenatu Bello Kudan to mention but a few, for their immense contribution throughout the period of this research.

Very special thanks to all other individuals that assisted me in one way or the other towards the achievement of this work.

## ABSTRACT

*Prosopis africana* seeds were processed to produce local fermented food condiment. The aim of this study was to deodorize laboratory prepared fermented food condiment from *Prosopis africana* seeds with potential chemical deodorant; *cyclodextrin (CD), and powdered activated carbon (PAC)* and determine its nutrients and antinutrients composition. Heterotrophic bacterial counts were determined using pour plate method and the growth increased exponentially from days 1- 6, with peak counts at day 7 (2.24 x $10^6$, 1.54 x $10^7$, 1.16 x$10^8$ and 0.77 x$10^9$) cfu/g and day 8 (2.25 x $10^6$, 1.56 x $10^7$, 1.18x$10^8$ and 0.78 x$10^9$) cfu/g respectively with steady growth, offensive odor and dark mash sticky cotyledon at days 7 - 9. Cyclodextrin treated sample 2.5g/100g and PAC treated sample 2.5g/100g had the least odor (Barely perceived odor) 73.4% and 86.6% respectively. In all, there were 27 volatile constituents in control sample using GC-MS, 19 in CD and 10 in PAC based on their retention time. The GC-MS of the control sample had 27 volatile constituents while the sample treated with PAC (2.5g/100g) which achieved 86.6% deodorization had only 10 volatile constituents suggesting that the 17 constituents which had been removed are largely responsible for the offensive odor. Treatment with deodorants reduced antinutritional constituents like alkaloids from control 7.21mg/100g to 4.10mg/100g and 4.95mg/100g respectively for CD and PAC treated samples as well as saponins from 47.45mg/100g to 34.55mg/100g and 35.86mg/100g for CD and PAC treated samples respectively. In conclusion, the present study depicted the potentiality of CD, *and PAC as* good chemical deodorants for the deodorization of fermented food condiment from *Prosopis africana* seed with PAC having the highest deodorization potential.

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background of Study

*Prosopis* trees are woody unceasing belonging to the family *Leguminosae*. The Prosopis genus comprises about 44 species of trees and shrubs; the number could be as high as more than 70 (Balogun and Oyeyiola, 2012). The species have been distributed in North America, Central/South America, Africa, and Asia (Ogunshe *et al.,* 2007). It occurs naturally in arid and semi-arid areas where it has been used by local populations as a good source of timber, fuel, fodder, food, gum, tannin or dyestuff. In Nigeria, it is predominant in North Central geopolitical zone around Kogi, Benue, Nasarawa, Kaduna, Niger and Plateau states (Ogunshe *et al.,* 2007). It is also sparsely growing in the South East geopolitical zones. The pod of *Prosopis* spp. consists of three separable components: exo and mesocarp (pulp), endocarp and the seed (Balogun and Oyeyiola, 2012). The pods are used as food for cattle and as fish poison by fishermen. Interestingly, these pods also contain seeds which can be processed to produce local fermented food condiment called "*okpehe*" by Idoma, Igala and other middle belts tribes. It is also called *Daddawa, Iru, Ogiri* in Hausa, Yoruba and Igbo respectively (Balogun and Oyeyiola, 2012).

In many countries in Africa including Nigeria, protein malnutrition is a major problem. The food diet of Nigerians is mostly from roots, tubers and cereals. The low protein content in the Nigerian diet contributes to the low nutrition security of the people (Ogunshe *et al.,* 2007). The high cost of animal protein has also directed interest towards several leguminous seed proteins as potential sources of vegetable protein for human food (Giri *et al.,* 2010). Most of the fermented vegetable proteins reported are from leguminous seeds (Balogun *et al.,* 2017). Quite often, seeds that are used for fermentation are inedible in their raw unfermented or cooked state (Akande and Fabiyi, 2010). The seeds of legumes

may account for up to 80% dietary protein and be the only source of protein for some groups of people (Adeniyi *et al.,* 2009). Although fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians exhibit an ambivalent attitude in terms of consumer tastes and preferences for such foods (Balogun *et al.,* 2017). *Prosopis africana* seed commonly known as African mesquite but also known by different native Nigerian names such as Kirya (Hausa), Kohi (Fulani), Sam chi lati (Nupe), Ayan (Yoruba), Kpaye (Tiv), Ubwa (Ibo) and *okpehe* (Idoma) is one of the lesser known legume seed crops which can be fermented and used as a food condiment known as *okpehe* (Ogunshe *et al.,* 2007). *Okpehe* is used as a food condiment in Nigeria by the Idoma and Igala people of the middle belt region and some parts of the Eastern and Southern Nigeria. It adds variety and pleasure to the otherwise monotonous traditional diet. It serves not only as a seasoning agent but also as a low-cost source of protein in the diet (Ogunshe *et al.,* 2007). *Okpehe* can serve as a substitute for meat for lowincome earners and can reduce protein-calorie malnutrition and essential fatty acid deficiencies (Akande and Fabiyi, 2010).

**1.1.1 Local Names** English (iron wood); Hausa (kirya);

**Scientific classification**

| **Binomial name*;*** | ***Prosopis africana*** |
|---|---|
| Kingdom; | *Plantae* |
| (unranked): | *Angiosperms* |
| (unranked): | *Eudicots* |
| (unranked): | *Rosids* |
| Order: | *Fabales* |
| Family: | *Fabaceae* |
| Subfamily: | *Caesalpinioideae* |
| (unranked): | *Mimosoid clade* |
| Genus: | ***P. africana*** |

Balogun *et al.,* (2017)

*Prosopis africana* (reaches 4-20 m in height); has an open crown and slightly rounded buttresses; bark is very dark, scaly, slash orange to red brown with white streaks (Apata and Olaghobo, 1994). Foliage drooping; leaves alternate, bipinnate; rachis 10-15 cm long with 3-6 pairs of opposite pinnae (5-8 cm long); 9-16 pairs of leaflets, oblonglanceolate, 12-30 mm, pubescent; a typical gland between pairs of pinnae and leaflets. Flowers greenish-white to yellow, fragrant, in dense 6-10 cm long axillary spikes; calyx pubescent but petals glabrous; 10 free-standing stamens; anthers with a small apical gland. Pods dark brown, cylindrical, thick and hard, shiny, up to 15 x 3 cm, with woody walls, compartmented; about 10 loose, rattling seeds per pod with a thin, inter-marginal line around (Borchers and Ackerson, 2015).

## 1.2 Statement of Research Problem

Fermented food condiments remain of interest since they do not require refrigeration during distribution and storage. The traditional fermented condiments have not attained commercial status due to the objectionable packaging materials, stickiness and the characteristic putrid and unpleasant odor (Culleré *et al.,* 2010). Fermented condiments often have a stigma attached to them despite serving not only as a nutritious non-meat protein, they are often considered as food for the poor due to the off odor associated (Difo *et al.,* 2015).

Malnutrition is considered to be of public health concern in Nigeria. Global nutrition survey indicates that deficiency in Macro and Micronutrients are most prevalent, because the sources of essential nutrients are expensive and beyond the reach of majority of the population (Difo *et al.,* 2015). Consumptions of monosodium glutamate and salts that are rich in sodium as flavour enhancers has been associated with health related cardiovascular disorders, thus there is need to substitute them with natural fermented condiments (Culleré *et al.,* 2010).

## 1.3 Justification

There is paucity of data on deodorization potential of cyclodextrin (CD) and powdered activated carbon (PAC) on fermented food condiments from *Prosopis africana* seeds. Fermented food condiments remain of interest since they are cheap, easily accessible and affordable by low income earners. However, acceptability of such condiments by the consumers is of serious challenge due to the offensive odor associated with them (Balogun *et al.,* 2017). Therefore, removing or reducing the odor may increase its acceptability and market range to people thus provide a cheap and healthier alternative to monosodium glutamate.

## 1.4 Aim and Objectives

### 1.4.1 Aim

The aim of this research study was to deodorize the fermented food condiments (*Okpehe*) from *prosopis africana* seeds and determine its nutritional composition.

### 1.4.2 Specific Objectives

The specific objectives of this research are to;

i. Prepare and deodorize the laboratory prepared fermented food condiment from *Prosopis africana* seeds with potential chemical deodorants**;** β-*Cyclodextrin, and powdered activated carbon.*

ii. Determine the effect of deodorizing agents on volatile fatty acid constituents of deodorized fermented condiment.

iii. Determine the effect of deodorizing agents on nutrient and antinutrient compositions of deodorized fermented condiment.

iv. Evaluate the physicochemical characteristics of the deodorized fermented condiment.

## 1.5 NULL HYPOTHESIS

There is no significant effect of potential chemical deodorants; cyclodextrin, and powdered activated carbon on the off-odor, nutrients and antinutrients of fermented food condiment from *Prosopis africana* seeds.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Nutritional Quality of *Okpehe*

Report shows that protein and carbohydrate have to be the most abundant nutritive macroelements, while lipids, ash and fiber occurred in minimal quantities (Balogun *et al.,* 2017). The crude protein of *okpehe* samples ranged between 36.88 - 40.05% (Ogunshe *et al.,* 2007). Alkaloids, steroids, flavonoids, saponins, resins and phenols were found to be present while anthraquinones, triterpenoids, tannins and oxalate were found to be absent *okpehe* as reported by Ogunshe *et al.,* (2007). The quantitative analysis of antinutritional factors in *okpehe* revealed the most abundant antinutritional factor to be saponins, with concentration ranging between 0.72-1.10% (Dirar, 1993). Alkaloids, steroids, flavonoids, resins and phenols were found in considerably low concentrations. Report shows that potassium was the most abundant element 2.101 - 3.496 mg/g in *okpehe*, while Copper, iron, calcium, manganese and zinc were found in relatively minute quantities, in addition lead and cadmium were found below detection limits in *okpehe* (Omafuvbe *et al.,* 2000).

### 2.2 Volatile Constituents of Local Fermented Condiment *Okpehe*

A report shows a total of 51 constituents were identified in *okpehe* (Onyenekwe *et al.,* 2014). On weight basis the aldehydes are the dominant group. The alkanolic constituents of *okpehe*, with the major alkanolic constituents such as decanol, octanol, ethanol, propanol, and dodecanol (Onyenekwe *et al.,* 2012). The alkanols present in the condiment help prevent *okpehe* from spoilage since alkanols are known to act as antifungal and prevent food spoilage. Most of these alkanols have been reported to be present in other fermented leguminous products (Dajanta *et al.,* 2011) It was also reported that *okpehe* contains 15 aldehydes, with hexanal, heptanal, 2-nonanal,

nonanal, 2,4 decadienal, Decanal, dodecanal, 2,4 – nonadienal, 2-butyloctenal, 2, 4-decadienal been the major.

The odor in the fermented food condiment could be due to the presence of these aldehydes (Onyenekwe *et al.,* 2014). Carbonyl compounds such as aldehyde and alcohol have strong impact on odor because of their sensitivity to olfactory receptors (Ullrich and Grosch, 1987). Hexanal, the dominant aldehyde, is a key organoleptic element of note that is found in both fragrances and flavours (Skrede *et al.*, 2002), such as traditional commercial shrimp paste kapi (Wittanalai *et al*., 2011) and fermented soybean, melon and locustbean (Onyenekwe *et al*., 2012). Hexanal is produced during advanced lipid oxidation, promoted by alkyl and alkoxyl radicals (Abdalla and Roozen, 1999). The effectiveness of hexanal as a metabolizable fungicide and enhancer of aroma production by its inter conversion to other aroma volatiles in minimally processed apples has been demonstrated (Song *et al.*, 1996; 1997). Report also shows that about eight ketones were identified and quantified in the fermented condiment *okpehe* from *Prosopis africana* and these include acetophone, acetophenone, 2-nonadecanone, 3-ethylhexanone, 3-ethyloctanone, ethylnonanone, 2,5-hexadione and 3-ethythepentanone (Ullrich and Grosch, 1987). Ketones are usually derived from lipid and amino acids degradation during microbial fermentation and have a high impact on food odor (Owens *et al*., 1997), ketones may contribute to the odor of *okpehe*.

Reports also shows that the ester constituents of the condiment, the higher concentration of esters compared with the levels reported by Onyenekwe *et al*. (2012) in ogi (fermented melon seed) and daddawa (fermented locustbean and soybean seeds) may be responsible for the ‘fruitiness’ associated with *okpehe*. The acetates of higher alcohols and the ethyl ester of fatty acids had been suggested to be the most desirable compounds in miso products to enhance the aroma of the

finished products and are responsible for the fruity tinge of freshly prepared miso (Giri *et al.*, 2010). Another report shows that the major benzene derivative present in the food condiment include toluene, benzaldehyde, benzeneacetaldehyde, 1, 2 – dimethyl benzene, 2 – ethyl 5 – methyl pyrazine, 2, 6 – dimethyl pyrazine, trimethyl pyrazine, tetramethyl pyrazine 2, 5 – dimethyl pyrazine, 2, 6 – diphenyl pyrazine (Sugawara *et al.,* 1985). Apart from its sensory attributes, tetramethyl pyrazine has been shown to have antioxidant activity (Wang *et al.*, 2012). This coupled with the fungicidal and bactericidal activities of other constituents may be responsible for non-spoilage of this condiment no matter how long it is stored without refrigeration (Afoakwa *et al.*, 2009).

**Plate 1: *Prosopis africana's* leaves and fruit**

**Sources:** www.westafricanplants.org

**Plate 2: (a): *Prosopis africana* pods and (b): *Prosopis africana* seeds**

Sources: www.westafricanplants.org

## 2.3 Traditional Preparation of *Okpehe* Condiment

The required quantity of harvested and dried seeds of *Prosopis africana* are soaked overnight and later boiled in a large earthen-ware pot for about 1-2 days with kerosene stove or fire wood, during which the seed coats become soft and the seeds swollen. Seed coats are removed by either pounding the boiled seeds in a big mortar for faster removal of the seed coats or by pressing between fingertips. The seeds coats are later decanted along with the washing water, leaving the clean seed cotyledons. The cotyledons are boiled again for another 1-2 hrs with fire wood. The cotyledons are later drained through a sieve and wrapped with paw-paw leaves, or clean cement papers. The wrapped cotyledons are stacked together and then covered by nylon. These are then kept in an incubating unit for about 5-6 days to produce the fermented *Prosopis* mash – *okpehe*, a strong-smelling mash of sticky brown cotyledons. (Ogunshe, *et al.,* 2007).

## 2.4 Laboratory Preparation of *Okpehe* Condiment

The boiling step in the traditional process is long tedious and wasteful; it also requires more volume of water. This boiling by fuel (kerosene) or fire wood was replaced boiling in an autoclave in the laboratory. At each stage 1kg of *Prosopis africana* seeds are boiled at 121°C for 2 hr in an autoclave and later dehulled. The cotyledons were separated from the coats and later rinsed in sterile water, before boiling again at same temperature in an autoclave for about 30 minutes to soften the cotyledons. The cotyledons are later drained through a sterile sieve and cooled to 35°C before wrapping in paw-paw leaves already cleaned with alcohol. The wrapped cotyledons are then incubated in an incubating unit for 5-6 days to produce the usual fermented mash of *Okpene* condiment (Ogunshe *et al.,* 2007; Balogun *et al.,* 2017).

Microorganisms associated with the production of *Okpehe* from *Prosopis africana* seeds are *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Staphylococcus aureus, Escherichia coli* and *Saccaromyces cerevisiae*. These microorganisms increased in number as fermentation progressed (Balogun *et al.,* 2017)

## 2.5 Potential Chemical Deodorants

These are compounds that encapsulates odor components into its cavities, and subsequently deodorizes the unpleasant odor. Some deodorants can oxidise into a quinone type structure (Onyenekwe *et al*., 2012). The phenolic groups react with compounds containing sulfur or nitrogen and eliminate the odor. This type of reaction is called chemical deodorisation. Some of these deodorants includes; cyclodextrin (CD), tert-butylhydroquinone, butylated hydroxyanisole (BHA), powdered activated carbon (PAC) and polyphenol oxidases (PPO).

### 2.5.1 Cyclodextrin (CD)

These are a family of cyclic oligosaccharides composed of α-(1,4) linked glucopyranose subunits. Cyclodextrins are useful molecular chelating agents. Cyclodextrins are cyclic oligosaccharides consisting of six α-cyclodextrin, seven β-cyclodextrin, eight ϒ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds (Harata *et al.,* 1981).

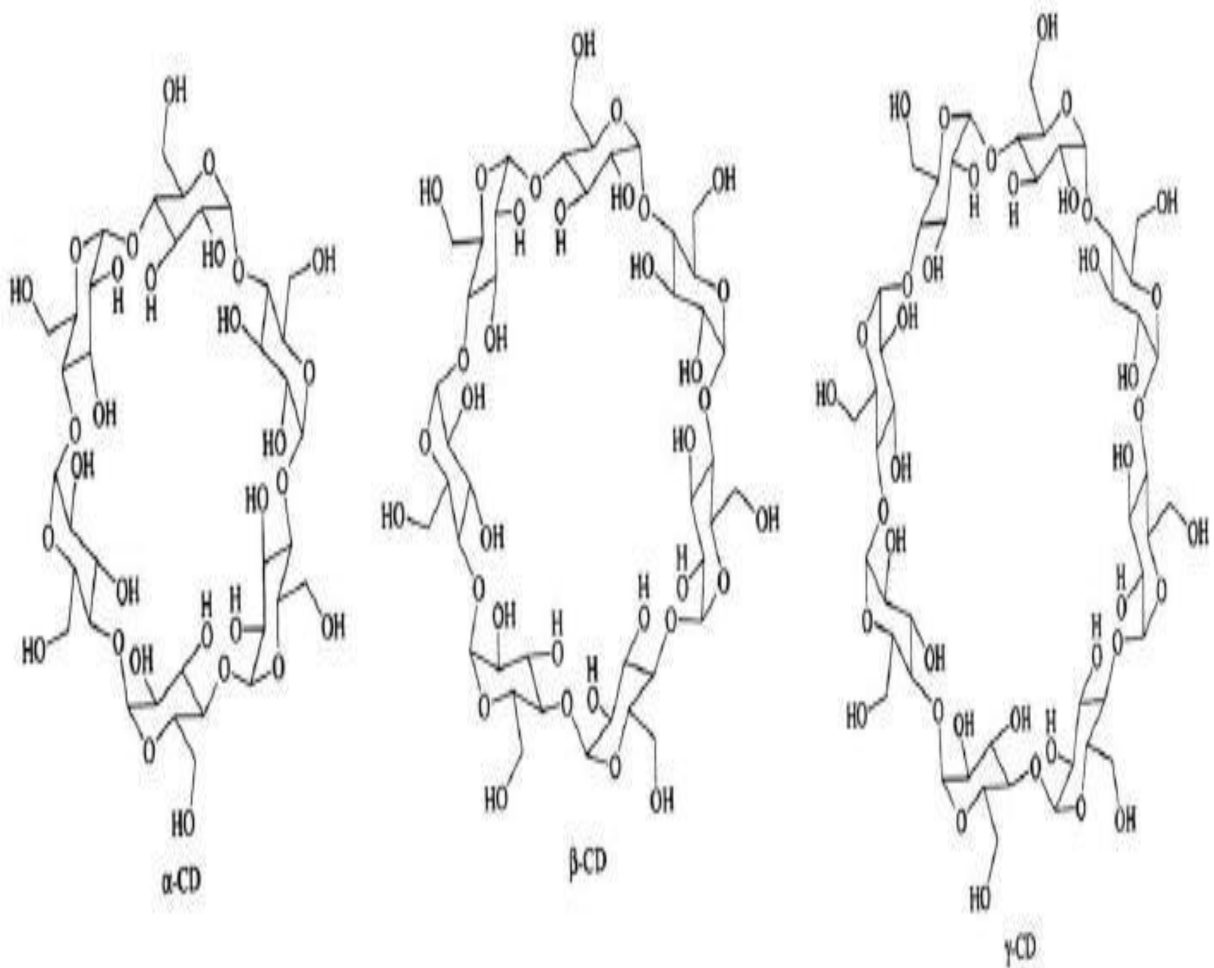



**Plate 3: Structures of α, β, and γ-cyclodextrins**

**Sources:** Harata *et al.,* (1981).

Cyclodextrins possess a cage-like supramolecular structure, which is the same as the structures formed from cryptands, calixarenes, cyclophanes, spherands and ethers (Horvat *et al.,* 1991). These compounds having supramolecular structures carry out chemical reactions that involve intramolecular interactions where covalent bonds are not formed between interacting molecules, ions or radicals (Harata *et al.,* 1981). The majority of all these reactions are of 'host– guest' type. Compared to all the supramolecular hosts mentioned above, cyclodextrins are most important (Tamaki *et al.,* 2007). As a result of molecular complexation phenomena. CDs are widely used in many industrial products, technologies and analytical methods. The negligible cytotoxic effects of CDs are an important attribute in applications such as rug carrier, food and flavours, cosmetics, packing, textiles, separation processes, environment protection, fermentation, odor control and catalysis (Tamaki *et al.,* 2007).

### 2.5.2 Powdered Activated Carbon (PAC)

Activated carbon is considered as crude form of graphite. But unlike graphite structure, it has random imperfect structure with macro and micro pores. The graphite structure gives the activated carbon it's very large surface area. Three (3g) of activated carbon can have a surface area of a football field. Activated carbon has the strongest physical adsorption forces The adsorbility of activated carbon increases with increasing molecular weight, higher number of functional group compounds, increasing polarizability. The surface area of Activated Carbon varies from 500 $m^2/g$ to 2000 $m^2/g$ (Wood, 1992). Total Pore Volume (TPV) refers to the total space of the pores in an Activated Carbon. The effectiveness of Activated Carbon is increased with increase of total pore volume. It is expressed in milliliters per gram(ml/g). The significance of Activated Carbon is its unique distribution of pore sizes. For decolourisation applications high distribution of mesopore is required.

Selection criteria for activated carbon includes resistance to abrasion, adsorption capacity, particle size distribution for optimal flow, rate of adsorption and desorption, consistency of product in applications and presence of other substances that may affect or interfere with loading of carbon (Bansal and Goyal, 2005). Activated carbon can be manufactured from many materials containing high carbon content such as coconut shell, wood, coal, coir, lignite, peat, petroleum pitch etc. Carbonaceous materials can be activated using two methods, steam activation and chemical activation (Wood, 1992).

#### 2.5.2.1 Steam Activation

Activated carbon is mainly used to adsorb organic compounds. Some large molecular weight inorganic compounds such as iodine and mercury can also be adsorbed. In this process the material is activated with steam at very high temperature. The chemical reaction between the carbon and the steam takes place at the internal surface of the carbon. Steam activation enlarges the pore structure and thus increases the internal surface area. It is the most widely used process to activate carbonaceous materials. It is considered as the best pollution free method of activation (Wood, 1992).

#### 2.5.2.2 Chemical Activation

This type of activation is only useful for the carbonization of wood, sawdust or peat. In chemical activation, the raw material is mixed with an activating agent such as phosphoric acid that swells the material and opens up the cellulose structure. It is then carbonized and dried in a rotary kiln at low temperature. It dehydrates the raw material resulting in the amortization of the carbon, thereby creating high porous structure. Steam activation method is preferred since it is the most environmentally friendly method of activation process (Bansal and Goyal, 2005).

## 2.6 Dehulling

It has also been shown that the effect of tannins can be eliminated or neutralized by dehulling (Edwards and Duthie, 1973; Ward *et al*., 1977). Bressani (2002) reported that dehulling improved protein quality of *Phaseolus vulgaris* and suggested that this could be due to the removal of the seed coat tannins which may have caused decrease in protein digestibility. When practically feasible, the recommended method for removal of condensed tannins is dehulling to remove the tannins rich seed coat.

## 2.7 Autoclaving

Autoclaving entails boiling under pressure. The time of cooking is shortened by this method. When leguminous seeds were autoclaved for 30 minutes at 125°C and 15 Ib pressure, thermolabile inhibitory substances such as cyanogenic glycosides, saponins, terpenoids and alkaloids could not be detected after autoclaving (Udedibie and Nwaiwu, 1988). The nutritive value of many legumes is enhanced by autoclaving and this effect is probably related to the destruction of haemagglutinins and other inhibitory factors. Preliminary soaking prior to autoclaving is required for complete elimination of the toxicity of kidney bean (Akande and Fabiyi, 2010). Kakade and Evans (1965) found that autoclaving for 5 min was sufficient to eliminate the toxicity of finely ground navy bean meal. Kessler *et al*. (1990) stated that there was little nutritional advantage in autoclaving for more than half an hour. They reported that autoclaving of jackbeans was a satisfactory technique for ensuring survival of birds receiving jackbean diets, confirming the findings of Jayne-Williams (1973). The absence of lesions on the small intestine cells of broilers fed autoclaved jackbean suggest the elimination of the lectins during the autoclaving of the jackbeans. Autoclaving is a more sophisticated, but effective method of processing seeds for eliminating antinutrients (Abeke and Out, 2008). However,

autoclaving is expensive, it requires adequate supply of electricity and technical knowledge. Pressure cooking**:** Udedibie et al., (1996) stated that since it is a common practice to use a pressure cooker to cook most legume grains to save time and cost, they decided to determine how long it would take the pressure cooker to completely inactivate the concanavalin A and trypsin inhibitors in *Canavalia ensiformis*. The beans were subjected to four different pressure cooking times: 15, 30, 45 and 60 mins. It took 30 mins of pressure cooking to completely inactivate the trypsin inhibitor in *Canavalia ensiformis* while concanavalin A required 45 min for complete inactivation, establishing the fact that concanavalin A was more resistant to heat treatment than trypsin inhibitor.

## 2.8 Fermentation

Fermentation is an age-long method of processing cereals and legumes (Siegel and Fawcet, 1978). It modifies some physical characteristics of cereals and legumes, increases the level of some nutrients, digestibility and bioavailability (Ojokoh, 2014), decreases levels of antinutrients and increases nutrient density (Nnam, 1999) and imparts some antimicrobial property (Mensah *et al*., 1990). According to Quinn *et al*. (1975), fermentation of grains and oil seeds results in increased nutritional value and wholesomeness over the starting material and it may also lead to changes in vitamin levels. Food fermentation, and especially lactic acid fermentation, is an important technology in Africa. Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some plant foods. The traditional processing of these foods therefore needs to be changed or modified to improve their nutritional status.

Fermentation of grains has been reported to yield a significant reduction in concentration of both phytic acid (Marklinder *et al*., 1996; Mukhopadhyay and Ray, 1999; Skrede *et al*., 2002) and α-galactoside oligosaccharide (Skrede *et al*., 2002; Rhabasa and Chiasson, 2004). Sandberg (1991) and Hurrell (2004) reported that fermentation can induce phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates. They further assert that such hydrolysis is important because myoinositol phosphates with five phosphate groups (i.e., IP1 to IP-4) do not have a negative effect on zinc absorption, and those with 3 phosphate groups do not inhibit non-heme iron absorption. Microbial phytases originate either from the micro-flora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg, 1991). Low-molecular-weight organic acids (e.g., citric, malic, lactic acids) are also produced during fermentation and have the potential to enhance iron and zinc absorption via the formation of soluble ligands while simultaneously generating a low pH that optimizes the activity of endogenous phytase from cereal or legume flours (Teucher *et al.,* 2004). Pusztai (1989) reported that fermentation helps to reduce raffinose oligosaccharides due to the alpha-galactosidase found in the bean seeds, and also phytic acid content due to the action of phytase. Microorganisms which are responsible for the fermentation also play a role (Pusztai, 1989).

Similarly, in germination, most of the changes occurring during the fermentation of foods are of catabolic nature, and they help in the hydrolysis of such components as proteins and carbohydrates. Fermentation of foods can also result in significant reduction in the quantity of certain antinutrients. Depending upon the type of legume as well as fermentation, phytic acid is also hydrolysed during fermentation to a variable degree (Deshpande, 2002). Fermentation of cereals reduces phytate content via action of phytase that catalyses the conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phosphate. A

3-phytase appears to be characteristic of microorganisms, while a 6-phytase is found in cereal grains and other plant food (Shahidi, 1997). According to study by Vin (2009), consumption of fermented food has been shown to improve the intestinal balance of beneficial lactic acid bacteria. Based on this and the phytase producing capability of the bacteria, the consumption of fermented foods may be another effective way to reduce the potential of dietary phytic acid impairing mineral absorption (Vin, 2009).

### 2.9 Biochemical Effects of Antinutrients in Legumes

The antinutritional factors can be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (such as inactivation of some nutrients or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992). The biochemical and adverse effects of plant's secondary metabolites (antinutritional factors) have been reviewed by several authors (Cheeke and Shull, 1985; Aletor, 1991; Fu *et al*., 2002). The adverse effects of antinutrients are as follows; Antinutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992). Oxalate, phytate and tannins are antinutrients, which could be toxic when consumed in an unprocessed food (Ojiako and Igwe, 2008). The bioavailability of the essential nutrients in plant foods could be reduced by the presence of some antinutritional factors such as oxalates and cyanogenic glycosides (Akindahunsi and Salawu, 2005).

#### 2.9.1 Cyanogenic glycosides

Cyanogenic glycosides on hydrolysis yield toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems, it depresses growth through interference with certain essential

amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Osuntokun, 1970; Fernando, 1987).

### 2.3.2 Alkaloids

Alkaloids cause gastrointestinal and neurological disorders (Aletor, 1993). The glycoalkaloids, solanine and chaconine present in potato and *Solanum spp*. as reported by Saito *et al*. (1990) and Aletor (1991) alkaloaids are haemolytically active and toxic to fungi and humans. Some of the toxicological manifestations of potato glycoalkaloids involve gastrointestinal upsets and neurological disorders, especially in doses in excess of 20 mg/100 g sample.

### 2.9.3 Tannins

Tannins are complex polyphenolic compounds found widely in the plant kingdom (Hagerman and Butler, 1978). They are found in leaves, twigs, flowers, fruits and tree barks. Tannins are usually subdivided into two major groups, the hydrolysable and condensed tannins.

#### 2.9.3.1 The hydrolysable tannins

These are split into sugars and phenolic carboxylic acids in acidic and alkaline conditions (White, 1957) and are further classified according to the products of hydrolysis into gallo tannins (gallic acid and glucose) and ellagic tannins (ellagic acid and glucose) (Mcleod, 1974). Two other categories, tara-gallo tannins (gallic and quinic acid plus glucose) and caffe-tannins (caffeic acid and quinic acid plus glucose) have also been suggested (Haslam, 1966). Hydrolysable tannins can be hydrolyzed upon heating by weak acid, weak base or enzymatically (Gemede and Ratta, 2014).

#### 2.9.3.2 Condensed tannins

Condensed tannins are referred to as proanthocyanidins because they produce red anthocyanidins when heated in acid (Haslam, 1966). Proanthocyanidins are phenylpropanoid polyphenols and are categorized by the type of monomer they contain: either flavan-3-ols or flavan-3,4-diols into catechin or lecoanthocyanidin (Horvath, 1981). Generally, the high molecular weight condensed tannins (i.e >10 flavonol units) have limited solubility and extractability and hence may have little nutritional significance (Mehansho *et al.*, 1987). Condensed tannins polymerize on heating in acids to amorphous phlobaphenes (Swain, 1979).

#### 2.9.3.3 The beta tannins

Beside hydrolysable and condensed tannins, beta tannins can be added (Swain, 1979; Horvath 1981). Beta-tannins are protein precipitating compounds which are insoluble in water. They form very stable bond with protein and appeared to have lower molecular weight than other tannins (Woodward and Reed, 1989). Tannins cause decreased consumption of feed in animals by binding dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). The precipitation can be as a result of hydrogen bonding (Loomies and Battaile, 1966), covalent bonding (Swain, 1979), ionic bonding or hydrophilic interaction (Oh *et al.,* 1980). The interaction between tannins and protein is very specific (Hagerman and Butter, 1981) and dependent on their molecular weight (Kumar and Horigome, 1986), water solubility (Addisu, 2016), conformation, compatibility of binding site and the solvent pH (Wooward and Reed, 1989) showed that condensed tannins seem to be more important in forming complex in feed than hydrolysable tannins. The effect of tannins on quality of forage includes reduction in palatability and feed intake of feeds in animals and also cause decrease in palatability and reduced growth rate (Roeder, 1995).

### 2.9.4 Saponins

The saponins are naturally occurring surface-active glycosides which are composed of carbohydrate and non-carbohydrate or aglycone portion. The aglycones are often referred to as sapogenins. The sapogenin nucleus may be either of steroid or triterpenoid structure. They are mainly produced by plants, but also by lower marine animals and some bacteria (Yoshiki *et al.,* 1998). They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature (Das *et al.,* 2012). The aglycone may contain one or more unsaturated C–C bonds. Saponins cause hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats (Johnson *et al*., 1986). Saponins from *Bulbostermma paniculatum* and *Pentapamax leschenaultii* have also been reported to have anti-spermal effects on human spermatozoa (Das *et al.,* 2012). They significantly inhibite acrosine activity of human sperms and the spermicidal effect might be attributed to strong damage of the spermal plasma membrane (Johnson *et al*., 1986).

### 2.9.5 Trypsin inbitors

Trypsin (a protese inhibitor) causes pancreatic enlargement and growthdepression (Aletor and Fetuga, 1987). Proteins like haemagluttinins are known for agglutinating red blood cells. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract (Aletor, 1991).

### 2.9.6 Phytates

Among all the antinutritional components, phytic acid is of a major concern for human nutrition and health management. The unique structure of phytic acid (myoinositol (1,2,3,4,5,6) hexakisphosphoric acid) offers it the ability to strongly chelate cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts. It therefore adversely affects the absorption and digestion of these minerals by animals (Raboy, 2001). Salts of phytic acid contains mono and divalent cations such as $K^+$, $Mg^{2+}$ and $Ca^{2+}$ which accumulates in the seeds during ripening and are the main storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2002). Besides, phytate has also been reported to form complexes with proteins at both low and high pH values. These complex formations alter the protein structure, which may result in decreased protein solubility, enzymatic activity and proteolytic digestibility. In spite of many negative aspects on human health, the consumption of phytate, however, has been reported to have some favourable effects, including its anticarcinogenic effect (Shamsuddin, 2002). The metal binding characteristics of phytate endow it with an antioxidant function, inhibiting the production of hydroxyl radicals that normalise cell homeostasis (Minihane and Rimbach, 2002) and it also acts as a natural food antioxidant (Raboy, 2003). Dietary phytate may have health benefits for diabetic patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying (Thompson, 1993). Likewise, phytate has also been shown to regulate insulin secretion (Barker and Berggren, 1999). It is believed that phytate reduces blood clots, cholesterol and triacylglycerols and thus prevents heart diseases (Jariwalla *et al*., 1990). It is also suggested that it prevents renal stone development (Selvam, 2002). Phytates is, therefore a common constituent of plant derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans on vegetarian diets, on an average,

is 2000–2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150–1400 mg (Reddy, 2002).

### 2.9.7 Oxalates

Oxalates like phytates also bind minerals like calcium and magnesium and interfere with their metabolism (Selvam, 2002). They also cause muscular weakness and paralysis. Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia (Adeniyi *et al.,* 2009). Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate binds the calcium ions to form insoluble calciumoxalate complexes (McDonald *et al.,* 1995). As a result of this, people with the tendency to form kidney stones are advised to avoid oxalate-rich foods (Adeniyi *et al*., 2009).

### 2.9.8 Alpha amylase

Alpha amylase is responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise (Rhabasa and Chiasson, 2004).

## 2.10 Nutritional Effect of Mineral Elements in Plants

Calcium, according to Vasudevan and Sreekumari (2007) is highly recommended for activation of enzymes, mediation of muscle excitation and contraction, bone and teeth formation, blood coagulation, transmission of impulses, regulation of heartbeat, maintenance of acid-base equilibrium in the body of animals and reduction of allergic exudates. Phosphorus plays more known functions in the body than other mineral elements (McDonald *et al.,* 1995). It associates

with Ca in bone and teeth formation, involved in energy metabolism and low dietary intakes are associated with infertility, poor growth and live weight gain in livestock (McDonald *et al.,* 1995). Phosphorus in the form of phosphate ion is needed for, production of high energy compounds e.g. ATP, DNA and RNA formation and activation of enzymes.

Sodium is present in the soft tissues and body fluids. It is associated with acid base equilibrium. A chief cation of blood plasma, it functions in nerve impulse transmission and absorption of sugars and amino acids from the gut. A deficiency of sodium leads to body dehydration, poor growth and reduced utilization of digested proteins (McDonald *et al*., 1995). Sodium also helps in maintaining body fluid pH, support nerve transmission and muscular contraction in livestock (McDonald *et al.,* 1995). Magnesium is necessary for efficient metabolism of carbohydrates and lipids, involved in cellular respiration and general cellular biochemistry and function (McDonald *et al.,* 1995). As reported by Vasudevan and Sreekumari (2007), beans and other vegetable foods like cereals are rich in Mg. Mg is also important in lowering the irritability of neuromuscular system and activates enzymes like phosphatase which require ATP (Banerjee *et al.,* 2004).

The requirement for Fe is most critical in young suckling animals because milk is a poor source of Fe, the deficiency which causes iron deficiency anemia (Vasudevan and Sreekumari, 2007), which could be prevented by feeding nursing animals with diets rich in Fe. Iron content was high compared to 11.5 and 8.4mg/100g for soy bean and mung beans (Vasudevan and Sreekumari, 2007). This indicates the possibility of *Senna obtusifolia* to feed livestock, it would improve transport of oxygen to the tissues as well as enzymes of electron transport chain (McDonald *et al.,* 1995). Chatterjea and Shinde (2007) reported that legumes, pulses, oil seeds and un-milled cereals are good sources of Zn. Zn is an antioxidant and aids in Cu absorption in some disease conditions. Many enzymes are dependent on Zn for proper functioning. This implies that feeding

animals with high level of Zn could improve electrolyte balance; hence production, storage and secretion of hormones; improve immune system and prevent parakeratosis, a skin ailment (McDonald *et al.,* 1995). Potassium is necessary for nerve transmission, maintenance of osmotic pressure and acid-base equilibrium, activation of certain enzymes uptake of certain amino acids as well as carbohydrates and protein metabolism (Banerjee *et al.,* 2004). The inclusion of Cu in livestock diets could protect the heart by increasing high density lipoprotein (HDL) tyrosinase activity and Fe absorption and incorporation into haemoglobin (Minihane and Rimbach 2002).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1. Materials

#### 3.1.1. Equipment and Reagents used

| S/N | Equipments | Reagents |
|---|---|---|
| 1 | Autoclave | Cyclodextrin (CD) |
| 2 | Petri dishes | Powdered Activate Carbon (PAC) |
| 3 | Oven | Nutrient Agar media |
| 4 | Incubator | Per-chloric Acid ($HCl0_4$) |
| 5 | Sieve | Dimethyl-sulfur-oxide (DMSO) |
| 6 | Bunsen burner | Tetraoxosulphate (IV) Acid ($H_2S0_4$) |
| 7 | 100ml Beaker | 4M Hydrochloric Acid HCl |
| 8 | 10ml Beaker | Petroleum Ether |
| 9 | 10ml Test tube | Diethyl ether |
| 10 | Kjeldahl apparatus | Chloroform $Ch(Cl)_3$ |
| 11 | Conical flask | Retinyl-acetate (Vitamin A standard) |
| 12 | Erlenmeyer Flask | α-tocopherol (Vitamin E standard) |
| 13 | Water bath | 5M Ammonium Hydroxide |
| 14 | Desiccator | Potassium permanganate ($KMnO_4$) |
| 15 | 100ml Volumetric flask | Potassium ferro cyanide |
| 16 | UV-Spectrophotometer | 20% Ethanol |
| 17 | UV-Cuvatte | 80% aqueous Methanol |

| 18 | Whatman #4 Filter paper | Sodium phosphate |
|---|---|---|
| 19 | Atomic Absorption Spectrometer | Phenolphthalein indicator |
| 20 | Sample Container | Sodium hydroxide |

**Note;** All chemicals and reagents used were of analytical grade**.**

### 3.1.2. Collection of seed samples

The *Prosopis africana* seeds and leaves were purchased from the new Monday market, popularly known as Kasuwan Amaru/new Katafawa market in Zaria city and identified with voucher number; V/N 06919 in Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

## 3.2 Methodology

### 3.2.1 Laboratory Preparation of Food Condiment

The boiling step in the traditional process is long, tedious and wasteful; it also requires more volume of water. Thus boiling by fuel (kerosene) or fire wood was replaced by boiling in an autoclave in the laboratory. Two kilograms (2kg) of *Prosopis africana* seeds were boiled in 2000ml of water at 121$^{o}$C for 2h in an autoclave and later dehulled. The cotyledons were separated from the coats and later rinsed in sterile water, before boiling again in an autoclave for about 30 min to soften the cotyledons. The cotyledons were drained through a sterile sieve and cooled to about 35$^{o}$C, then wrapped in paw-paw leaves already disinfected with alcohol and wrapped in a foil. The wrapped cotyledons were then incubated in an incubator for 5-9 days to produce the fermented mash of food condiment. A modified method of Balogun *et al.,* (2017).

### 3.2.2 Serial Dilution and Microbial Counts Determination

Serial dilution was carried out by weighing 1g of laboratory fermented condiment and dissolved into 90ml of diluents water to form a stock culture ratio (1:90). From the stock solution 1ml was transferred into 9ml sterile distilled water, 1ml diluents from the stock culture ratio (1:90) was transferred into 9ml sterile distilled water (1:9) making it to dilution factors of $10^1$, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, and $10^9$ respectively. Also 1ml each from dilution factors of $10^1$, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, and $10^9$ were inoculated in nutrient agar plates and incubated at 37˚C for 24h. The bacterial populations of the fermented condiment samples were estimated from a pour plate technique (Omafuvbe *et al.,* 2000).

$$\text{Colony Forming Unit Per gram (cfu/g)} = \frac{\text{Total number of Colony counted x Dilution Factor}}{\text{Volume of Inocula}}$$

The stock preparation and process were repeated every day for nine consecutive days.

### 3.2.3 Deodorizing Process

The powdered activated carbon (PAC) had a surface area of $1077m^2/g$, specific pore volume of 0.64ml/g and an average pore diameter of 2.37nm. A varying amount of (0.5, 1.0, 1.5, 2.0, and 2.5g) of deodorizing agents: cyclodextrin and powdered activated carbon in 100g of fermented condiment each of the deodorizing agents were mixed with the sample fermented condiment and deodorized the off odor separately for 24h at room temperature (Onyenekwe *et al.,* 2012).

Fermented condiments were grouped as follows:

GROUP 1: Fermented condiment without treatment (Negative control)

GROUP 2: Fermented condiment mixed with cyclodextrin

GROUP 3: Fermented condiment mixed with powdered activated carbon

### 3.2.4 Odor Ranking Test

Descriptive word category scale method was applied and it determines how the samples differ on the basis of intensity of the off odor. Odor was observed by sniffing; odor testing was conducted within a nominal 24h time period after deodorization. A panel of fifteen (15) trained panelists were set up, each panelist was presented with coded samples and asked to rank according to their preference.

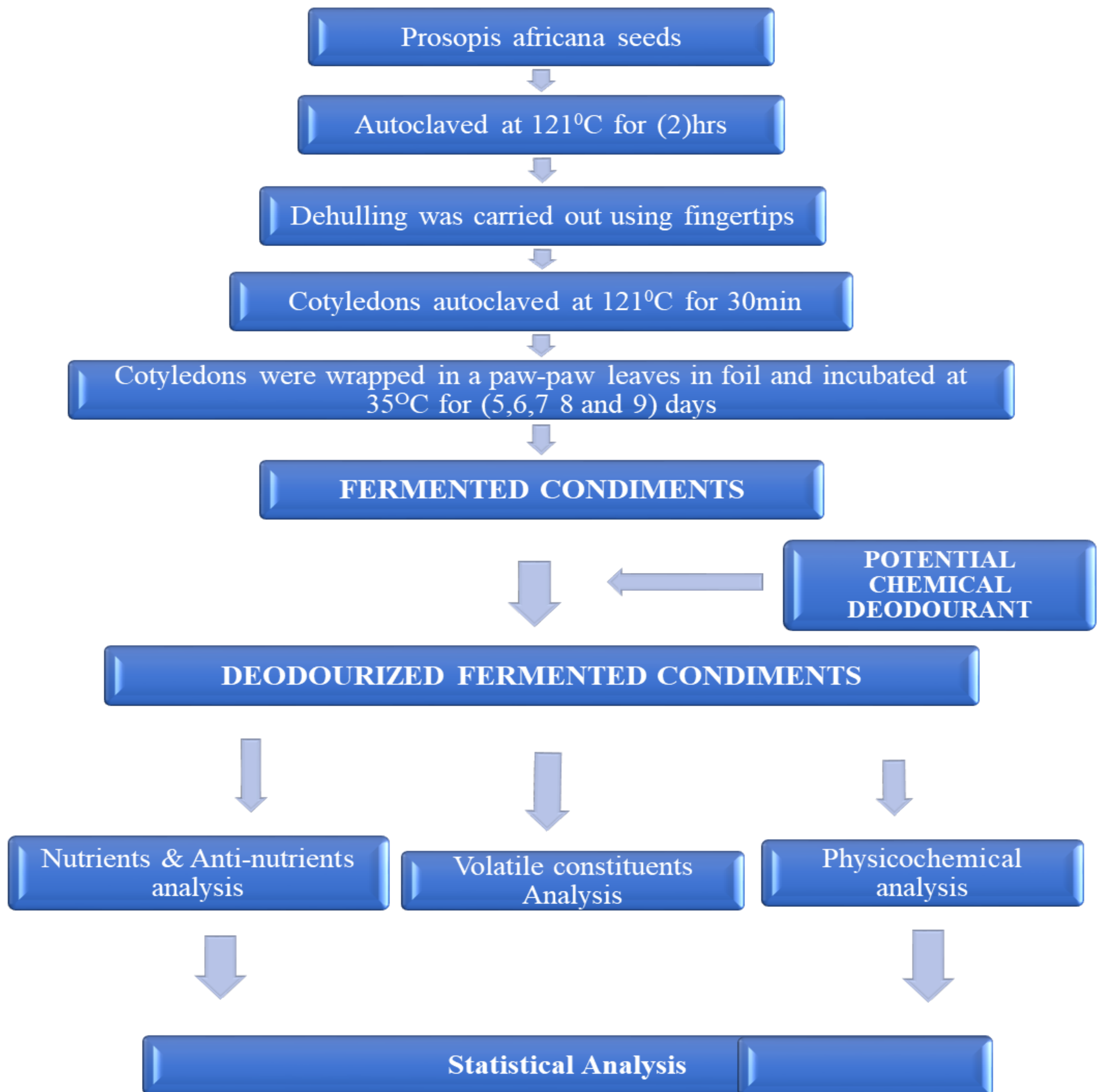



**Figure 3.1; Flow Chart of Laboratory Preparation and Deodorization of Fermented Condiment from *Prosopis africana* Seeds *Okpehe***

Judging was done individually, this assures independent judgments and communication between panel members was not allowed except for consultation with the panel observer on any point of

doubt. The time for odor ranking test was between morning 10.00 am to 12.00 noon on 23$^{rd}$ March 2020.

Descriptive word category scale was used to give the assessors to rate the odor on a scale, using 5-point scale as follows:

1 ______Odorless

2 ______Barely perceives odor

3 ______Mild off odor

4 ______Moderate odor

5 ______Strong odor

A score card was developed for this experiment. All score cards contain date and name of the judge as described by Ibe and Orabuike (2009).

### 3.2.5 Identification of Volatile Components of Deodorized Fermented Condiment from *Prosopis africana* Seeds

The volatile constituents were analyzed by gas chromatography using the PDMS-SPME head space technique. To extract the volatile constituents, 5g of the mashed deodorized fermented condiment was transferred into capped glass and 100% Dimethylsulfuroxide (DMSO) was added for extraction. The mixture was incubated in an oven for 30 min at a temperature of 37$^{o}$C. The constituents of the extract were analysed using GC-MS by direct injection method in the split mode with (split ratio 20:1) under the following conditions; Hewlett – Packard 6890 GC equipped with an ionization detector and a quartz capillary column; 30 m x 0.25 nm x 0.25 nm, nitrogen was used as carrier gas, hydrogen pressure and compressed air were 28 and 35 psi, respectively. A digital integrator was then used to integrate the area of the signal from the detector. The integrated area, retention time and constituents were printed automatically at the end of each peak. The

identification of the different constituents in the extract was performed by the comparison of their retention times and mass spectra with those of the database of National Institute Standard and Technology (NIST) having more than 32,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The names of the components of the test materials were ascertained (Onyenekwe *et al.,* 2014).

### 3.2.6 Proximate Analysis

Proximate analysis was carried out on deodorized fermented condiment to determine the nutritional values with regards to moisture, ash, crude protein, fat, crude fiber and total carbohydrate, using standard methods as described by AOAC (2006).

#### 3.2.6.1 Determination of Moisture content

Moisture evaporation, oven drying method was used to determine the moisture content of the deodorized fermented condiment, one hundred grams (100g) of the sample was heated at (70-100°C) for 1–2h to evaporate the water, with weight loss equal to the moisture content, measured in percentage. The moisture content was determined by the formula:

$$\% \text{ Moisture} = \frac{M_{INITIAL} - M_{DRIED}}{M_{INITIAL}} \text{ X } 100\%$$

Where; $M_{INITIAL}$ and $M_{DRIED}$ are the mass of the sample before and after drying, respectively. (AOAC, 2006)

#### 3.2.6.2 Determination of Ash Content

Dry ashing method was used to determine the ash content of the deodorized fermented condiment.

Hundred grams (100g) each of the treated sample and control were placed differently in a crucible and heated in a muffle furnace to a high temperature 500– 600°C, to incinerate all the organic matter, leaving inorganic material to be quantitated gravimetrically.

$$\% \text{ Ash (dry basis)} = \frac{M_{Ash}}{M_{Dry}} \times 100\%$$

Where; $M_{Ash}$ refers to the mass of the ashed sample, and $M_{Dry}$ refer to the original mass of the dried sample (AOAC, 2006).

### 3.2.6.3 Determination of Crude Protein

Kjeldahl method was used to determine the amount of protein in the deodorized fermented condiment and control sample, using a conversion factor of 6.25. This was conveniently divided into three steps: digestion, neutralisation and titration (AOAC, 2006).

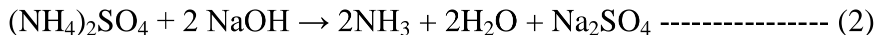
#### 3.2.6.3.1 Digestion of the Sample

One hundred grams (100g) each of the treated samples and control were weighed into a digestion flask and digested by heating it in the presence of 5M sulfuric acid ($H_2SO_4$) (an oxidizing agent which digests the condiment). Anhydrous sodium sulfate was used in raising the boiling point and a mercury as catalyst, to speed up the reaction and convert all nitrogen in the condiment (other than that which is in the form of nitrates or nitrites) into ammonium ion ($NH_4^+$) which binds to the sulfate ion ($SO_4^{2-}$), other organic matters are converted to $CO_2$ and $H_2O$.

N (food condiment) → $(NH_4)_2SO_4$ ------------------ (1)

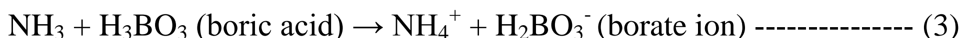
#### 3.2.6.3.2 Neutralization

The solution in the digestion flask was then made alkaline by adding sodium hydroxide, to convert the ammonium sulfate into ammonia gas:



$(NH_4)_2SO_4 + 2\ NaOH \rightarrow 2NH_3 + 2H_2O + Na_2SO_4$ ---------------- (2)

The ammonia gas formed was collected into the receiving flask, which contains an excess boric acid and then the ammonia gas converted into the ammonium ion, and simultaneously the 2M boric acid converted to the borate ion:



$NH_3 + H_3BO_3$ (boric acid) $\rightarrow NH_4^+ + H_2BO_3^-$ (borate ion) --------------- (3)

### 3.2.6.3.3 Titration

The nitrogen content was then estimated by titration of the ammonium borate formed with sulfuric acid using a phenolphthalein indicator.

$H_2BO_3^- + H_2SO_4 \rightarrow H_3BO_3 + HSO_4^-$------------ (4)

The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original food condiment (Equation 3).

$$\%\ N_2 = ((14 \text{ x } M \text{ x } V_t \text{ x } V) / (\text{Weight of Sample (mg) x } V_a)) \text{ x } 100$$

$$\%\ \text{Crude Protein} = \%\ N_2 \text{ (Nitrogen) x } 6.25$$

Where;

M = Molarity of Acid, V = Titre Value of $H_2S0_4$ used, $V_t$ = Total Volume of diluted digest

$V_a$ = Aliquot Volume distilled (AOAC, 2006).

### 3.2.6.4 Determination of Crude Fat

The lipid content of the food deodorized condiment and control were determined by solvent extraction methods. The fat content was extracted from the food sample with (diethyl ether). The

fat content was measured by weight loss of the initial weight of condiment treated samples. Two grams (2g) each of dried treated samples and control (*W1*) were placed differently in a 250 ml Erlenmeyer flask and 2ml alcohol was added, stirred to moisten all particles (moistening of sample with alcohol, prevents lumping on addition of acid). Ten milliliters (10ml) of the diluted 4M of HCl was added and mixed thoroughly. The flask was then set on the heater and refluxed for 30 min after which placed in water bath held at 70–80°C and stirred at frequent intervals until sample was dissolved. Ten milliliters (10ml) alcohol was poured into the extraction tube with 25ml of diethyl ether. Twenty-five (25ml) petroleum ether was added and shaken vigorously for 1min and left to stand until upper liquid was practically clear. Ether-fat solution was transferred into a pre-weighed 125ml flask by filtering through a funnel containing a plug of cotton packed firmly and allowed free passage of ether into the flask and allowed to dried in an oven at 100±5°C and then cooled in a desiccator and weighed (*W2*). The extraction of the liquid sample was repeated using the same solvent. Each time, clear ether solution was transferred through the same funnel into the same flask. When finished, it was rinsed inside and outside of the funnel into the same flask. The solvent was evaporated on a water bath at 70-80°C, dried in an oven at 100±5°C, allowed to cool in a desiccator and weighed (*W3*).

$$\text{Total Fat (g/100 g)} = \frac{(W3 - W2)}{W1} \times 100$$

Where: W1 = Weight of sample, W2 = Weight of dried flask before fat extraction W3 = Weight of dried flask after fat extraction (AOAC, 2006).

**3.2.6.5 Determination of Total Carbohydrate**

Total carbohydrate content of deodorized fermented condiment was calculated by difference after determining all other components of proximate composition.

Total carbohydrate (%) = 100% - (%moisture + % ash + %lipid + %protein)

#### 3.2.6.5.1 Dietary fibre

A dried, defatted deodorized samples and control were enzymatically digested with a-amylase, amyloglucosidase, and protease to remove the starch and protein. The soluble fibre components was precipitated by adding 10ml of 5M ethanol. Soluble and insoluble fiber components were collected as the residue upon filtration and weighed (AOAC, 2006).

### 3.2.7 Vitamins

The standard methods of the association of official analytical chemists (AOAC, 2006) was used for Vitamin A, Vitamin B, Vitamin C, and Vitamin E analysis.

#### 3.2.7.1 UV-Spectrophotometry

After homogenisation and saponification of each of the treated condiments and control sample in a solution of ethanolic potassium hydroxide, the retinol (vitamin A alcohol) and tocopherol (vitamin E) released were totally extracted with organic solvents. (AOAC, 2006).

#### 3.2.7.2 Standard Vitamin A

One milligram (1mg) of retinol was weighed into a 100 ml volumetric flask, dissolved with 5ml of 2M chloroform until all retinol dissolved, then diluted to the mark with ethanol and mixed. The solution was diluted ten times in another volumetric flask. Absorbance of the final solution was read with UV-spectrophotometer at 325 nm and concentration of retinol was calculated by extinction coefficient E (1% in ethanol at 325 nm) = 1850 (1835). The final solution was further diluted to a concentration of 0.2-1.0 µg/ml (AOAC, 2006).

### 3.2.7.2.1 Determination of Vitamin A

Twenty-five grams (25g) each of deodorized samples and control were weighed into a 5ml test tube differently and dissolved to concentration of 4µg/ml with 99.8% ethanol. One milliliter (1ml) each of treated condiments and control were diluted to 50ml with 99.8% ethanol. Absorbance of final solution was read with UV-spectrophotometer at 325 nm and concentration of retinol acetate was calculated by extinction coefficient E (1% in ethanol at 325 nm) = 1550. The content of total vitamin A in the samples being analysed were calculated from the peak height (or areas) of retinol in the injected volume (20µl) of sample solution and standard solution:

$$\text{Retinol } (\mu g/100g) = \frac{100 \times V \times D}{W}$$

Where; V = total volume (ml), W = weight of sample (g). D = dilution factor Calibration curve was prepared, the amount of retinol (µg/ml) was read from calibration curve and calculated to mg per 100g of sample. The following conversion factor was used to report in (RAE):

$$\text{Vitamin A} = \frac{(\text{Retinol } (\mu g/100\ g) / 0.3)}{12} = \text{Vitamin A Retinol Activity Equivalent (RAE)}$$

(AOAC, 2006).

### 3.2.7.3 Determination of Vitamin E

Twenty-five grams (25g) each of deodorized samples and control were weighed into different 25ml volumetric flasks, dissolved with 10ml of 2M $CH(Cl)_3$ until condiment were dissolved and then diluted to the mark with 5M ethanol and mixed. The solution was diluted ten times in another volumetric flask (100ml). Absorbance of final solution was read with UV-spectrophotometer at 292nm and concentration of tocopherol was calculated by extinction

coefficient E (1% in ethanol at 292nm) = 71 and the solution was further diluted to concentration of 2-50μg/ml (AOAC, 2006).

**Calculations**

$$\text{Tocopherol (mg/100g)} = \frac{100 \times V \times D}{W}$$

Where; V = total volume (ml), W = weight of sample (g). D = dilution factor

Calibration curve was prepared, the amount of tocopherol (μg/ml) was read from calibration curve and calculated to mg per 100 g of sample. The following conversion factors was used to report in IU (AOAC, 2006)

$$\text{Vitamin E (IU/100 g)} = \text{Tocopherol (mg/100 g)} / 0.909$$

#### 3.2.7.4 Determination of Vitamins B

##### 3.2.7.4.1 Buffer Solutions

Phosphate buffer, acetate buffer, pH 3–8, and borate buffer solution, pH 4–10 were prepared in freshly boiled and cooled distilled water. The solutions were refrigerated in light-protected flasks and used only for one week.

##### 3.2.7.4.2 Sodium Hydroxide Solution

Sodium hydroxide solution (1.0 M) was prepared by dissolving 4g pure sodium hydroxide pellets in 100ml carbon dioxide free distilled water.

##### 3.2.7.4.3 Iodine Solution

An aqueous iodine solution (0.03 M) was freshly prepared daily by dissolving 0.42g pure iodine and 1.08g potassium iodide in 100ml distilled water.

### 3.2.7.4.4 Preparation of Standard Solutions

Twenty-five milligram (25.0mg) standard Vitamins $B_1$, $B_2$, $B_3$, $B_5$, $B_6$ and $B_9$, each were transferred into a 50ml volumetric flask differently, dissolved in about 30 ml distilled water, then diluted to the mark with the same solvent to obtain the stock standard solution of $B_1$, $B_2$, $B_3$, $B_5$, $B_6$ and $B_9$ vitamins containing 0.5mg/ml each. The working standard solutions were prepared by further dilution of a suitable volume of the stock solution with the same solvent to obtain concentration ranges of 200–5000ng/ml for thiamine HCl vitamin $B_1$, riboflavin vitamin $B_2$, nicotinamide $B_3$, and 100–1200ng/ml for pantothenic Acid $B_5$, pyridoxine HCl vitamin $B_6$ and Folate vitamin $B_9$. The stock and working standard solutions were kept refrigerated in light protected flasks.

### 3.2.7.4.5 Preparation of Sample Solutions

Twenty grams (20g) were weighed, finely powdered, and mixed thoroughly. An accurately weighed quantity of the powdered sample equivalent to 25mg was transferred into a 50ml volumetric flask containing 30ml distilled water. The contents of the flask were shaken well for at least 10 min, further diluted to the mark 50ml with distilled water. The resulting solution was filtered and a suitable aliquot of the obtained solution was diluted quantitatively with the same solvent to obtain a concentration within the linearity range of each vitamin and suitable for the determinations. The general assay procedure was applied according to the vitamin determined.

### 3.2.7.4.6 Determination of Vitamin $B_1$ (Thiamine HCl)

One millilitre (1ml) of sample solution was transferred into a 10 ml volumetric flask, followed by addition of 1ml iodine solution (0.030 M) and 1ml sodium hydroxide solution (0.053 M). The

solution was shaken well, then diluted with ethanol to the mark. The absorbance of the solution was measured at wavelengths of 360 nm and 430 nm against a blank solution treated similarly.

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard. (AOAC, 2006).

**3.2.7.4.7 Determination of Vitamin $B_2$ (Riboflavin)**

One millilitre (1ml) of sample solution was transferred into a 10ml volumetric flask, followed by 3ml acetate buffer, pH 6. The flask contents were shaken and diluted to the mark with the same buffer solution. The absorbance of the solution was measured at wavelengths of 457nm and 527nm.

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

**3.2.7.4.8 Determination of Vitamin $B_3$ (Nicotinamide)**

One millilitre (1ml) of sample solutions were pipetted into 10ml calibrated volumetric flasks. Then 1.0ml of KIO3 (0.20M), 3.0ml of KI (0.20M) solutions were added. The volume was made up to the mark with distilled water and the absorbance was measured at 288, 350 nm against a similar reagent blank. The amount of vitamin $B_3$ was calculated from standard curve (AOAC, 2006).

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

#### 3.2.7.4.9 Determination of Vitamin $B_5$ (Pantothenic Acid)

One gram of each food sample was accurately weighed into a 50ml centrifuge tube, and 1ml methotrexate solution (10ng/ml) and 9ml ammonium acetate solution (9mmol/ml) were added. After thoroughly shaking the mixture for 5 min over a magnetic stirring plate and extracted using an ultrasonic bath, 10ml of chloroform was added and shaken again for 1min over a magnetic stirring plate, the mixture was centrifuged for 10min at 10,000rpm. Eventually, the supernatant was filtered through a 0.22-μm filter and the filtrate collected was transferred in 10ml volumetric flasks and absorbance was measured at wavelengths of 200-350nm.

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

#### 3.2.7.4.10 Determination of Vitamin $B_6$ (Pyridoxine HCl)

One millilitre (1ml) of sample solution was transferred into a 10ml volumetric flask, followed by 3ml acetate buffer, pH 6. The flask contents were shaken and diluted to the mark with the same buffer solution. The absorbance of the solution was measured at wavelengths of 325nm and 415nm.

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

### 3.2.7.4.11 Determination of Vitamin $B_9$ (Folic Acid)

One hundred milligram (100mg) each of dried treated samples and control were transferred to 100ml volumetric flask differently. The final volume was made up with distilled water to get the solution of 1000 µg/ml. From stock solution 0.1ml was further diluted to 10ml with distilled water in a 10ml volumetric flask to get the solution of 100µg/ml and the sample was scanned in UV Spectrophotometer in the range 400-200nm using distilled water as a blank and the wavelength corresponding to maximum absorbance (λ max) was found to be 281nm.

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

### 3.2.7.5 Determination of Vitamin C

The condiment treated samples and control were homogenised in 3% metaphosphoric acid separately and centrifuge at 7000rpm for 10min. The sample extract, obtained after filtering the homogenate were transferred into volumetric cuvette and absorbance was measured at 250-300nm using distilled water as blank by means of UV-spectrophotometer. Evaluation was carried out by comparing the peak area against an ascorbic acid standard (AOAC, 2006).

### 3.2.7.5.1 Sample Preparation

Two and half grams (2.5g) weighed portion of food condiment was transferred into 100ml volumetric flask with a 10ml of 3% metaphosphoric acid. Shaken vigorously for 2min and

sonicate in ultrasonic bath for 5min. The sample extract was made up to the mark with 3% metaphosphoric acid. Filter through filter paper Whatman #4 and further passed through membrane filter 0.45µm. The sample solution was transferred into a cuvatte and analysed using UV-spectrophotometer at 250-300nm (AOAC, 2006).

**Calculation, Unit of Expression and Test Report**

Standard test values of concentrations of standard ascorbic acid per ml was plotted and calculated by linear regression. The values of sample in micrograms of ascorbic acid per ml was read off the standard calibration curve and calculated. The ascorbic acid content in 100g of the treated samples and control were calculated.

$$\text{Vitamin C (mg/100 g)} = \frac{A_2 \text{ x } C_1 \text{ x } V}{A_1 \text{ X } 10 \text{ X } W}$$

$A_1$ = peak area of standard solution

$C_1$ = concentration of standard solution (µg/ml)

$A_2$ = peak area of sample

V = final volume of sample (ml)

W = weight of sample (g)

The result of the determination in mg/100 g sample (AOAC, 2006).

### 3.2.8 Determination of Mineral Elements

Organic matter in the treated samples and control were ignited. The trace elements in the sample were quantitatively measured by atomic absorption spectrophotometer (AAS) at a specific wavelength (AOAC, 2006). Zinc (Zn), iron (Fe), calcium (Ca), and magnesium (Mg) were determined by atomic absorption spectrometry while potassium (K) was determined by flame photometry according to the method of AOAC (2003). An aliquot portion 5ml of the acidified

sample was diluted to mark10ml volume of the cuvette and read by AAS using deionised water as reagent blank (AOAC, 2006).

Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) were used at the wavelengths below.

| ***Element*** | ***Wavelength (nm)*** |
|---|---|
| **Fe** | 248.3 |
| **Zn** | 213.9 |
| **Ca** | 236.3 |
| **Mg** | 251.0 |

The order of measurements is: - water (set to zero), reagent blank (0 ppm, auto zero), standard set, sample blank and test solution. Reagent blank and a standard were read every 5 measurements to check the stability of the instrument (AOAC, 2006).

**CALCULATIONS**

$$\text{Trace element (mg /100g sample)} = \frac{C_o \ x\ V\ x\ D\ x\ 100}{W\ x\ P\ x\ 1000}$$

Where: $C_0$ = concentration of the sample in mg/L

$V$ = total volume, ml

$D$ = Dilution factor

$W$ = weight sample, g

$P$ = sample solution taken, ml

1000 = conversion of mL to L

Test results were reported in mg per 100g sample (AOAC, 2006).

### 3.2.8 Determination of Potassium (K)

One gram of powdered sample (1g) was taken in to digesting glass tube. Twelve milliliters (12ml) of 2M $HNO_3$ was added to the sample and the mixture was kept overnight at room temperature. Then, 4.0ml of 1M per-chloric acid ($HClO_4$) was added to the mixture and kept in

the fumes block for digestion. The temperature was increased gradually starting from 50°C and increased up to 250 – 300°C for about 70-85min and terminated as white fumes appeared. The mixture was left to cool and the contents of the tubes were transferred to 100ml volumetric flasks and the volumes was made to 100ml with distilled water. The wet digested solution was then transferred to plastic bottles labeled accurately and stored for potassium determination. The absorption measurement of the element was read out.

The concentrations of minerals were determined using the formula below:

$$Mw = \frac{(\text{absorbance (ppm)} \times \text{dry wt.} \times D)}{\text{wt. of sample} \times 100}$$

Where; Mw = Conc. of minerals, D = Dilution factor Standard solutions of 20, 40, 60, 80 and 100mls equivalent/L were used for K.

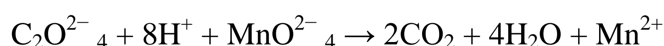
### 3.2.9 Determination of Antinutritional Factors

The methods as described by AOAC (2006) were used for the determination of the antinutrients.

#### 3.2.9.1. Determination of Oxalates

Oxalate quantitative determination was carried out using the method reported by (Gemede and Ratta, 2014). Exactly 20ml of 0.3 M HCl in the sample (2.50 g) was extracted three (3) times repeatedly by warming at a temperature of 50°C for 1 hour with constant stirring using a magnetic stirrer. One milliliter (1.0ml) of 5 M ammonium hydroxide was added to 5.0ml of extract to ensure alkalinity and 2 drops of phenolphthalein indicator was added, 3 drops of glacial acetic acid, and 1ml of 5% calcium chloride to make the mixture acidic and allowed to stand for 3 hours, followed by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded; the precipitate was washed three times using hot water by mixing thoroughly. Two milliliter (2ml) of 3M tetraoxosulphate (VI) acid was added and the precipitate dissolved by warming in a water

bath at 70°C. Freshly prepared 0.01 M potassium permanganate ($KMnO_4$) was titrated against the content of tube at room temperature until the first pink colour appeared throughout the solution. The solution was allowed to stand until it returned colourless, after which it was warmed on an electric hot plate at 70°C for 3 minutes, and retitrated again until a pink colour appeared and persisted for at least 30 seconds (AOAC, 2006).



$$C_2O^{2-}{}_4 + 8H^+ + MnO^{2-}{}_4 \rightarrow 2CO_2 + 4H_2O + Mn^{2+}$$

Ratio of reacting ions = 1:1

From $M_1V_1 = M_2V_2$,

Where $M_1$ is molarity of $KMnO_4$, $M_2$ is molarity of extract (oxalate), $V_1$ is volume of extract (oxalate), and $V_2$ is volume of $KMnO_4$ (Titre Value). The oxalates were calculated using the formula as describe by AOAC (2006)

Molecular Weight of $CaCO_3$ = 100

Weight of oxalate in titre = $M_2 \times$ molecular weight = $Xg$

Weight of oxalate in titrant 2ml = $(Xg/1000) \times 2 = Y$

100 ml of oxalate extract will contain = $(Y/\ 2.5) \times 100$ g = $W$

% oxalate composition g/100 g = $(W/\ 2.5) \times 100$

### 3.2.9.2 Determination of Tannins

Five hundred milligrams (500mg) of the sample was weighed into plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. The mixture was filtered into 50ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a tube and mixed with 3ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The

absorbance was measured in a spectrophotometer at 120nm wavelength, within 10 minutes. A blank sample was prepared and the colour developed was read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (AOAC, 2006).

$$C_X = \frac{A_X}{A_S} \times C_S$$

Where; $C_X$ = Concentration of the sample, $A_X$ = Absorbance of the sample $C_S$ concentration of sample, $A_S$ = absorbance of standard (AOAC, 2006).

### 3.2.9.3 Determination of Saponins

Twenty grams (20g) each of the treated samples and control were dissolved differently in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was collected while the ether layer was discarded. The purification process was repeated. Sixty milliliter (60ml) of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven to a constant weight. The Saponins content was calculated in percentage (AOAC, 2006).

$$\%\text{Saponins} = (\text{Weight of Saponins}/\text{Wight of Sample}) \times 100$$

### 3.2.9.4 Determination of Alkaloids

Quantitative determination of alkaloids was done according to the methodology described by Harborne and Mabry, (2013). Exactly 200ml of 10% acetic acid in ethanol was added to the deodorized fermented condiment sample (2.5g) in a 250ml beaker and allowed to stand for 4

hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop-wise to the extract until the precipitation completed and filtered. The supernatant was discarded and the precipitate was washed with 20ml of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5cm) (AOAC, 2006). The residue was dried in an oven and the percentage of alkaloid was expressed mathematically as

% Alkaloids = (Weight of Alkaloids/Weight of Sample) X 100

#### 3.2.9.5 Determination of Flavonoids

Flavonoids were determined according to the method reported by Harborne and Mabry, (2013). Exactly 50ml of 80% aqueous methanol was added to 2.5g of sample in a 250ml beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol and transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoids was calculated (AOAC, 2006)

% Flavonoids = (Weight of Flavonoids/Weight of Sample) X 100

#### 3.2.9.6 α-Amylase inhibition assay

The assay was carried out according to a modified procedure described by McCue and Shetty, (2004). A total of 250μL of extract was placed in a tube and 250μL of 0.02M sodium phosphate buffer pH 6.9 containing α-amylase solution was added. The solution was pre-incubated at 25$^{o}$C for 10minutes, after which 250μL of 1% starch solution in 0.02M sodium phosphate buffer pH

6.9 was added and then further incubated at 25$^{o}$C for 10mins. The reaction was terminated after incubation by adding 500µL of dinitro-salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 minutes and cooled to room temperature. The reaction mixture was diluted with 5ml distilled water and the absorbance was measured at 540nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. Individual blanks were prepared for correcting the background absorbance. In this case, the colour reagent solution was added prior to the addition of starch solution and the mixture then placed in the water bath immediately. The α-amylase inhibitory activity was calculated as percentage inhibition.

$$(\%\text{Inhibition} = \{(A_{control} - A_{sample})/A_{control}\} \times 100)$$

Where; $A_{control}$ is the absorbance of each control and $A_{sample}$ is the net absorbance of each sample. The net absorbance of each sample was calculated using the following equation:

$$A_{sample} = A_{test} - A_{blank}$$

Where $A_{test}$ is the absorbance of each test and $A_{blank}$ is the absorbance of each blank.

Concentrations of extract resulting in 50% inhibition of enzyme activity were determined.

#### 3.2.9.7 Trypsin inhibition assay

Trypsin inhibition was determined according to the method described by Onwuka, (2005). Extraction of sample: A known weight (1g) of ground sample was weighed and dispersed into 50ml of 0.5M NaCl solution. The mixture was stirred for 30mins at room temperature and centrifuged at 10,000rpm for 5min. The supernatant was filtered through Whatman filter paper. The filtrate (extract) was used for the assay. Preparation of standard trypsin and substrate: 1mg/ml of trypsin in 0.1M HCl was prepared, 1% casein substrate in 0.1M phosphate buffer pH7.7 was

prepared. To 2ml of trypsin standard solution, $1cm^3$ of trypsin inhibitor was added and incubated at $37^oC$ for 10min. A blank of 5ml substrate was prepared in a test-tube (with no trypsin inhibitor extract added). The contents in the test-tube was left for 10 minutes and the reaction was stopped by adding 3ml 5% TCA. It was then filtered and absorbance was measured spectrophotometrically at 410nm. The trypsin inhibitor activity was expressed as the number of trypsin unit inhibited (TUI) per unit weight of the sample analyzed.

$$\text{Calculation: TUI/mg} = (b-a)/0.1 \times F$$

Where b = absorbance of test sample solution, a = absorbance of the blank

$$F = (1 \times Vf / Va \times D) / W$$

Where; W = weight of sample Vf = total volume of extract used in the assay, D = dilution factor and Va = volume of standard

### 3.2.10 Determination of Physicochemical Properties

#### 3.2.10.1 Determination of pH

The pH of the deodorized fermented condiments was measured using pH meter. Ten grams (10g) of sample was diluted with 10ml distilled water and mixed thoroughly to measure pH. The pH meter was calibrated with standard buffer solutions pH=4.01 and pH=6.01 before measuring the pH.

#### 3.2.10.2 Determination of Titratable Acidity (TA)

Twenty grams (20g) of powdered deodorized fermented condiment was weighed into a 25ml conical flask and 200ml of distilled water was added. The flask was allowed to stand in a water

bath maintained at 40°C for 1hour. Phenolphthalein was added as indicator and titrated with 0.1M

NaOH solution.

$$\text{Acidity (\% Lactic acid)} = \frac{(\text{Volume of 0.1M NaOH x 0.9) x 100}}{\text{Weight of the sample}}$$

### 3.2.10.3 Determination of Water and Oil Absorption Capacity

One gram (1g) of the sample was mixed with 10ml distilled water for 30 seconds. The sample was allowed to stand at room temperature 25 ± 2°C for 30 minutes after which it was centrifuged at 3000 rpm for 30 minutes. The volume of the supernatant was taken in a 10ml graduated cylinder. Water absorption in mg/ml was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The same procedure was carried out to determine the oil absorption capacity.

$$\text{Absorption Capacity} = V_{Initial} - V_{Supernatant}$$

Where: $V_{Initial =}$ initial volume of water added while $V_{Supernatant}$ volume of the supernatant

### 3.2.10.4 Determination of Bulk Density

The bulk density of deodorized fermented condiment was determined by gently pouring 15g samples into a cylindrical container of known volume ($10cm^3$) while striking excess samples off the brim without compacting it. And gently taping the bottom of the cylinder on the lab bench several times until there is no further diminishing of the sample level after filling to the $10cm^3$ mark. The weighted of the sample was recorded. The bulk density was calculated using the relationship in the equation below.

$$D_b = W/V$$

Where; $D_b$ is bulk density of deodorized fermented food condiment (g $m^{-3}$); W is weight of sample (g) and V is volume ($m^3$). (AOAC, 2006).

## 3.3 STATISTICAL ANALYSIS

Results were expressed as mean ± SD. The data were analysed using one way (ANOVA) where applicable. Differences between mean were determined using Dunnett post-hoc test ($p \leq 0.05$). Statistical analysis was carried out with the Statistical package for social science (SPSS version 21).

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Heterotrophic Bacterial Count of Laboratory Fermented *Prosopis africana* Seeds

Heterotrophic bacterial count of laboratory fermented *Prosopis africana* seeds is presented in Figure 4.1. The microbial counts increased exponentially from days 1- 6, with peak count at day 7 (2.24 x $10^6$, 1.54 x $10^7$, 1.16 x$10^8$ and 0.77 x$10^9$cfu/g) and day 8 (2.25 x $10^6$, 1.56 x $10^7$, 1.18 x$10^8$ and 0.78 x$10^9$cfu/g) observed with steady growth, offensive odor and dark mash sticky cotyledon was observed at days 7 - 9.

### 4.2 Changes in Physical Sensory Parameters of Laboratory Fermented *Prosopis africana* Seeds

Changes in physical sensory parameters of laboratory fermented *Prosopis africana* seeds are presented in Table 4.1. The fermentation period was nine days and there was no change observed in colour of creamy cotyledon from Days 1 – 3, but at days 4 - 6 a slight change in colour was observed from creamy brownish to brown colour and a persisted dark brown colour was observed at days 8 – 9. At day 1 of the fermentation, there was no odor observed, but a slight change from odorless to barely perceived odor at days 2 – 4. Mild odor was observed at day 5, moderate odor at day 6 and persisted strong odor was observed at days 7 – 9. At days 1 - 4 of the fermentation, there was no change observed in the texture of mash cotyledon, but a stick mash cotyledon was observed at day 5, and persisted mashy sticky cotyledon was observed at days 6 – 9.

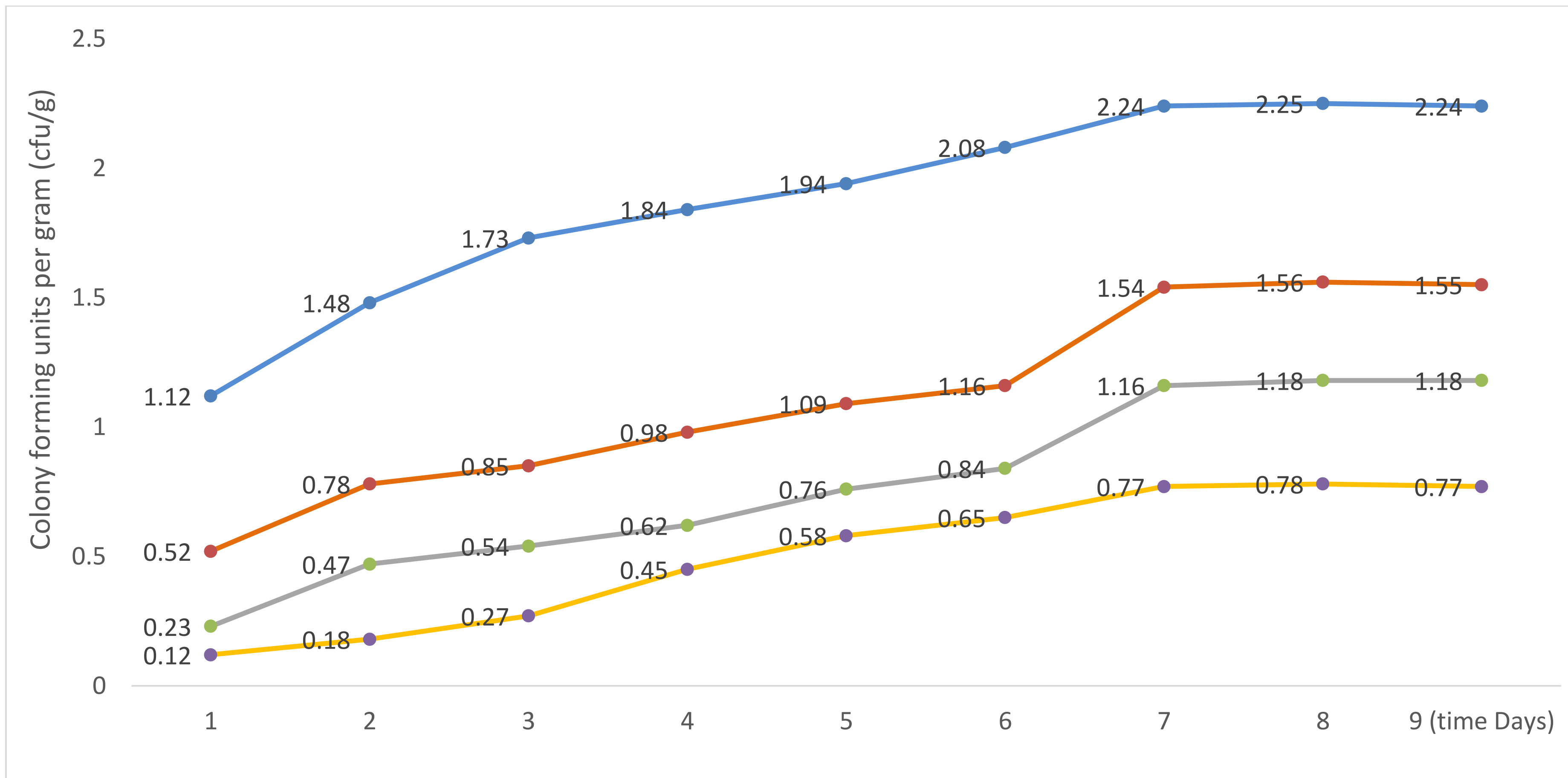



**Figure 4.1; Heterotrophic Bacterial Count of Laboratory Fermented *Prosopis africana* Seeds (cfu/g)**
Where cfu/g refers to Colonies forming unit per gram.

**Table 4.1; Changes in Physical Sensory Parameters of Laboratory Fermented *Prosopis africana* Seeds**

| DAYS | COLOUR | ODOR | TEXTURE |
|---|---|---|---|
| 1 | Creamy | Odorless | Mash cotyledon |
| 2 | Creamy | Non offensive Odor | Mash cotyledon |
| 3 | Creamy | Non offensive Odor | Mash cotyledon |
| 4 | Creamy/brownish | Barely odor | Mash cotyledon |
| 5 | Brownish | Mild odor | Stick mash cotyledon |
| 6 | Brown | Moderate odor | Mashy sticky cotyledon |
| 7 | Brown | Offensive odor | Mashy sticky cotyledon |
| 8 | Dark brown | Very offensive odor | Mashy sticky cotyledon |
| 9 | Dark brown | Very offensive odor | Mashy sticky cotyledon |

### 4.3 Odor Evaluation of Deodorized Fermented Condiment from *Prosopis africana* Seeds

Results for odor evaluation of deodorized fermented condiment from *Prosopis africana* seeds are presented in Figures 4.2. and 4.3. Cyclodextrin (CD) treated sample (2.5g/100g) and powdered activated carbon (PAC) treated sample (2.5g/100g) were observed to have the least odor (Barely perceived odor) with 73.4% and 86.6% respectively. The (0.5g/100g) of both CD treated sample and PAC treated sample were observed to have 100% strong odor when compared with control (NC). The samples treated with PAC (1.5g/100g), CD (2.0g/100g) and PAC (2.0g/100g) were observed to have 53% barely perceived odor, while samples treated CD (1.0g/100g) and PAC (1.0g/100g) were observed to have 10% and 40% (barely perceived odor) and CD treated sample (1.5g/100g) have 20% (barely perceived odor).

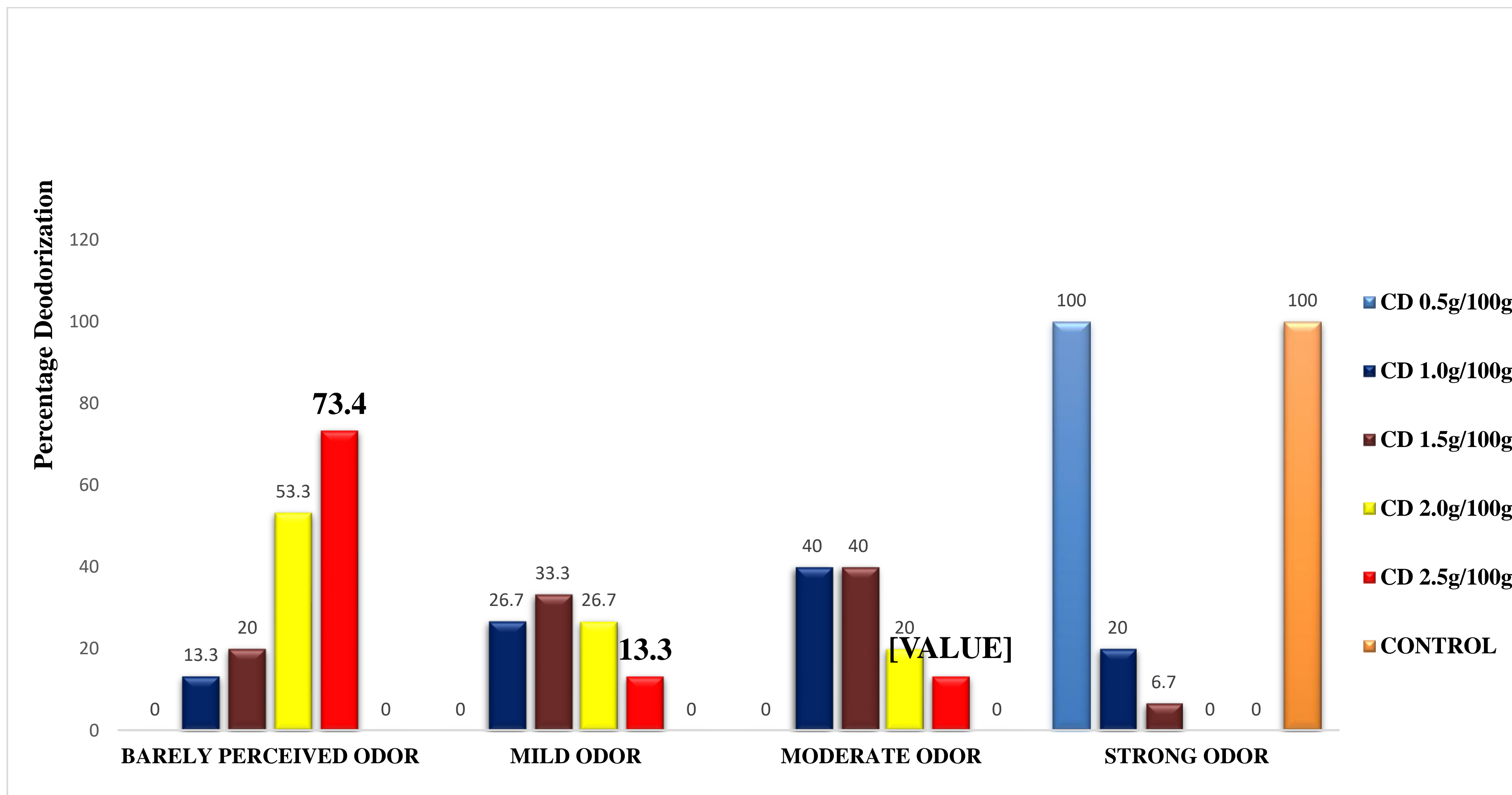



**Figure 4.2; Odor Evaluation of Deodorized Fermented Condiment from *Prosopis africana* Seeds Treated with Cyclodextrin (CD)**

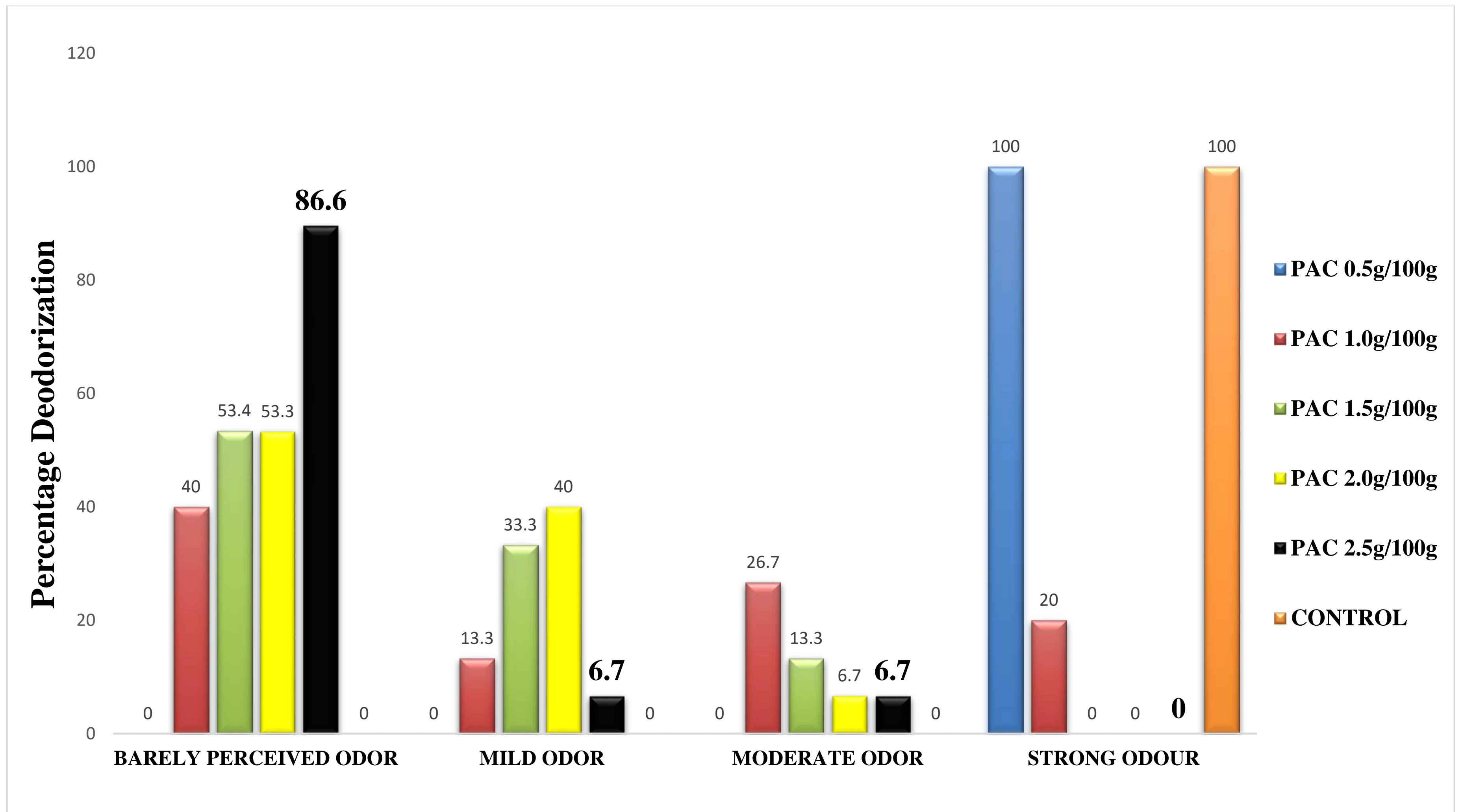



**Figure 4.3; Odor Evaluation of Deodorized Fermented Condiment from *Prosopis africana* Seeds Treated with Cyclodextrin (PAC)**

### 4.4 Volatile Constituents of Deodorized Fermented Condiment from *Prosopis africana* Seeds

Volatile constituents of deodorized fermented condiment from *Prosopis africana* Seeds are presented in Table 4.2. The presence of 27 volatile constituents in control sample, 19 in CD and 10 in PAC based on their retention time were observed. The predominant compounds responsible for the offensive odor are the carbonyl compounds aldehydes and aetones as well as amines and amides. These includes compounds such as hexadecanoic acid, methyl ester, n-hexadecanoic acid, docosylpropylether, hexacosylpropylether, propyltriacontylether, 9-Octadecenoic acid, 2,3dihydroxypropylester, 11-octadecenoic acid, methylester, cis-13-Octadecenoic acid, octadecanoic acid, oleic acid, Fumaric acid, 4-heptyltridecyl ester, 11-hexadecenoic acid, benzenamine, 4-(2-phenylethenyl)-N-(3,5-dimethyl-1-pyrazolylmethyl)-l-(+)-Ascorbic acid, anthracene, 9,10-dihydro-9,9,10-trimethyl-Benzo[h]quinolone, 1-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-isopropylester, 2,4-dimethyl-2Ethylacridine, octasiloxane, 1,5-hexadecamethyl-acetic acid, 2-Ethylacridine, 2-Methyl-7phenylindole, 1,2 benzisothiazol-3-amine, 2-ethylacridine, benzo[h]quinoline, 2,4-dimethyl-Thymol, 2,4-cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-thymol, 2,4 cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-benzo[h]quinolone, 1,2-benzenediol, 3,5-bis(1,1-dimethylethyl)-1H-Indole-2-carboxylic acid, isopropylester, 1H-Indole, 1-methyl-2-phenyl-1h-Indole-2-carboxylic acid, 2-methyl-3-phenyl-4-(4 Hydroxyphenyl)-4methyl-2-pentanone, 1h-Indole-2-carboxylic acid,

6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7tetrahydro-, isopropyl ester, 1,2-benzenediol,3,5-bis(1,1-dimethylethyl)-5-methyl2phenylindolizine, octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,hexadecamethyl-n,ndimethyl-4-nitroso-3 (trimethyn,n-dimethyl-4-nitroso-3-(trimethy2,4-cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl) 4-hydroxy-1,2-benzenediol, 5-methyl-2-phenylindolizine, 5-methyl2-phenyl-2,4-cyclohexadien 1-one, 1,2-benzisothiazol-3-amine, 5-methyl-2 phenylindolizine and others.

The volatile compounds responsible of the of odor were; anthracene, 9,10-dihydro9,9,10-trimethyl-benzo[h]quinolone, 1,5-hexadecamethyl-2,4-cyclohexadienone, 2,4-cyclohexadienone, 2'-hydroxypropiophenone, 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzo[h]quinolone, 2-methyl-3-phenyl-4-pentanone, acetophone, heptanal, acetophenone, nonadecanone, 2-butyl-octenal, 2,4-nonadienal, ethyl hexanone, ethyl heptanone, 2,4undecadienal, 2,4,6-dodecatrienal, 5-methyl-2-phenyl-2,4-cyclohexadienone, ethyl octanone, 2,4-decadienal, hendecanal and 2-nonenal. Powdered activated carbon (PAC) was observed to have the highest deodorization potential with 63% deodorization of the volatile constituents while cyclodextrin (CD) with least deodorization potential with 30% deodorization of the volatile constituents respectively.

**Table 4.2; Volatile Constituents of Deodorized Fermented Condiment from *Prosopis africana* Seeds**

| S/N | CONTROL | | CD | | PAC | |
|---|---|---|---|---|---|---|
| | RT (min) | Volatile Constituents | RT (min) | Volatile Constituents | RT (min) | Volatile Constituents |
| 1 | 8.177 | Hexadecanoic acid, methyl ester | 8.146 | Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester | 11.593 | 9-Octadecenoic acid, methyl ester Dodecahydropyrido[1,2b]isoquinolinone, Cyclopentene, carboxylic acid |
| 2 | 8.548 | n-Hexadecanoic acid | 9.821 | Oleic Acid, 6-Octadecenoic acid, | 14.418 | 1,2-Benzisothiazol-3-amine, 5-Methyl-2-phenylindolizine |
| 3 | 9.275 | Docosyl propyl ether, Hexacosylpropyl ether, Propyltriacontylether | 10.987 | Heptadecanolide, | 14.695 | 1,2-Benzisothiazol-3-amine, isopropyl ester |
| 4 | 9.853 | 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 11-Octadecenoic acid, methyl ester | 11.589 | Oxirane, tetradecyl-6-Octadecenoic acid | 14.983 | 2'-Hydroxy-5'-methylacetophenone, 2-Ethylacridine |
| 5 | 10.257 | Oleic Acid, cis-13-Octadecenoic acid | 11.890 | 6-Octadecenoic acid, Oleic Acid | 15.213 | Benzendehyde |
| 6 | 10.452 | Octadecanoic acid | 12.993 | Octasiloxane | 15.438 | Octasiloxane |
| 7 | 11.740 | Oleic Acid, Fumaric acid, 4-heptyl tridecyl ester, 11-Hexadecenoic acid | 11.930 | Benzenamine, 4-(2-phenylethenyl)-N-(3,5-dimethyl-1-pyrazolylmethyl)-l-(+)-Ascorbic acid | 16.025 | 1,5-hexadecamethyl-acetic acid |
| 8 | 11.930 | Benzenamine, 4-(2-phenylethenyl)-N-(3,5-dimethyl-1-pyrazolylmethyl)-l-(+)-Ascorbic acid | 14.192 | 3,5-bis(1,1-dimethylethyl)-Heptasiloxane | 16.249 | Octasiloxane, 13,13,15,15-hexadecamethyl-1,2-Benzisothiazol-3-amine, |
| 9 | 14.918 | Anthracene, 9,10-dihydro-9,9,10-trimethyl-Benzo[h]quinolone | 14.725 | Octasiloxane, 1,5-hexadecamethyl-2,4-Cyclohexadienone | 16.769 | 2-Ethylacridine, Benzo[h]quinoline, 2,4-dimethyl-1,2-Benzisothiazol-3-amine |
| 10 | 15.044 | 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4 oxo-4,5,6,7-tetrahydro-, isopropyl ester | 14.881 | 2-Ethylacridine, Cyclotrisiloxane | 16.892 | 1-methyl-2-phenyl-5-Methyl-2-phenylindolizine |
| 11 | 15.213 | Benzendehyde 2,4-Cyclohexadienone | 15.156 | 2,4-Cyclohexadienone | | |
| 12 | 15.438 | Octasiloxane, 1,5-hexadecamethyl-acetic acid | 15.366 | 2'-Hydroxypropiophenone, | | |
| 13 | 15.552 | 2-Ethylacridine, 2-Methyl-7-phenylindole | 15.438 | Octasiloxane | | |
| 14 | 15.737 | 1,2-Benzisothiazol-3-amine, Benzeneacetaldehyde | 16.016 | 1,2-Benzisothiazol-3-amine, 1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-Derivative | | |
| 15 | 16.302 | 2-Ethylacridine, Benzo[h]quinoline, 2,4-dimethyl-Thymol | 16.312 | 1H-Indole-2-carboxylic acid, isopropyl ester, 1,2-Benzisothiazol-3-amine | | |
| 16 | 16.777 | 2,4-Cyclohexadienone, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-Thymol, | 16.550 | 2,4-Cyclohexadienone, 2,4-dimethyl-2-Methyl-7-phenylindole | | |
| 17 | 16.923 | 2,4-Cyclohexadienone, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-Benzo[h]quinolone | 16.713 | Octasiloxane, isopropyl ester, acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide | | |
| 18 | 17.774 | 1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-1H-Indole-2-carboxylic acid, isopropyl ester | 17.066 | 1,2-Benzisothiazol-3-amine, 13,13,15,15-hexadecamethyl-5-Methyl-2-phenylindolizine | | |
| 19 | 17.971 | 1H-Indole, 1-methyl-2-phenyl-1H-Indole-2-carboxylic acid | 17.616 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-1H-Indole | | |
| 20 | 18.259 | 2-methyl-3-phenyl-4-pentanone, | | | | |
| 21 | 18.120 | Acetophone, heptanal | | | | |
| 22 | 18.391 | Acetophenone | | | | |
| 23 | 22.313 | Nonadecanone, 2-Butyl-octenal, 2,4-Nonadienal | | | | |
| 24 | 22.505 | Ethyl hexanone | | | | |
| 25 | 22.202 | Ethyl heptanone, 2,4-Undecadienal, 2,4,6-Dodecatrienal | | | | |
| 26 | 22.255 | 5-methyl-2-phenyl-2,4-Cyclohexadienone, | | | | |
| 27 | 23.213 | Ethyl octanone, 2,4-Decadienal, Hendecanal, 2-Nonenal | | | | |

- **CONTROL 27**
- **CD 19**
- **PAC 10**

**NOTE; RT: Retention Time, CD: Cyclodextrin and PAC: Powdered Activated Carbon**

## 4.5 Effect of Deodorizing Agents on Proximate Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds

The proximate compositions of deodorized fermented condiment from *Prosopis africana* seeds are presented in Table 4.3. The crude protein content of control sample was (40.30±0.14g/100g) while for deodorized fermented condiment CD treated sample and PAC treated sample were (33.18±0.23g/100g) and (54.50±0.25g/100g) respectively. The crude protein content of the control sample differed significantly with CD and PAC ($p \leq 0.05$). The protein content of CD treated sample was significantly lower (33.48±0.23g/100g) while PAC treated sample significantly higher (54.50±0.25g/100g) than the control (40.33±0.14g/100g). The crude lipid content of the control sample was (39.60±0.33g/100g), while the CD treated sample (17.46±0.54g/100g) and PAC treated sample was (13.73±0.11g/100g) and the two samples had a lower lipid content than the control with significant differences ($P \leq 0.05$), about 2/3 reduction 65.48% of lipid content of PAC treated sample and more than a half 56.10% reduction of lipid content of CD treated sample. The ash content of the CD treated sample (5.72±0.12g/100g) and PAC treated sample (7.12±0.04g/100g) were significantly higher ($p \leq 0.05$) compared with control (5.28±0.02g/100g) and PAC treated sample recorded the highest value of the ash content. The crude fiber content of control sample was (5.82±0.32g/100g) and significantly differed ($p \leq 0.05$) from CD treated sample (14.06±0.13g/100g) and PAC treated sample (14.58±0.29g/100g). There was no significant difference ($p>0.05$) between CD treated sample and PAC treated sample crude fibre contents. The moisture content of CD treated sample and PAC treated sample were significantly lower than that of control ($P \leq 0.05$); CD treated sample was (2.44±0.14g/100g) while PAC treated sample was (5.08±0.26g/100g), with CD treated sample recorded the lowest moisture content compered to control (8.79±0.04g/100g) and PAC

treated sample. The carbohydrate content of control (6.03±0.17g/100g) was significantly differed ($P \leq 0.05$) from CD treated sample (41.20±0.24g/100g) and PAC treated sample (19.57±1.04g/100g). The CD treated sample recorded the highest carbohydrate content and significantly differed ($p \leq 0.05$) from PAC treated sample.

### 4.6 Effect of Deodorizing Agents on Vitamins Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds

The vitamin compositions of deodorized fermented condiment from *Prosopis africana* seeds are presented in Table 4.4. The vitamin A content of CD treated sample and PAC treated sample were significantly ($p \leq 0.05$) higher than that of control sample (639.17±16.67RAE/100g); CD treated sample was (975.83±25.83RAE/100g) while PAC treated sample was (881.67±39.17RAE/100g). The CD treated sample recorded the highest value of vitamin A content. The vitamin $B_1$ content of CD treated sample and PAC treated sample were also significantly ($p \leq 0.05$) higher than control sample (276.65±2.33mg/100g), CD treated sample was (308.61±1.57mg/100g) while PAC treated sample was (291.61±2.27mg/100g). The vitamin $B_2$ content of control sample was (5.79±0.15mg/100g) and differed significantly ($p \leq 0.05$) from CD treated sample (13.51±0.60mg/100g) and PAC treated sample (13.73±0.98mg/100g). But there was no significant difference ($p > 0.05$) of vitamin $B_2$ contents between the CD treated sample and PAC treated sample. In addition, there was no significant difference ($p > 0.05$) between vitamin $B_3$ contents of all the samples; control sample (3.06±0.04mg/100g), CD treated sample (3.26±0.22mg/100g) and PAC treated sample (3.26±0.06mg/100g).

**Table 4.3; Effect of Deodorizing Agents on Proximate Compositions of Deodorized Fermented Condiment from *Prosopis africana* seeds**

| SAMPLES | MOISTURE (g/100g) | ASH (g/100g) | LIPID (g/100g) | PROTEIN (g/100g) | Crude FIBRE (g/100g) | TOTAL CARBOHYDRATE (g/100g) |
|---|---|---|---|---|---|---|
| **CONTROL** | $8.79 \pm 0.04^a$ | $5.28 \pm 0.02^c$ | $39.60 \pm 0.33^a$ | $40.30 \pm 0.14^b$ | $5.82 \pm 0.32^b$ | $6.03 \pm 0.17^c$ |
| **CD** | $2.44 \pm 0.14^c$ | $5.72 \pm 0.12^b$ | $17.46 \pm 0.54^b$ | $33.18 \pm 0.23^c$ | $14.06 \pm 0.13^a$ | $41.20 \pm 0.24^a$ |
| **PAC** | $5.08 \pm 0.26^b$ | $7.12 \pm 0.04^a$ | $13.73 \pm 0.11^c$ | $54.50 \pm 0.25^a$ | $14.58 \pm 0.29^a$ | $19.57 \pm 1.04^b$ |

Values are expressed as Mean ± SD of triplicate determination and values with different superscripts on the same column are significantly different ($p \leq 0.05$)

There was a significant difference ($p \leq 0.05$) in vitamin $B_5$ content between CD treated sample (733.56±0.09mg/100g) with PAC treated sample (481.62±2.30mg/100g) and control sample (471.92±0.82mg/100g). Statistically, there was no significant difference ($p > 0.05$) between the control (471.92±0.82mg/100g) and PAC treated sample (481.62±2.30mg/100g) in their vitamin $B_5$ contents. There was significant difference ($p \leq 0.05$) in vitamin $B_6$ contents of CD treated sample (22.69±0.40mg/100g) and PAC treated sample (16.63±0.66mg/100g), with the PAC treated sample recorded the highest value of vitamin $B_6$ content compared with control sample (12.15±0.22mg/100g). Similarly, there was significant difference ($p \leq 0.05$) in vitamin $B_9$ content of CD treated sample (37.25±0.35mg/100g) and PAC treated sample (15.05±0.01mg/100g), with CD treated sample recording the highest value of vitamin $B_9$ content compared with control sample (2.80±0.42mg/100g). The vitamin C content of CD treated sample (19.43±0.59mg/100g) and PAC (17.41±0.49mg/100g) were significantly different ($p \leq 0.05$), with CD treated sample and recorded the highest value of vitamin C content compared with control (12.46±0.68mg/100g). Lastly, there were significant differences ($p \leq 0.05$), in vitamin E content of CD treated sample (33.37±0.57IU/100g) and PAC treated sample (31.12±1.42IU/100g), with CD treated sample recorded the highest value of vitamin E content compared with control sample (12.39±0.06IU/100g).

**Table 4.4; Effect of Deodorizing Agents on Vitamins Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds**

| SAMPLE | Vitamin A (RAE/100g) | Vitamin $B_1$ (mg/100g) | Vitamin $B_2$ (mg/100g) | Vitamin $B_3$ (mg/100g) | Vitamin $B_5$ (mg/100g) | Vitamin $B_6$ (mg/100g) | Vitamin $B_9$ (mg/100g) | Vitamin C (mg/100g) | Vitamin E (mg/100g) |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | 639.17±16.67$^{c}$ | 276.65±2.33$^{c}$ | 5.79±0.15$^{b}$ | 3.06±0.04$^{a}$ | 471.92±0.82$^{b}$ | 12.15±0.22$^{b}$ | 2.80±0.42$^{c}$ | 12.46±0.68$^{c}$ | 12.39±0.06$^{b}$ |
| **CD** | 975.83±25.83$^{a}$ | 308.61±1.57$^{a}$ | 13.51±0.60$^{a}$ | 3.26±0.22$^{a}$ | 733.56±0.09$^{a}$ | 22.69±0.40$^{a}$ | 37.25±0.35$^{a}$ | 19.43±0.59$^{a}$ | 33.37±0.57$^{a}$ |
| **PAC** | 881.67±39.17$^{b}$ | 291.61±2.27$^{b}$ | 13.73±0.98$^{a}$ | 3.26±0.06$^{a}$ | 481.62±2.30$^{b}$ | 16.63±0.66$^{b}$ | 15.05±0.01$^{b}$ | 17.41±0.49$^{b}$ | 31.12±1.42$^{a}$ |

Values are expressed as Mean ± SD of duplicate measurement and values with different superscripts in the same column are significantly differ ($p \leq 0.05$). Where **CD**; Cyclodextrin treated sample, **PAC**; Powdered Activated Carbon treated sample and **Control;** sample without treatment.

### 4.7 Effect of Deodorizing Agents on Mineral Elements Compositions of Deodorized Fermented Condiment from *Prosopis africana* seeds

The results for effect of deodorizing agents on mineral elements compositions of deodorized fermented condiment from *Prosopis africana* seeds are presented in Table 4.5. The calcium (Ca) content of CD treated sample (292.5±1.80mg/100g) and PAC treated sample (257.67±1.95mg/100g) were higher and significantly differed ($p \leq 0.05$) from that of control fermented condiment (222.50±2.50mg/100g). Similarly, the magnesium (Mg) content of CD treated sample (192.52±1.50mg/100g) and PAC treated sample (187.17±2.52mg/100g) were significantly increased ($p \leq 0.05$), from that of control sample (143.25±1.00mg/100g). Meanwhile, there were significant differences ($p \leq 0.05$) between potassium (K) content of the deodorized fermented condiments CD treated sample (594.83±2.50mg/100g) and PAC treated sample (652.57±2.10mg/100g) from control sample (754.5±25.60mg/100g). Statistically, there was significant difference ($p \leq 0.05$) of iron content. The iron (Fe) content of PAC treated sample was lower (72.50±2.10mg/100g) than the control sample (96.57±0.80mg/100g) and CD treated sample (94.70±2.50mg/100g), but there was no significant difference ($p > 0.05$) between (Fe) contents of CD treated sample (94.70±2.50mg/100g) and control sample (96.57±0.80mg/100g). Also, there were no significant differences ($p \leq 0.05$) in Zinc (Zn) contents of the deodorized fermented condiments of both CD treated sample (16.60±0.75mg/100g) and PAC treated sample (14.81±0.18mg/100g) with control sample (18.25±1.56mg/100g). There was decrease in phosphorus of PAC treated sample (536.24±1.85mg/100g) and increased in CD treated sample (894.26±4.70mg/100g) from phosphorus content of control sample (617.19±1.53g/100g) and significantly differed ($p \leq 0.05$).

**Table 4.5; Effect of Deodorizing Agents on Mineral Elements Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds**

| Sample | Ca (mg/100g) | Mg (mg/100g) | K (mg/100g) | Fe (mg/100g) | Zn (mg/100g) | P (mg/100g) |
|---|---|---|---|---|---|---|
| **Control** | 222.50±2.50[c] | 143.25±1.00[c] | 754.50±2.56[a] | 96.57±0.80[a] | 18.25±1.56[a] | 617.19±1.53[b] |
| **CD** | 292.50±1.80[a] | 192.52±1.50[a] | 594.83±2.50[c] | 94.70±2.50[a] | 16.60±0.75[a] | 894.26±4.70[a] |
| **PAC** | 257.67±1.95[b] | 187.17±2.52[b] | 652.57±2.10[b] | 72.50±2.10[b] | 14.81±0.18[a] | 536.24±1.85[c] |

Values are expressed as (Mean ± SD) of triplicate determinations and values with different superscripts in the column are significantly different (p≤0.05). Where **CD**; Cyclodextrin treated sample, **PAC**; Powdered Activated Carbon treated sample and **Control;** sample without treatment

## 4.8 Effect of Deodorizing Agents on Antinutrients Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds

The results for effect of deodorizing agents on antinutrients composition of deodorized fermented condiment from *Prosopis africana* seeds are presented in Table 4.6. There was significant difference ($p \leq 0.05$) in antinutritional compounds between control and the treated samples, the levels of these compounds reduced significantly. The alkaloids concentration in the CD treated sample (4.10±0.02mg/100g) and PAC treated sample (4.95±0.01mg/100mg), were significantly decreased ($p \leq 0.05$) from control sample (7.21±0.01mg/100g), with CD treated sample recorded the lowest alkaloids value. Similarly, the Saponins content of CD treated sample (34.55±0.13mg/100g) and PAC treated sample (35.86±0.06mg/100g) were significantly reduced ($p \leq 0.05$) from of control sample (47.45±0.07mg/100g), and CD treated sample had the lowest value of saponins content. The flavonoids content of CD treated sample (61.35±0.22mg/100g) and PAC treated sample (56.18±0.00mg/100g) were significantly increased ($p \leq 0.05$) compared with control sample (46.21±0.02mg/100g), and CD treated sample recorded the highest value of flavonoids content. Similarly, the tannins content of CD treated sample (3.90±0.21mg/100g) and PAC treated sample (4.69±0.02mg/100g) were increased significantly ($p \leq 0.05$) with control sample (2.70±0.03mg/100g), and PAC treated sample recorded the highest value of tannins concentration. There was no significant difference between the control, CD and PAC treated samples in their oxalates contents ($p > 0.05$); PAC treated sample recorded (0.84±0.02mg/100g), CD treated sample recorded (0.68±0.06mg/100g) while control sample recorded (0.55±0.01mg/100g). The trypsin inhibitors content of CD treated sample (9.92±0.01TUI/100g) and PAC treated sample (6.72±0.01TUI/100g) were significantly reduced ($p \leq 0.05$) compared to control value of (11.23±0.03TUI/100g) with PAC recorded the

lowest value of trypsin inhibitors. Similarly, the α-Amylase inhibitors of CD treated sample (6.40±1.57mg/ml) and PAC treated sample (5.77±0.50mg/ml) were also significantly reduced ($p<0.05$) compared to control sample (13.05±0.88mg/ml) with the PAC treated sample recorded lowest α-amylase inhibitors.

### 4.9 Physicochemical Characteristics of Deodorized Fermented Condiment from *Prosopis africana* Seeds

The results for effect of deodorizing agents on physicochemical characteristics of deodorized fermented condiment from *Prosopis africana* seeds are presented in Table 4.7. The pH value of CD treated sample (7.20±1.00) was increased significantly ($p\leq0.05$) compared control sample (6.56±1.50) and PAC treated sample (6.76±1.50), but there was no significant difference ($p>0.05$) in pH value between the control sample (6.56±1.50) and PAC treated sample (6.76±1.50). The Titratable acidity of CD treated sample (8.50±1.60) and PAC treated sample (13.80±0.50) were increased significantly ($p\leq0.05$) from the control sample, with PAC treated sample recorded the highest value of titratable acidity. The water absorption capacity of CD treated sample (2.05±0.10g/ml) and PAC treated sample (3.24±0.06g/ml) were also significantly increased ($p\leq0.05$) from control sample (1.80±0.01g/ml) with PAC treated sample recorded the highest value of water absorption capacity, and there was no significant difference ($p>0.05$) between the CD treated sample (2.05±0.10g/ml) and control sample (1.80±0.01g/ml). The oil absorption capacity of CD treated sample (1.52±0.40g/ml) and PAC treated sample (1.10±0.11g/ml) were also reduced significantly ($p\leq0.05$) from control (2.05±0.05g/ml), and PAC treated sample recorded the lowest value of oil absorption capacity. There was no

significant difference ($p>0.05$) between the bulk density of control sample ($1.25\pm0.05 g/m^3$), CD treated sample ($1.00\pm0.95 g/m^3$) and PAC treated sample ($1.13\pm0.57 g/m^3$).

**Table 4.6; Effect of Deodorizing Agents on Antinutrients Composition of Deodorized Fermented Condiment from *Prosopis africana* Seeds**

| SAMPLES | ALKALOIDS (mg/100g) | FLAVONOIDS (mg/100g) | SAPONINS (mg/100g) | TANNINS (mg/100g) | OXALATES (mg/100g) | TRYPSIN INHIBITORS (TUI/mg) | α-AMYLASE INHIBITORS (mg/ml) |
|---|---|---|---|---|---|---|---|
| **CONTROL** | 7.21±0.01[a] | 46.21±0.02[c] | 47.45±0.07[a] | 2.70±0.03[c] | 0.55±0.01[a] | 11.23±0.03[a] | 13.05±0.88[a] |
| **CD** | 4.10±0.02[c] | 61.35±0.22[a] | 34.55±0.12[c] | 3.90±0.21[b] | 0.68±0.06[a] | 9.92±0.01[b] | 6.40±1.57[b] |
| **PAC** | 4.95±0.01[b] | 56.18±0.06[b] | 35.86±0.06[b] | 4.69±0.02[a] | 0.84±0.12[a] | 6.72±0.01[c] | 5.77±0.50[c] |

Values are expressed as Mean ± SD of duplicate measurement and values with different superscripts in the same column are significantly differ ($p \leq 0.05$). Where **CD**; Cyclodextrin treated sample, **PAC**; Powdered Activated Carbon treated sample and **Control;** sample without treatment.

**Table 4.7; Physicochemical Characteristics of Deodorized Fermented Condiment from *Prosopis africana* Seeds**

| SAMPLES | pH Value | Titratable acidity | Water Absorption Capacity (g/ml) | Oil Absorption Capacity (g/ml) | Bulk Density ($g/m^3$) |
|---|---|---|---|---|---|
| **Control** | 6.56±1.50$^{b}$ | 3.80±0.20$^{c}$ | 1.80±0.01$^{b}$ | 2.05±0.05$^{a}$ | 1.25±0.05$^{a}$ |
| **CD** | 7.20±1.00$^{a}$ | 8.50±1.60$^{b}$ | 2.05±0.10$^{b}$ | 1.52±0.40$^{b}$ | 1.00±0.95$^{a}$ |
| **PAC** | 6.76±1.50$^{b}$ | 13.80±0.50$^{a}$ | 3.24±0.06$^{a}$ | 1.10±0.11$^{c}$ | 1.13±0.57$^{a}$ |

Values are expressed as (Mean ± SD) of triplicate determinations and values with different superscripts in the column are significantly different ($p \leq 0.05$). Where **CD**; Cyclodextrin treated sample, **PAC**; Powdered Activated Carbon treated sample and **Control;** sample without treatment.

# CHAPTER FIVE

## 5.0 DISCUSSION

*Prosopis africana* seeds are processed to produce local fermented food condiment. In this study, the laboratory prepared fermented food condiment from *Prosopis africana* seeds was deodorized with potential chemical deodorants β-Cyclodextrin (CD), and powdered activated carbon (PAC) and their effects on nutrients and antinutrients composition were investigated. Heterotrophic bacterial count of laboratory prepared fermented *Prosopis africana* seeds increased exponentially from day 1 - 6, with peak count at day 7 and steady growth was observed from day 7 – 9. Also, changes in physical sensory parameters of laboratory fermented *Prosopis africana* seeds was observed during the fermentation period. The fermentation period was nine days. There was no change observed in colour of creamy cotyledon during the first three days of fermentation, but subsequently a slight change from odorless to non-offensive odor with mash cotyledon was observed. At day 4 and 5, a slight change from creamy-brownish to brownish colour and barely odor to mild odorous stick mash cotyledon were observed. At day 6 – 9, brown to dark brown colour was observed with increased offensive odor and mashy sticky cotyledon. The result obtained corroborates with the study reported by Ajayi (2014) that demonstrated some changes in physical sensory parameters of fermented condiments as a result of microbial activities and related environmental factors. It is expected that the longer the period of fermentation (5 - 7) days, the better the product due to more activities of the fermenting bacterial flora but period of fermentation is another major factor to assess the quality of production of fermented condiments (Balogun *et al.,* 2017).

A total of 27 volatile constituents were identified in this study. The alkanols present in the condiment may help prevent them from spoilage since alkanols are known to act as antifungal

and prevent food spoilage. Most of these alkanols had been reported present in other fermented leguminous products (Dajanta *et al*., 2010; Onyenekwe *et al.*, 2012). Alcohols also contribute to the flavour of the condiments. This is consistent with previous work where alcohol was reported in soy sauce, Miso (Ku *et al*., 2000) and Korea doenjang (Park *et al*., 1994) as important contributor of flavour. Eight ketones were identified in the sample in this study. Ketones are usually derived from lipid and amino acid degradation during microbial fermentation and have a high impact on food odor (Owens *et al*., 1997), ketones contribute to the odor of *okpehe.* The result corraborates with the study reported by Stephan and Steinhart (1999) that identified 17 ketones in soya bean condiment.

The ester constituents of the condiment may be responsible for the 'fruitiness' associated with *okpehe*. The acetates of higher alcohols and the ethyl ester of fatty acids had been suggested to be the most desirable compounds to enhance the aroma of the finished products and are responsible for the fruity tinge of freshly prepared miso (Giri *et al.*, 2010). The majority of benzene derivatives present in the fermented food condiment are the most important flavor constituents of daddawa (fermented locust bean seeds) after aldehydes. Microbial fermentation of legumes is known to increase the free amino acids content by five folds (Shu, 1998) and some of these amino acids are precursors of pyrazines. Threonine could be the precursor of 2, 5 – dimethyl pyrazine while tetramethyl pyrazine can be produced from different amino acids such as glycine, alanine, valine, isoleucine and leucine via Strecker degradation and deamination (Shu, 1998). This coupled with the fungicidal and bactericidal activities of other constituents may be responsible for non-spoilage of this condiment no matter how long it is stored without refrigeration. Other volatile constituents present in the fermented food condiment include anthracene, fumaric acid, 1h-Indole and 5-Methyl-2-phenylindolizine.

Powdered activated carbon (PAC) treated sample had the highest deodorization potential which deodorized 63% of the volatile constituents while Cyclodextrin (CD) treated sample had the least deodorization potential which deodorized only 30% of the volatile constituents. This is consistent with the study conducted by Tamaki *et al.,* (2007) who reported that, PAC has higher deodorization potential in sweet potato juice while CD treated sample showed least effect in reducing the off-flavour of the sweet potato juice. It is expected that all the odor component molecules are small enough to enter the microspores of the PAC (Wittanalai *et al.,* 2011). However, PAC differed in its adsorption removal ability for individual odor components. It is thought that the odor removal ability depends on the difference in physical and/or chemical interactions between the PAC surface and the odor components. Since, several kinds of coexisting odor components in water were removed by the PAC adsorption, it is difficult to evaluate the potential physical and/or chemical interactions between the PAC surface and the respective odor components (Tamaki *et al.,* 2007).

Cyclodextrin removed only a small amount of acetone although acetone being small in size could be enveloped easily in its cavity, the inner hydrophobic environment prevents hydrophilic acetone from being adsorbed (Tamaki *et al.,* 2007). Ethyl acetate, with a relatively high hydrophobicity, showed a higher value and the peak intensity remained high after activated carbon treatment. When treated with apple polyphenols AP deodorant, the peak intensity for ethyl acetate was approximately four times larger than that for acetone. On the contrary, the peak intensity for acetone was slightly larger than that for ethyl acetate during PAC treatment (Wittanalai *et al.,* 2011). In comparison to the apple polyphenol (AP) treatment a conspicuous reduction in peak intensity was noted for the activated carbon treatment. The most probable

reason for such observations is the hydrophilic and hydrophobic nature of acetone and ethyl acetate, respectively (Tamaki *et al.,* 2007).

In addition, the results of crude protein revealed that the CD treated sample recorded the lowest protein content while PAC treated sample recorded the highest value of protein content of the condiment. Meanwhile, the CD and PAC treated samples had a lower lipid content compared to the control sample, this corraborate with study reported by Balogun *et al*, (2017), in which the crude protein content of *Prosopis africana* seeds inoculated with mono- and mixed cultures of bacterial isolates were increased as the days of fermentation increases. The crude protein increased significantly for both the mono and mixed culture inoculated samples, *B. subtilis* had the highest while *B. pumilus* had the lowest crude protein for the mono inoculated seeds. The highest crude protein content was obtained when *B. subtilis* and *B. licheniformis* were combined, while the lowest protein content was obtained for combination of *B. megaterium* and *B. pumilus.* The fermented seeds obtained from the mixed culture had a higher crude protein than both the monoculture inoculated and control samples.

The ash content of the CD and PAC treated samples were increased compared to control. Similarly, the crude fiber contents of CD and PAC treated samples were increased compared to control sample. The increased was associated with additions of chemical deodorants (CD and PAC). The crude fibre content of the CD treated sample was lower than that of PAC treated sample. The moisture content of CD and PAC treated samples were reduced, with CD treated sample recorded the least moisture content. The reduced moisture content of CD and PAC treated samples is of remarkable significance because of the consequential impartment in shelf-life of the condiment. Very low moisture content increases shelf-life as microbial spoilage is minimized. The carbohydrate content of control sample was lower from that of CD and PAC

treated samples. The CD treated sample recorded the highest carbohydrate content and the increase was associate with the fact that cyclodextrin is a hydrolytic form of starch.

The vitamins A, B, C and E contents of the CD and PAC treated samples increased significantly compared to control sample. CD treated sample recorded the highest value of these vitamins. The increase in these vitamins are associated with the fact that, cyclodextrins create a conducive environment for microbial activities and formed inclusion complexes with degraded products of protein, lipids and the polyglucopyranose units of cyclodextrin (Tamaki *et al.,* 2007). Therefore, the noticeable increase in the vitamin A, B vitamins, vitamins C and E content of the fermented condiment makes it a good source of these vitamins.

The calcium (Ca), magnesium (Mg), and phosphorus (P) content of CD treated sample were increased significantly and this noticeable increase makes the condiment a good source of these essential minerals. The increased calcium content in the condiment is good because the element is needed for bone development and strong teeth. Zinc was also found in an appreciable amount and this is desirable as it is known to aid digestion, body functions and lower the risk of diarrhea in children. Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system. The appreciable amount of potassium is good because the element helps in regulation of body fluids and maintenance of normal body pressure. It helps in controlling kidney failure, heart oddities and respiratory flaw (Balogun *et al.,* 2017).

In addition, there was significant difference in antinutritional compounds between control and the treated samples, the levels of these compounds reduced in an appreciable amount. The alkaloids, Saponins, oxalates, trypsin inhibitors and α-amylase inhibitors concentrations in the CD and PAC treated samples were reduced, with CD treated sample recorded the least alkaloids

and Saponins, while PAC treated sample recorded lowest value of trypsin and α-Amylase inhibitors. The flavonoids content of CD and PAC treated samples were increased and CD treated sample recorded the highest value. These bring out the fact that reduction of antinutritional factors Saponins, alkaloids and inhibitors such as trypsins and α-Amylase improve the nutritional quality of the condiment while the increase in the flavonoids content enhances the antioxidant capacity of the condiment.

The result also revealed that the pH value of CD treated sample was higher compared to control and PAC treated samples. The rise in pH is due to the high proteinase activity of the microorganisms involved which ultimately results in the liberation of ammonia as is reported for some other fermenting protein foods (Ogunshe *et al.,* 2007). The increased in titratable acidity of both CD and PAC treated samples makes it a good condiment. Desirable flavour components of the condiment are presumably developed after an alkaline titratable acidity is reached. Organic acids which may result from protein decomposition may contribute to the darkening of colour (Balogun *et al.,* 2017). The titratable acidity of okpehe being alkaline agrees with earlier reports of Ogunshe *et al.* (2007), that recorded alkaline titratable acidity in fermented food condiments from vegetable proteins. Most fermented vegetable-protein condiments are characterized by a very strong pungent smell. The increase in titratable acidity is generally due to the production of ammonia, which is characterized by the pungent smell of alkaline fermented condiments (Ogunshe *et al.,* 2007).

The increased water absorption capacity of CD and PAC treated samples and also the decreased oil absorption capacity of both CD and PAC treated samples may probably increase its acceptability and market range. The appreciable amount of oil absorption capacity suggests that cyclodextrin treated sample has more hydrophobic interaction sites than powdered activated

carbon. Variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the condiments, could explain differences in the oil binding capacity of the treated samples (Balogun *et al.,* 2017). However, the condiments are potentially useful for their structural interactions in food, especially for flavor retention, and to improve palatability and extension of shelf life, in which fat absorption is desirable (Dirar, 1993).

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

### 6.1 Summary

This study revealed that fermented food condiment from *Prosopis africana* seeds was prepared in the laboratory with microbial counts increasing exponentially from days 1 – 6 with peak count at day 7 and steady growth, offensive odor and dark mash sticky cotyledon at days 7 - 9. There was no change observed in colour of creamy cotyledon from days 1 – 3, a slight change from odorless to non-offensive odor with mash cotyledon was observed. At days 4 – 5, a slight change from creamy-brownish to brownish colour, barely odor to mild odorous sticky mash cotyledon was observed. At days 6 – 9, brown to dark brown colour was observed with increased offensive odor and mashy sticky cotyledon.

Cyclodextrin (CD) and powdered activated carbon (PAC) treated samples 2.5g/100g had the least odor (Barely perceived odor) of 73.3% and 86.6% deodorization respectively, while 0.5g/100g of both CD and PAC treated samples had 100% strong offensive odor when compared with normal control (NC). The PAC treated samples 1.5g/100g, 2.0g/100g and CD treated sample 2.0g/100g had 53.3% barely perceived odor.

In all, there were about 27 volatile constituents in control sample, 19 in CD treated sample and 10 in PAC treated sample based on their retention time. Powdered activated carbon had the highest deodorization potential, which deodorized 63% of the volatile constituents while

cyclodextrin had a lower deodorization potential which deodorized 30% of the volatile constituents respectively.

The crude protein content for the control sample differed significantly ($p \leq 0.05$), the protein content of CD treated sample was decreased while PAC treated sample significantly increased compared with control sample. The crude fiber contents of control sample significantly differed ($p \leq 0.05$) from CD and PAC treated samples. There was no significant difference ($p > 0.05$) between CD and PAC treated samples crude fibre contents. The total carbohydrate content of control was significantly differed ($p \leq 0.05$) with that of CD and PAC treated samples. The CD treated sample recorded the highest carbohydrate content.

The vitamins A, B, C and E contents of the CD and PAC treated samples increased significantly compared to control sample. CD treated sample recorded the highest value of these vitamins. The vitamin A content of CD and PAC treated samples were significantly ($p \leq 0.05$) higher than that of control sample content. The vitamin $B_1$ content of CD and PAC treated samples were also significantly ($p \leq 0.05$) higher than control sample. The vitamin $B_2$ content of control sample was differed significantly ($p \leq 0.05$) from CD and PAC treated samples. But there was no significant difference ($p > 0.05$) of vitamin $B_2$ contents between the CD and PAC treated samples. In addition, there was no significant difference ($p > 0.05$) between vitamin $B_3$ contents of all the samples; control, CD and PAC treated samples. The vitamin C content of CD and PAC treated samples were significantly different ($p \leq 0.05$). Lastly, there was significant differences ($p \leq 0.05$), in vitamin E content of CD and PAC treated samples.

The calcium content of CD and PAC treated samples were higher and significantly differed ($p \leq 0.05$) from that of control fermented condiment. Similarly, the magnesium content of CD and

PAC treated samples were significantly increased ($p \leq 0.05$), from that of control sample. Meanwhile, there were significant differences ($p \leq 0.05$) between potassium content of the deodorized fermented condiments CD and PAC treated samples from control sample. Statistically, there was significant difference ($p \leq 0.05$) of iron content. The iron content of PAC treated sample was lower (72.50±2.10mg/100g) than the control and CD treated sample, but there was no significant difference ($p > 0.05$) between iron contents of CD treated sample and control sample. Also, there were no significant differences ($p \leq 0.05$) in Zinc contents of the deodorized fermented condiments of both CD and PAC treated sample with control. There was decrease in phosphorus of PAC treated sample and increased in CD treated sample from phosphorus content of control and significantly differed ($p \leq 0.05$).

The pH value of CD treated sample was increased significantly ($p \leq 0.05$) compared with control and PAC treated sample, but there was no significant difference ($p > 0.05$) in pH value between the control and PAC treated samples. The Titratable acidity of CD and PAC treated samples were increased significantly ($p \leq 0.05$) from the control sample, with PAC treated sample recorded the highest value of titratable acidity. The water absorption capacity of CD and PAC treated samples were also significantly increased ($p \leq 0.05$) from control sample with PAC treated sample recorded the highest value of water absorption capacity, and there was no significant difference ($p > 0.05$) between the CD treated sample and control. The oil absorption capacity of CD and PAC treated samples were also reduced significantly ($p \leq 0.05$) from control and PAC treated sample recorded the lowest value of oil absorption capacity. There was no significant difference ($p > 0.05$) between the bulk density of control, CD and PAC treated samples.

There was significant difference ($p \leq 0.05$) in antinutritional compounds between control and the treated samples, the levels of these compounds reduced significantly. The alkaloids

concentration in the CD and PAC treated samples were significantly decreased ($p \leq 0.05$) from control sample, with CD treated sample recorded the lowest alkaloids value. Similarly, the Saponins content of CD and PAC treated samples were significantly reduced ($p \leq 0.05$) from of control, and CD treated sample had the lowest value of saponins content. The flavonoids content of CD and PAC treated samples were significantly increased ($p \leq 0.05$) compared with control, and CD treated sample recorded the highest value of flavonoids content. Similarly, the tannins content of CD and PAC treated samples were increased significantly ($p \leq 0.05$) with control sample, and PAC treated sample recorded the highest value of tannins concentration. There was no significant difference between the control, CD and PAC treated samples in their oxalates contents. The trypsin inhibitors content of CD and PAC treated samples were significantly reduced ($p \leq 0.05$) compared to control. Similarly, the α-Amylase inhibitors of CD and PAC treated samples were also significantly reduced ($p \leq 0.05$) compared to control sample.

## 6.2 CONCLUSION

In conclusion, the present study depicted the potentiality of cyclodextrin (CD) and powdered activated carbon (PAC) as good chemical deodorants for the deodorization of fermented food condiment from *Prosopis africana* seeds. Powdered activated carbon exhibited the highest deodorization potential. The cyclodextrin and powdered activated carbon are suitable and effective in reducing various antinutritional compounds most especially the inhibitors except flavonoids and tannins, with significant increase in the nutrient contents of fermented condiment from *Prosopis africana* seeds.

### 6.3 RECOMMENDATIONS

1) It is recommended to use 2.5g/100g concentration of cyclodextrin (CD) or powdered activated Carbon (PAC) on fermented food condiment to achieved higher deodorization.
2) It is recommended that deodorization with powdered activated carbon to achieved a high yield of ash and protein contents as well as least trypsin and α-Amylase inhibitors while deodorization with cyclodextrin to achieve least moisture content and high yield in vitamins and total carbohydrate contents.
3) Further studies should be carried out on the organoleptic properties of the food recipe prepared with deodorized fermented condiment.

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| **CHEMICAL DEODOURANTS** | | **CD** | | | | | **PAC** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **S/N** | **DOSAGE g/100g** | **0.5** | **1.0** | **1.5** | **2.0** | **2.5** | **0.5** | **1.0** | **1.5** | **2.0** | **2.5** |

## APPENDICES

**INSTRUCTIONS; Tick the appropriate box to reflect your preference**
**NAME**_____________________________________________ DATE______________

**Appendix 1: Score Card for Deodourized Fermented Condiment**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **ODOURLESS** | | | | | | | | | | |
| **2** | **BARELY PERCEIVED ODOUR** | | | | | | | | | | |
| **3** | **MILD ODOUR** | | | | | | | | | | |
| **4** | **MODARATE ODOUR** | | | | | | | | | | |
| **5** | **STRONG ODOUR** | | | | | | | | | | |

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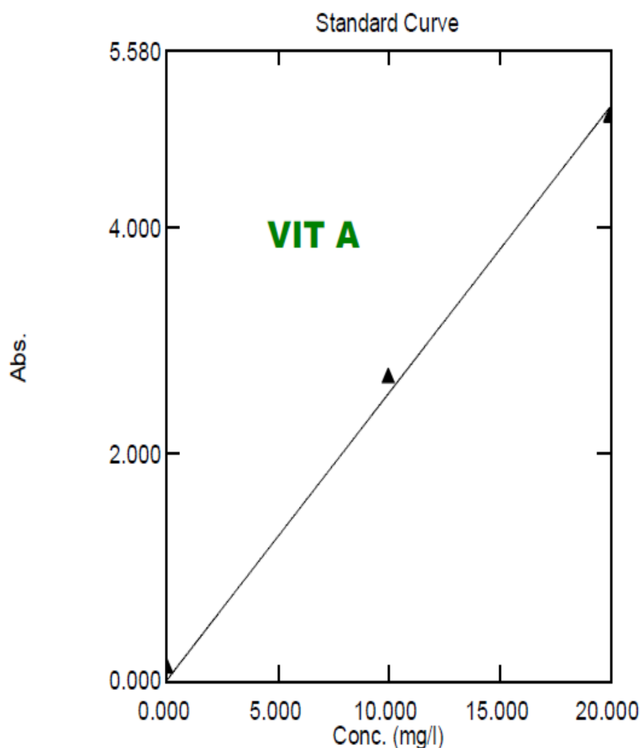



y = 0.25387 x + 0.00000
Correlation Coefficient r2 = 0.99895

Standard Table

| | Sample | Type | Ex | Conc | WL325 | Wgt.F |
|---|---|---|---|---|---|---|
| 1 | Blank | Standard | | 0.000 | 0.113 | 1.0 |
| 2 | 1 | Standard | | 10.000 | 2.693 | 1.0 |
| 3 | 2 | Standard | | 20.000 | 5.000 | 1.0 |
| 4 | | | | | | |

Sample Table

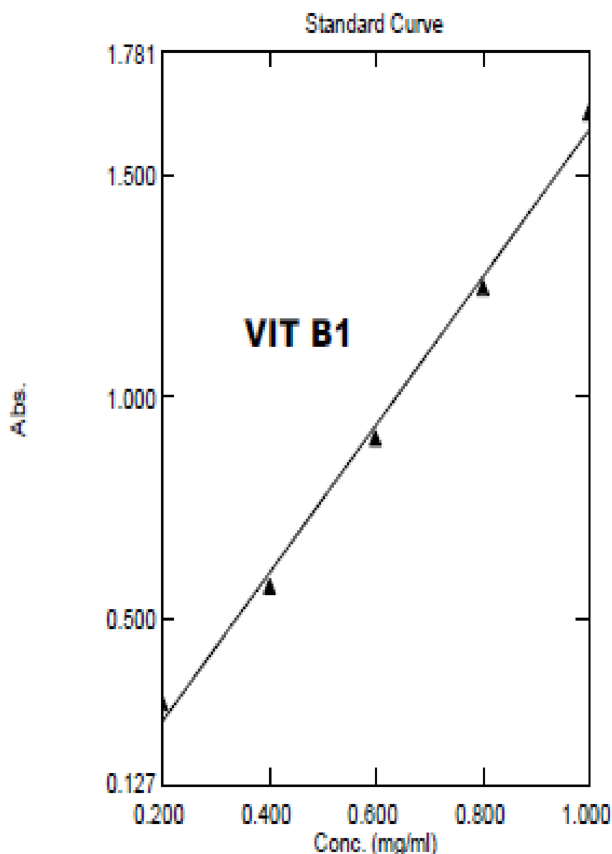
| | Sample ID | Type | Ex | Conc | WL325 | Comments |
|---|---|---|---|---|---|---|
| 1 | PAC | Unknown | | 4.099 | 1.041 | |
| 2 | CD | Unknown | | 4.599 | 1.167 | |
| 3 | CTRL | Unknown | | 3.010 | 0.764 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL325
Wavelength: 325.00 nm

Calibration Curve
Column for Cal. Curve: WL325
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL325
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Selected

**Appendix 2: Calibration Curve for Determination of Vitamin A in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




Standard Table

| | Sampl | Type | Ex | Conc | WL540.0 | Wgt.F: |
|---|---|---|---|---|---|---|
| 1 | 1 | Stan | | 0.200 | 0.307 | 1. |
| 2 | 2 | Stan | | 0.400 | 0.570 | 1. |
| 3 | 3 | Stan | | 0.600 | 0.908 | 1. |
| 4 | 6 | Stan | | 0.800 | 1.248 | 1. |
| 5 | 7 | Stan | | 1.000 | 1.643 | 1. |
| 6 | | | | | | |

Sample Table

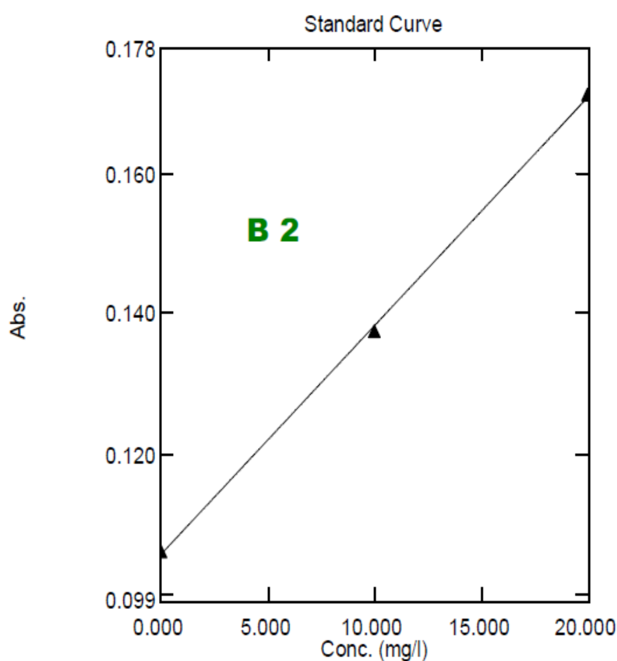
| | Sample ID | Type | Ex | Conc | WL540.0 | Comments |
|---|---|---|---|---|---|---|
| 1 | CTRL | Unknown | | 0.110 | 0.114 | |
| 2 | PAC | Unknown | | 0.116 | 0.124 | |
| 3 | CD | Unknown | | 0.123 | 0.137 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL540.0
Wavelength: 540.00 nm

Calibration Curve
Column for Cal. Curve: WL540.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/ml
Selected Wavelength: WL540.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 3: Calibration Curve for Determination of Vitamin B1 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 0.00326 x + 0.10571
Correlation Coefficient r2 = 0.99950

Standard Table

| | Sample I | Type | Ex | Conc | WL530.0 | Wgt.F |
|---|---|---|---|---|---|---|
| 1 | BLANK | Standard | | 0.000 | 0.106 | 1 |
| 2 | 1 | Standard | | 10.000 | 0.137 | 1 |
| 3 | 2 | Standard | | 20.000 | 0.171 | 1 |
| 4 | | | | | | |

Sample Table

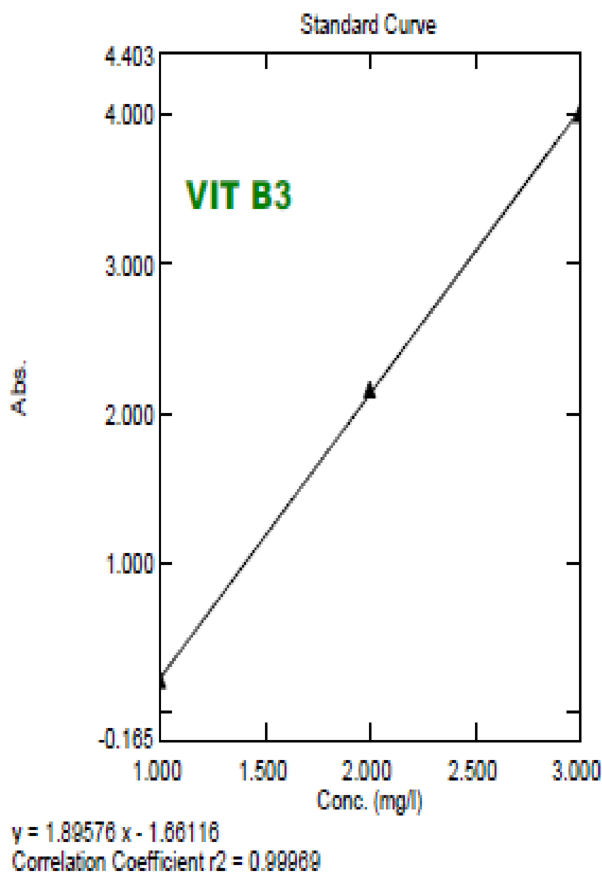
| | Sample ID | Type | Ex | Conc | WL530.0 | Comments |
|---|---|---|---|---|---|---|
| 1 | CTRL | Unknown | | -2.275 | 0.098 | |
| 2 | CD | Unknown | | 5.573 | 0.124 | |
| 3 | PAC | Unknown | | 5.465 | 0.124 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL530.0
Wavelength: 530.00 nm

Calibration Curve
Column for Cal. Curve: WL530.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL530.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 4: Calibration Curve for Determination of Vitamin B2 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 1.89576 x - 1.66116
Correlation Coefficient r2 = 0.99969

Standard Table

| | Sample ID | Type | Ex | Conc | WL261.0 | Wgt.Fa |
|---|---|---|---|---|---|---|
| 1 | BLANK | Blank | | 0.000 | 0.112 | 1.000 |
| 2 | 1 | Standard | | 1.000 | 0.215 | 1.000 |
| 3 | 2 | Standard | | 2.000 | 2.169 | 1.000 |
| 4 | 3 | Standard | | 3.000 | 4.007 | 1.000 |
| 5 | | | | | | |

Sample Table

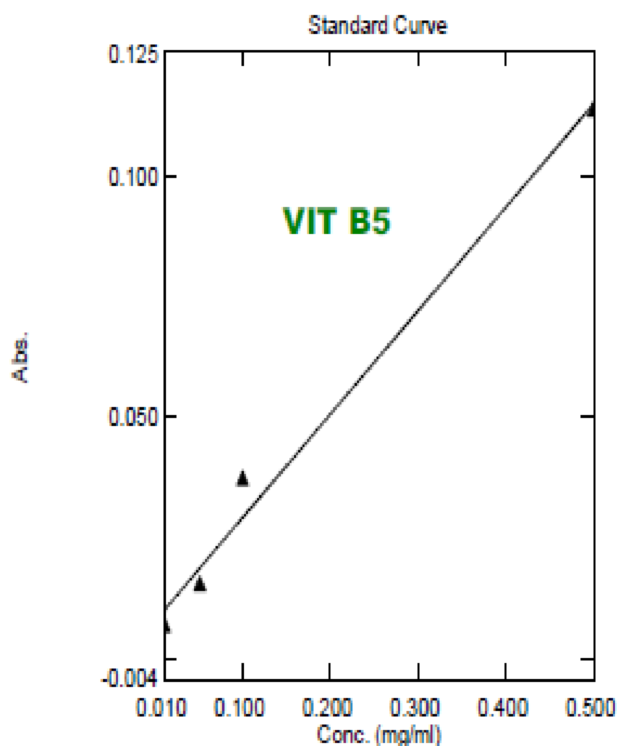
| | Sample ID | Type | Ex | Conc | WL261.0 | Co |
|---|---|---|---|---|---|---|
| 1 | CD | Unknown | | 1.368 | 0.933 | |
| 2 | PAC | Unknown | | 1.288 | 0.781 | |
| 3 | CTRL | Unknown | | 1.217 | 0.645 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL261.0
Wavelength: 261.00 nm

Calibration Curve
Column for Cal. Curve: WL261.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL261.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 5: Calibration Curve for Determination of Vitamin B3 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 0.21307 x + 0.00813
Correlation Coefficient r2 = 0.98830

Standard Table

| | Sample ID | Type | Ex | Conc | WL478.5 |
|---|---|---|---|---|---|
| 1 | 1 | Blank | | 0.000 | 0.04 |
| 2 | 3 | Standard | | 0.010 | 0.00 |
| 3 | 4 | Standard | | 0.050 | 0.01 |
| 4 | 5 | Standard | | 0.100 | 0.03 |
| 5 | 6 | Standard | | 0.500 | 0.11 |
| 6 | | | | | |

Sample Table

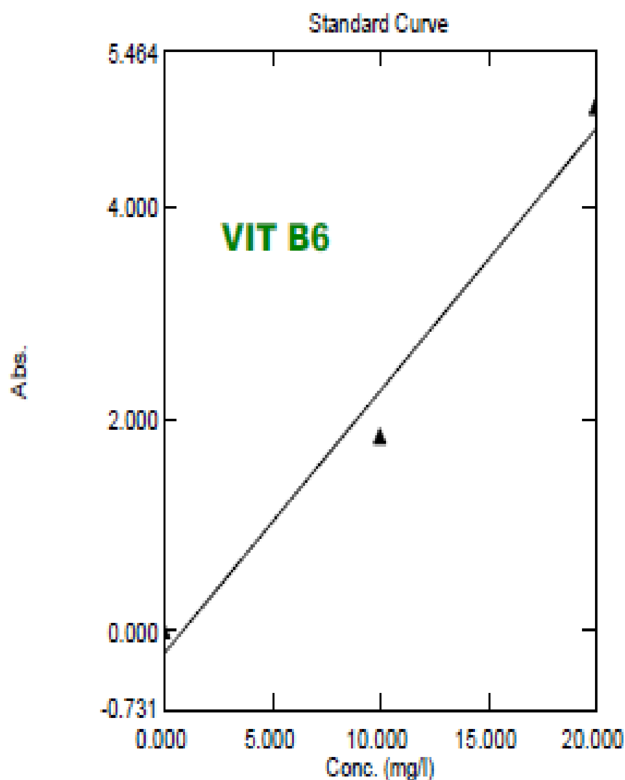
| | Sample ID | Type | Ex | Conc | WL478.5 | Comments |
|---|---|---|---|---|---|---|
| 1 | PAC | Unknown | | 0.192 | 0.049 | |
| 2 | CTRL | Unknown | | 0.189 | 0.048 | |
| 3 | CD. | Unknown | | 0.294 | 0.071 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL478.5
Wavelength: 478.50 nm

Calibration Curve
Column for Cal. Curve: WL478.5
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/ml
Selected Wavelength: WL478.5
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 6: Calibration Curve for Determination of Vitamin B5 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 0.24738 x - 0.21448
Correlation Coefficient r2 = 0.97791

Standard Table

| | Sample | Type | Ex | Conc | WL291.0 |
|---|---|---|---|---|---|
| 1 | BLANK | Standard | | 0.000 | 0.00 |
| 2 | 1 | Standard | | 10.000 | 1.83 |
| 3 | 2 | Standard | | 20.000 | 4.94 |
| 4 | | | | | |

Sample Table

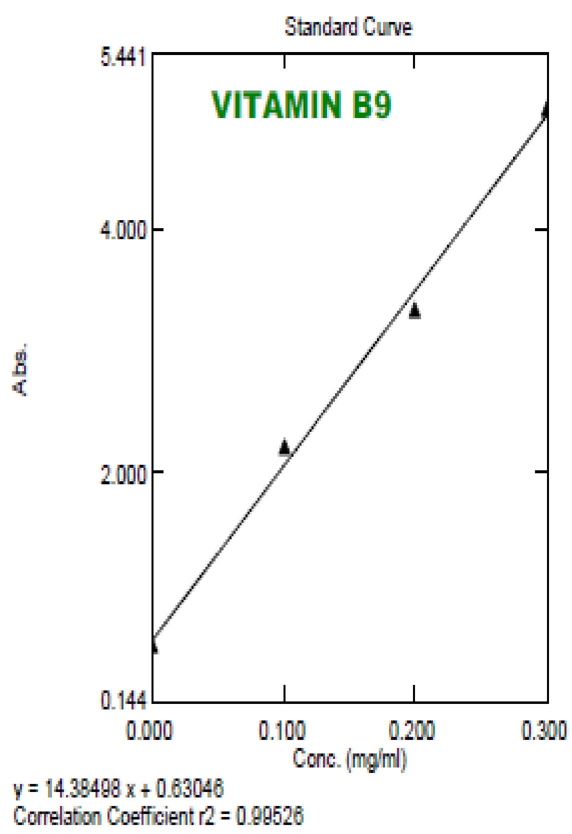
| | Sample ID | Type | Ex | Conc | WL291.0 | Comments |
|---|---|---|---|---|---|---|
| 1 | PAC | Unknown | | 6.586 | 1.415 | |
| 2 | CD | Unknown | | 8.965 | 2.003 | |
| 3 | CTRL | Unknown | | 4.924 | 1.004 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL291.0
Wavelength: 291.00 nm

Calibration Curve
Column for Cal. Curve: WL291.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL291.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 7: Calibration Curve for Determination of Vitamin B6 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 14.38498 x + 0.63046
Correlation Coefficient r2 = 0.99526

Standard Table

| | Sample ID | Type | Ex | Conc | WL: |
|---|---|---|---|---|---|
| 1 | Blank | Standard | | 0.000 | |
| 2 | 1 | Standard | | 0.100 | |
| 3 | 2 | Standard | | 0.200 | |
| 4 | 3 | Standard | | 0.300 | |
| 5 | | | | | |

Sample Table

| | Sample ID | Type | Ex | Conc | WL282.0 | Con |
|---|---|---|---|---|---|---|
| 1 | CTRL | Unknown | | -0.001 | 0.617 | |
| 2 | CD | Unknown | | 0.015 | 0.846 | |
| 3 | PAC. | Unknown | | 0.006 | 0.714 | |
| 4 | | | | | | |

Wavelengths

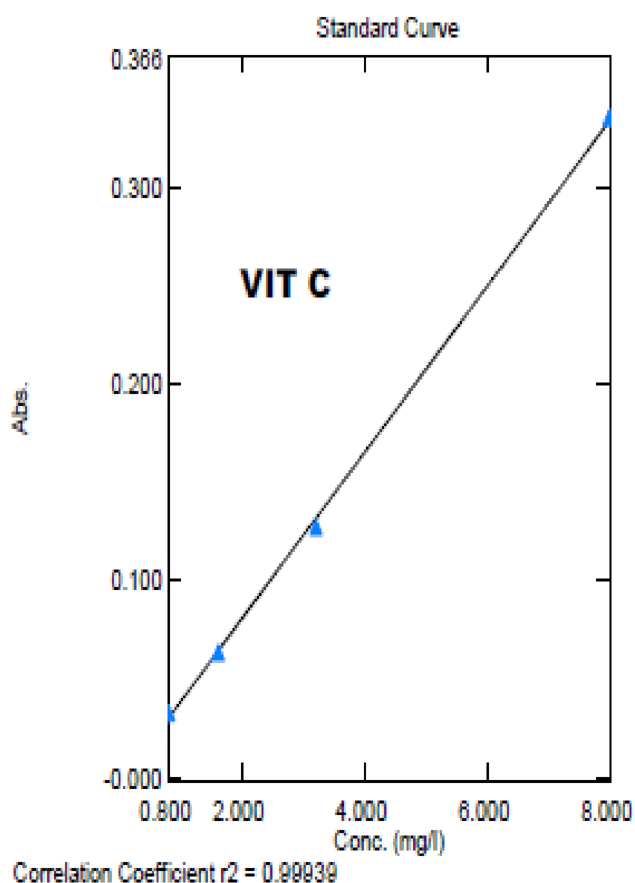
| | |
|---|---|
| Wavelength Name: | WL282.0 |
| Wavelength: | 282.00 nm |

Calibration Curve

| | |
|---|---|
| Column for Cal. Curve: | WL282.0 |
| Cal. Curve Type: | Multi Point |
| Cal. Curve Unit: | mg/ml |
| Selected Wavelength: | WL282.0 |
| Calibration Equation: | Abs = K1*(Conc) + K0 |
| Zero Interception: | Not Selected |

**Appendix 8: Calibration Curve for Determination of Vitamin B9 in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




Standard Table

| | Sample I | Type | Ex | Conc | WL452.0 | Wg |
|---|---|---|---|---|---|---|
| 1 | STD 1 | Standard | | 0.800 | 0.033 | |
| 2 | STD 2 | Standard | | 1.600 | 0.064 | |
| 3 | STD 3 | Standard | | 3.200 | 0.127 | |
| 4 | STD 4 | Standard | | 8.000 | 0.336 | |
| 5 | | | | | | |

Sample Table

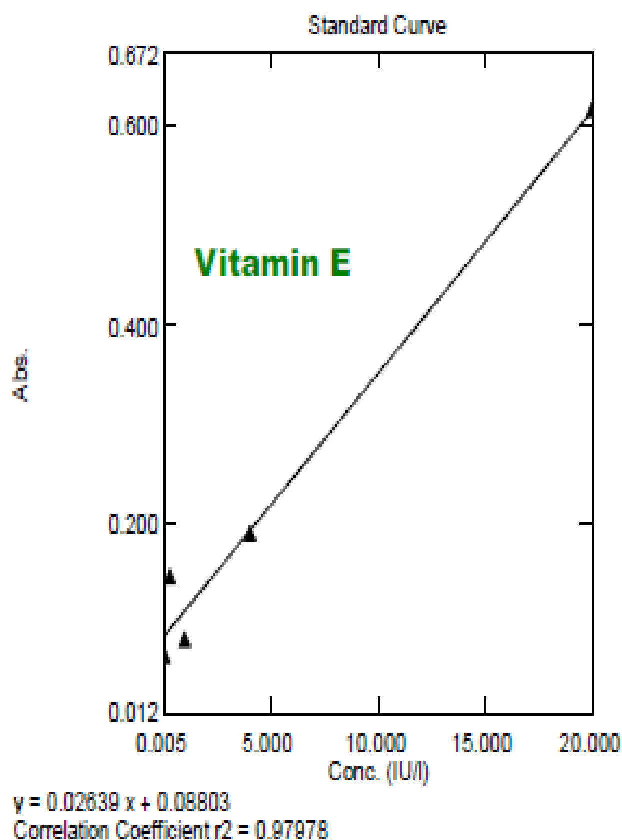
| | Sample ID | Type | Ex | Conc | WL452.0 | Comments |
|---|---|---|---|---|---|---|
| 1 | PAC | Unknown | | 6.825 | 0.285 | |
| 2 | CTRL | Unknown | | 5.174 | 0.215 | |
| 3 | CD. | Unknown | | 7.938 | 0.332 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL452.0
Wavelength: 452.00 nm

Calibration Curve
Column for Cal. Curve: WL452.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL452.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 9: Calibration Curve for Determination of Vitamin C in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples**

File Name: C:\Documents and Settings\NARICT\Desktop\STANDARDS\SULEMAN UBANGIDA




y = 0.02639 x + 0.08803
Correlation Coefficient r2 = 0.97978

Standard Table

| | Sample ID | Type | Ex | Conc | WL295.0 | Wgt.F |
|---|---|---|---|---|---|---|
| 1 | 1 | Standard | | 20.000 | 0.617 | 1.00 |
| 2 | 2 | Standard | | 4.000 | 0.190 | 1.00 |
| 3 | 3 | Standard | | 1.000 | 0.085 | 1.00 |
| 4 | 4 | Standard | | 0.250 | 0.148 | 1.00 |
| 5 | 5 | Standard | | 0.005 | 0.067 | 1.00 |
| 6 | | | | | | |

Sample Table

| | Sample ID | Type | Ex | Conc | WL295.0 | Comments |
|---|---|---|---|---|---|---|
| 1 | PAC | Unknown | | 12.213 | 0.410 | |
| 2 | CD | Unknown | | 13.511 | 0.445 | |
| 3 | CTRL | Unknown | | 4.970 | 0.219 | |
| 4 | | | | | | |

Wavelengths
Wavelength Name: WL295.0
Wavelength: 295.00 nm

Calibration Curve
Column for Cal. Curve: WL295.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: IU/l
Selected Wavelength: WL295.0
Calibration Equation: Abs = K1*(Conc) + K0
Zero Interception: Not Selected

**Appendix 10: Calibration Curve for Determination of Vitamin E in Deodorized Fermented Condiments from *Prosopis africana* Seeds for Control, Cyclodextrin and Powdered Activated Carbon Treated Samples.**