EFFECTS OF DIFFERENT SOLVENTS ON THE PROXIMATE COMPOSITION OF LIQIUD STATE FERMENTED *Citrullus colocynthis* FRUITS.

BY

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CERTIFICATION PAGE

This is to certify that this project work was indeed carried out by **OLORUNSOLA, TEMIDAYO O'BUKUNMI (MCB/14/2330)** supervised by **DR. R.A.O GABRIEL-AJOBIEWE.**

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DEDICATION

This project work is dedicated to the Almighty God and my loving family and friends.

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I appreciate the Almighty God for the grace and privilege he bestowed upon me throughout the tenure of the project work. I appreciate my loving parents in person of **Mr Temitope Lawrence and Mrs Comfort Bolaji Olorunsola** for granting me the opportunity to be educated and all the care they have given to me since I was given birth to.

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ABSTRACT

Citrullus colocynthis, commonly known as the colocynth, bitter apple, bitter cucumber was alongside with *Adansonia digitata* (*Epo Igi Ose*), *Syzigium aromaticum* (*konafuru*) divided into groups; variable 1: all ingredients and palmwine (CPA), variable 2: all ingredients and distilled water (CWA), variable 3: fruits and palmwine only (CP), variable 4: fruits and distilled water only (CW), variable 5: fruit only (CO) depending on the solvents (palmwine and water) and fermented for five (5) days. The physioco-chemical analysis (pH, temperature, total titrable acidity) readings were taken every 24 hours following standard procedures. At the end of fermentation, proximate analysis were carried out following standard procedures on day 0 and day 5 for the wet and dry fermented samples. The results obtained from the proximate analysis were: moisture content (WCON: 9[illegible].5 %) and (DCWA: 9.40%), protein (WCW: 2.56%) and (DCW: 14.47%), fat (WCW: 6.76% and DCW: [illegible]%), fiber (WCW: 17.46%) and (DCWA: 47.85%), total ash (WCWA: 1.04% and DCON: [illegible]%), carbohydrate (WCWA: 7.85%) and (DCPA: 28.47%) were the parameters with the highest values recorded for the wet and dry fermented samples. The results of the proximate composition revealed that *Citrullus colocynthis* fruit is a good source of protein, fat, fiber and carbohydrate.

CHAPTER ONE

1.0 INTRODUCTION.

Citrullus colocynthis (cucurbitaceae) is a native of arid soils. It is commonly found in Saudi Arabia, Syria, Jordan, Egypt, Iran, India and Pakistan. It is commonly known as Hanzal, Indrian, Tumma or Bitter apple. It is a large creeping herb, with deeply dissected lobulate leaves. Fruits rounded 7-9cm in diameter, green and white, striped, become yellow when ripe. The dried pulp of mature fruits freed from rind and seeds constitute the drug "Colocynth" formerly official in Indian pharmacopeia and currently official in Homoeopathic pharmacopeia; which was included in United States pharmacopeia between 1829 to 1910 (Rizvi *et al.*, 2007). Colocynth is a powerful hepatic and intestinal stimulant. It is also found useful in asthma, rheumatism, sciatica, gout, paralysis, leprosy, epilepsy and in expulsion of intestinal parasites. It is also used as purgative for chronic constipation and is a very well-known abortifacient (Usmanghani *et al.*, 1997).

Almost all "melons" have nutritional, therapeutic and economic value. The edible family member, *Momordica charantia*, called bitter melon or bitter gourd in English, is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. This fruit has been reported to have quite a number of medicinal uses ranging from antiviral, antidiabetic, antiulcerogenic, antioxidant and hepatoprotective (Semiz and Sen, 2007) to antihelmintic (Beloin *et al.*, 2005), antimalarial (Waako *et al.*, 2005), to cardio protective properties (Gadang *et al*, 2011).

1.2 Botanical Classification of *Citrullus colocynthis*

Kingdom: plantae

Subkingdom: Tracheobionta

Division : Magnoliophyta

Class: Mangnoliopsida

Order: cucurbitales

Family : cucurbitaceae

Genus : Citrullus Schrad

Specie : Citrullus colocynthis(L) Schrad.

(Rani *et al.*, 2017)

1.3 The *Cucurbitaceae* Family

Other Species of the Genus *Citrullus*

Cucurbitaceae, the gourd family of flowering plants belonging to the order cucurbitales and containing 98 genera and about 975 species of food and ornamental plants. Members of the family are annual or perennial herbs native to the temperate and tropical. It includes cucumbers, gourds, melons, squashes and pumpkins.

Etymology: The word *Citrullus* is the diminutive of the Greek word *citrus*, referring to the fruit sometimes called 'citron'. Some of the other species that can be found in the genus *Citrullus* is:

***Citrullus lanatus*:**

The species name *lanatus* in Latin means "woolly", referring to the plant's pubescence, (i.e. covered with soft hair) (Grubben, 2004; Lu and Jeffrey, 2011). It is a monoecious plant, simple toothed leaves, alternate, pinnately lobed. It has its male and female reproductive part

borne on different flowers on the same plant independently with a villous calyx and yellow corolla. It is large, and has a round to oblong fleshy berries, most commonly green watermelons can grow to a height of 0.5m. Aside from cultivated land, *C. lanatus* grows on disturbed sites, landfills, shores, and sewage plant fill. It thrives in well-drained, sandy loam soils and is shade intolerant. *C. lanatus* grows up to 2000m above sea level in Africa (Grubben, 2004).

Citrullus vulgaris:

The roots are large, fleshy, and perennial, leading to a high survival rate due to the long tap root. It has climbing vinyl stems. The leaves are palmate and angular with three to seven divided lobes resembling that of the watermelon. The flowers are yellow and solitary in the axes of leaves and are borne by yellow-greenish peduncle and are also monoecious, so the male (stamens) and the female reproductive parts (pistils and ovary) are borne in different flowers on the same plant. The fruit is smooth, spherical and with an extremely bitter taste. The mesocarp is filled with a soft, dry, and spongy white pulp, in which the seeds are embedded. The seeds are grey and edible but similarly bitter, nutty-flavored, and rich in fat and protein. They are eaten whole or used as an oilseed. The seeds contain a high amount of arginine, tryptophan, and the sulfur containing amino acids (Oluba *et al.*, 2010).

1.4 Morphology and Features

Roots:

The roots are large perpetual, long and delicate, bifurcate, tenacious and rocky vine-like (Rani *et al.*, 2017).

Stems:

The stems spread on the soil and have an affinity to mount over herbs and shrubs by their auxiliary branching tendrils (Rani *et al.*, 2017).

Leaves

The leaves are acutely divided, lobes slender, thick and barren. The slanted leaves are alternately positioned on prolonged petioles. Leaves are almost 5-10 cm in length and have approximately 3-7 lobes (Rani *et al.*, 2017).

Flowers

The flowers are mendacious, blonde, males and females are both separate, corolla beige. The yellow-colored flowers emerge individually at leaf axioms (Rani *et al.*, 2017)

Fruits

The fruits are bulbous or ovoid, corpulent, indehiscent berry, 5-7.5 cm in width and assorted with green and white. Each *egusi* harvest around 15-30 globoid fruit having a diameter of almost 7-10 cm. The outmost segment of the fruit is enclosed with an emerald coat having yellow bands (Rani *et al.*, 2017).

Seeds

The seeds are grey and 5 mm long by 3 mm wide. They are edible but similarly bitter, nuttyflavored, and rich in fat and protein. They are eaten whole or used as an oilseed. The oil content of the seeds is 17–19% (w/w), consisting of 67–73% linoleic acid, 10–16% oleic acid, 5–8% stearic acid, and 9–12% palmitic acid. The oil yield is about 400 l/hectare (Schafferman *et al.*,

1998). In addition, the seeds contain a high amount of arginine, tryptophan, and the sulfur containing amino acid (Gurudeeban *et al*, 2010).

1.5 Cultivation

C. colocynthis, a perennial plant, can propagate both by generative and vegetative means. However, seed germination is poor due to the extreme xeric conditions vegetative propagation is more common and successful in nature. In the Indian arid zone, growth takes place between January and October, but the most favorable period for the vegetative growth is during summer, which coincides with the rainy season. Growth declines as soon as the rains and the temperature decrease and almost stops during the cold and dry months of December and January. Colocynth prefers sandy soils and is a good example of good water management which may be useful also on research to better understand how desert plants react to water stress (Zhang, 2009).

To enhance production, an organic fertilizer can be applied. *Citrullus colocynthis* is also commonly cultivated together with cassava (intercropping) in Nigeria. Cultivated *C. colocynthis* suffers of climatic stress and diseases such as cucumber mosaic virus, melon mosaic virus *Fusarium* wilt, etc. as any other crop. To improve it, a relatively new protocol for regeneration has been developed with the aim of incorporating disease and stress resistance to increase yield potential and security avoiding interspecific hybridization barriers (Ntui *et al*, 2009).

Plate 1. Egusi melon - *Citrullus colocynthis*

(Source: Zell, 2012)

1.6 General Uses of *Citrullus colocynthis* fruit

Traditional medicine and research

Colocynth has been widely used in traditional medicine for centuries (Mora *et al*, 1991). In premodern medicine, it was an ingredient in the electuary called *confection hamech*, or diacatholicon, and other laxative pills, as documented in ancient times: for example, the following recipe was found in the Ebers medical papyrus in Egypt, dated to about 1550 BCE: "To cause a woman to stop [terminate] pregnancy in the first, second, or third period [trimester]: unripe fruit of acacia; colocynth; dates; triturate with the powder of colocynth was sometimes used externally with aloes, unguents, or bandages, 6/7th pint of honey, moisten a pessary of plant fiber [with the mixture] and place in the vagina." – — Ebers papyrus, .

In laboratory studies, aqueous and methanol extracts of colocynth showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*. Extracts of fruits, leaves, roots and stems were also under basic research for possible activity against Gram positive bacilli and fungi (Meena and Patni, 2008).

Culinary uses

The desert Bedouin are said to make a type of bread from the ground seeds. Some confusion exists between this species and the closely related watermelon (*Citrullus lanatus*), whose seeds may be used in much the same way. In particular, the name "egusi" may refer to either or both plants (or more generically to other cucurbits) in their capacity as seed crops, or for a soup made from these seeds and popular in West Africa. The seed flour is rich in micronutrients, and could therefore be used in food formulations especially in regions with low milk consumption, such as West Africa (Gurudeeban *et al*., 2010).

Practical uses

The oil obtained from the seeds (47%) can be used for medicinal and soap production. The production is not very time- and energy consuming due to the ability of colocynth to grow on poor soils with just a little moisture and organic fertilizer. The fruits are harvested still unripe by hand, the rind is removed by peeling and the inner pulp filled with seeds is dried in the sun or in ovens. The seeds yield is about 6.7-10 t/ha, which means that for an oil profit of 31-47%, oil yields may reach up to 3 t/ha. Oleic and linoleic acids isolated from *C. colocynthis* petroleum ether extracts show larvicidal activity against mosquitoes (Giwa *et al.*, 2010).

1.7 Aim of the study.

- To study the effect of the palm-wine micro floral as fermenting agents on the proximate composition of *Citrullus colocynthis* fruit.
- To ascertain the proximate composition of the fresh palm-wine fermented *Citrullus colocynthis* fruit

1.8 Objectives of the study.

The objectives of this research are to:

- analyze the proximate composition of unfermented *Citrullus colocynthis* during fermentation
- determine the proximate composition of naturally fermented *Citrullus colocynthis*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Proximate Composition

Proximate composition is a term used in the field of food/feed for the partitioning/approximation of compounds in a feed into six categories based on the chemical properties of the compounds. The six categories are:

i. Moisture content

ii. Ash

iii. Ether extract

iv. Crude protein

v. Crude fibre

vi. Carbohydrate

2.2 Components of Proximate Composition

The six (6) components into which proximate composition is being partitioned into are being briefly discussed below.

i. Moisture Content: this can be defined as the quantity of water present in a material. It may be soil, rock, food, e.t.c. Presence of moisture in food materials gives room microbial activities which might be beneficial as in fermentation of products into desirables and non beneficial as in spoilage of food.

ii. Ash Content: this carried out to measure the total amount of mineral contents present within a food.

iii. Ether Extract: this refers to the part of a complex organic material that insoluble in ether and is chiefly made up of fats and fatty acids. Ether extract is also known as the crude fat or the free lipid content.

iv. Crude Protein: is a chemical analysis carried out on food to determine the amount of nitrogen present in a food which is used for the estimation of protein amount within the food material.

v. Crude Fiber: this is the chiefly cellulose material obtained as a residue in the chemical analysis of vegetable substances.

vi. Carbohydrate; this is any of a large group of organic compounds occurring in foods and living tissues and including sugars, starch, and cellulose. They contain hydrogen and oxygen in the ratio (2:1) and can be broken down to release energy in the body.

2.3 Effects of Fermentation in the Processing of Foods.

Fermentation may be defined as an energy-yielding metabolism in which an energy substrate is metabolized without the involvement of an *exogenous* electron acceptor; fermentation typically occurs under anaerobic or micro-aerobic conditions. The absence of an exogenous electron acceptor (=oxidizing agent, 'electron sink') necessarily means that the products of fermentation – collectively – have the same oxidation state as that of the substrate, the oxidation of any intermediate in the fermentation pathway being balanced by equivalent reduction of other intermediate(s) in the pathway; since the substrate undergoes no *net* oxidation, energy derived from the fermentation of a given substrate is less than that obtainable by the respiration of that substrate (Tortora *et al.*, 2002; Paul and Diana, 2006).

As commonly used in industrial microbiology, fermentation is *any* of a wide range of processes carried out by microorganisms, regardless of whether fermentative or respiratory metabolism is involved (Tortora *et al.*, 2002; Paul and Diana, 2006).

Fermentation is one of the oldest ways of food processing. It is an operation carried out by using microorganisms and their enzymes to achieve desirable quality characteristics of food and food products (Campbell-Platt, 2004). During the process, microorganisms utilize microbial enzymes. This process enhances the palatability, increases protein value, vitamin content and mineral levels of such foods. It also improves food preservation, food safety, enhances flavour and acceptability. It increases variety in the diet, improves nutritional value, reduces anti-nutritional compounds and in some cases, it improves functional properties (Achi, 2005; Obute *et al.*, 2007).

Fermentation plays a pivotal role in the processing of different foods and condiments to improve the nutritional quality of foods. Many investigators have reported that fermentation can be effectively used for improving nutritional quality of food products by increasing protein content and digestibility and available lysine content and relative nutritive value. Fermentation was also found to decrease trypsin inhibitory activity (TIA), amylase inhibitor, activity, phytic acid, and tannins (Tweyongyere *et al.*, 2002; Omafuvbe *et al.*, 2004; Oladunmoye *et al.*, 2007; Ogunleye *et al.*, 2009; Fasoyiro *et al.*, 2012). Fermentation procedures have been used to prepare food owing to the action of micro-organisms (Tweyongyere *et al.*, 2002). This has resulted in the characteristics textures, color, flavors and the nutritional properties of food condiments and products (Omafuvbe *et al.*, 2004). Well-monitored fermentation process would greatly reduce, if not get rid of the anti-nutrients present in under-utilized legumes and as a result bring about new products as protein sources (Gabriel-Ajobiewe, 2012).

2.4 Proximate Composition of Unfermented *Citrullus colocynthis* Seeds.

The *Cucurbitaceae*, also called cucurbits, are a plant family known for its high proteins and oil content. Seeds of cucurbits are rich sources of oils and protein as prescribed by the work of Schafferman *et al*, 1998. The oil content of the seeds was found to be in the range of 17 – 19% (w/w). It has also been reported that its oil composition is similar to safflower one with a total of 80 – 85% of unsaturated fatty acids. The estimated oil yield value upon harvesting ranged between 250 – 400 L/ hectare (Schafferman *et al*, 1998) and composed of up to 35% protein, mainly, arginine, tryptophan, and sulfur-containing amino acids (Achu *et al*, 2005). Additionally, *Egusi* seeds are rich in fat and protein and thus are eaten as whole or even used as an oil seed (Gurudeeban *et al*, 2010). Therefore, due to the above mentioned reasons, they are cultivated and consumed over the world.

The protein content of seeds of *Colocynthis* (transitional weed) was found to be 8.25% and rich in lysine, leucine and sulfo-amino acids viz., methionine (Shaheen *et al*., 2003). Egusi (colocynthis) kernels contain oil (52%), protein (28.4%), fiber (2.7%), ash (3.6%) and carbohydrate (8.2%).

The results for the proximate composition analysis carried out by Ojieh *et al*., (2008) on the dry weight of *Citrullus colocynthis* fruit were: moisture content (4.6 ± 0.3), ash content (3.7 ± 0.1), crude protein (23.4 ± 0.2), ether extract (45.7 ± 0.1), crude fibre (12.0 ± 0.1), and carbohydrate (10.6 ± 0.2). The results revealed that the fruit is rich in protein, crude fibre and carbohydrate.

A comparative study carried out by Taiwo *et al* (2016) on two members of the family *Cucurbitaceae* namely *Lagenaria breviflora* and *Colocynthis citrullus* observed that the fruits are highly nutritious and the seeds and fruits are used in folk medicine. Both *C. citrullus and L. breviflora* seeds are of same family, their seeds look alike, the medicinal properties of the latter has been exploited but it's nutritive value has not been adequately documented in literatures, hence the research by Taiwo and co-worker (2016) was designed to determine the nutrient and anti-nutrient contents of *L. breviflora* seed which may be another local source of nutrients to ascertain it's edibility. The fruits of *L. breviflora* are used to treat cold in man, *Coccidiosis* in birds, *Schistomaisi*, anti-fertility and hermatinic effects in man. The pulp is bitter due to the presence of tetra cyclic Triterpenoids and saponins (Elujoba *et al.*, 1996). *C. citrullus* are grown as subsidiary crops interplanted with early maize, they are among important vegetable crops which are edible and are generally referred to as melon, less expensive, widely distributed and they can contribute to balanced diets (Fokou *et al.* 2004). Mercy and his group (2005), observed that melon seeds are highly nutritive, likely to be good sources of oil and protein. The oil content and protein content were found to be 50% and 30% respectively in the seeds, most of the oils are made up of unsaturated fatty acids thus they are of high nutritive value.

The results of the proximate analysis of *L. breviflora* and *C. citrullus* are represented were: the moisture content observed was 10.35 ± 0.03% in *L. breviflora* and 4.61 ± 0.08% in *C. citrullus*, ash content 6.13± 0.73% was observed in *C. citrullus* and 4.16 ± 2.00% in *L. breviflora*. *C. citrullus* had the highest carbohydrate content 10.53 ± 0.50% while *L. breviflora* was 4.13 ± 0.03%. Crude fibre content observed was 11± 0.01% in *L. breviflora* and 12 ± 4.0% in *C. Colocynthis*. The protein content was 20.40 ± 0.13% in *L. breviflora* and 23.23 ± 6.01% in

C. citrullus, and the fat content was 47.60 ± 5.43% in *L. breviflora* and 45.60 ± 3.03% in *C. citrullus*.

***Adansonia digitata* (*EPO IGI OSE*)**

Adansonia digitata (the baobab tree commonly known as *Igi ose* in Yoruba) tree is one of the most intriguing trees growing on the African continent and is often referred to as monkey bread tree, Senegal Calash (fruit), bottle tree or upside down tree which can have a life span of up to 6,000 years. The baobab tree is tolerant to high temperature and drought, and is mostly found in the northern part of Nigeria. Baobab or *Adansonia digitata* L. belongs to the Malvaceae family (Bremer *et al.*, 2003).. Every part of the baobab tree is reported to be useful (Igboeli *et al.*, 1997 and Gebauer *et al.*, 2002).

The bark and roots are cut and used as traditional medicine. (Sidibe and Williams, 2002; Shukla *et al.*, 2001). The fruit consists of large seeds embedded in a sour acidic pulp and shell. The pulp can be dissolved in water or milk and the liquid is then used as a drink, a sauce for food, a fermenting agent in local brewing or as a substitute for cream of tartar in baking (Sidibe and Williams, 2002; Obizoba, 1983). Fermented seeds are used as flavouring for soup, and the roasted seeds are used as a side dish, substituting peanut (Addy and Eteshola, 1984). The seeds are also pressed for oil but the by-product, baobab seed cake is typically underutilized. The leaves are used to make soup (Yazzie *et al.*, 1994). The plant also provides forage for wildlife and domestic animals (Nkafamiya *et al.*, 2007).The consumption of baobab seeds in different forms has therefore been going on for quite a long time with little or no knowledge about the composition and nutritional value of the seeds, hence the need to investigate the mineral and nutritional content of the seed. A number of studies on the proximate values and mineral composition of baobab seeds and other indigenous plants have been carried out several times in

different geographical locations because plants nutrient and mineral contents do vary with soil type, as well as with climate type.

CHAPTER THREE

3.0. MATERIALS AND METHODOLOGY

3.1. Materials

Agar: Nutrient agar (NA), Sabouraud dextrose agar (SDA), peptone water, mannitol salt agar (MSA).

Ingredients: *Citrullus colocynthis* fruits, *Syzigium aromaticum* (*kanafuru*), *Adansonia digitata* (*epo igi ose*), freshly tapped palmwine, distilled water.

Reagents: Distilled water, 3% hydrogen peroxide (H_2O_2), TE buffer, 3% Acid alcohol, Malachite green, 70% ethanol, 90% ethanol, peptone water, Carbol fuschin Gram's iodine, crystal violet, acetone, safranin, 0.1% peptone water, streptomycin antibiotics, 0.85% NaCl, 80% peptone water, immersion oil.

Equipments: Microscope, incubator, autoclave, sterile spatula, weighing balance, vortex mixer, test tubes, petri dishes, glass slide, syringes, inoculating loop, Micropipettes, micropipette tips, Bunsen burner, sterile transparent buckets with cover, test tube racks, mercury thermometer rod, pH tester, hot air oven, burette, G-clamp stand, phenopthalein.

3.2. Methodology

3.2.1 Sample Collection

Whole, healthy fruits with no physical damage and injury were obtained from the Oja Oba market in Ado-Ekiti, the capital of Ekiti state. So also were the *ede bara* (*Citrullus colocynthis* fruit), *Adansonia digitata* (bark of African baobab tree, epo igi ose) and *Syzigium aromaticum* (*kanafuru*) a day to the sample preparation. Freshly tapped palmwine was obtained very early on

the day of sample preparation to ensure freshness and minimal contamination and poured in a tightly capped keg. They were transferred aseptically to the laboratory.

3.2.2. Sample Preparation and fermentation

Fruits and the remaining ingredients were washed under a clean running water to minimize risk of contamination. The buckets were also washed and rinsed under clean water and later disinfected with 70% ethanol. Fruits and other ingredients were chopped aseptically to reduce surface area in presence of a burning flame to minimize risk of contamination.

The fruits were prepared into five different buckets with two of the buckets having the other ingredients. The three other buckets had the fruits only. Into the two buckets having all the ingredients, palm wine was added into one while distilled water was added into the other in a ratio of 1:2.5. To the other three buckets, palm wine was added into one, distilled water was added into one while the last one was left without any solvent. These gave rise to five variables as follows which was allowed to undergo liquid state natural fermentation for 5 days.

Variable 1: all ingredients and palmwine **(CPA)**

Variable 2: all ingredients and distilled water **(CWA)**

Variable 3: fruits and palmwine only **(CP)**

Variable 4: fruits and distilled water only **(CW)**

Variable 5: fruit only **(CO)**

Solvents was added in a ratio of **1:2.5** while other ingredients were added in a ratio of **1:10:50** with **1** belonging to the fruits, **10** belonging to the epo igi ose and **50** belonging to the kanafuru.

3.3 Physico-chemical Analysis of Samples

3.3.1 Total Titrable Acidity (TTA)

Total titrable acidity of the fermenting melon seeds as described by (Padmashree *et al.*, 1987) was adopted. A small quantity (2.5g) of fermenting melon seed samples were macerated with 50ml of distilled water. Three (3) drops of phenolphthalein indicator were added. This suspension was titrated with 0.1N NaOH to a pink end point which persisted for 15sec.

3.3.2 Temperature

The temperature of the sample was determined by immersing a glass mercury thermometer into the container with the mother broth of each sample and left for 5minutes. Subsequently the temperature value of each sample was noted.

3.3.3 ph

The sample was homogenized with distilled water and the pH was determined using HANNA Instrument pH meter (S N: 5358236, H_2O: 5.70) (Akinyele and Oloruntoba, 2013)

3.4 Preparation of Reagents/Media

All chemicals used in this study were of analytical grade. The reagents, buffers and agar medium used were prepared following standard methods as shown in the appendix. All media used was prepared according to the manufacturer's specifications.

3.5 Sterilization of Media and Apparatus

3.5.1 Autoclaving

The culture media used for the isolation and cultivation of the bacterial isolates were sterilized by autoclaving at 121^0C for 15 minutes under pressure. Pipette tips were always wrapped with foil paper before sterilizing in the autoclave.

3.5.2 Flaming

The mouth of the conical flasks, test tubes, McCartney bottles and Bijou bottles were sterilized by flaming with a Bunsen burner before and after taking/pouring media into them. Inoculating loops and needles were sterilized using the Bunsen burner flame before and after inoculation. Streaking, inoculation and pouring of molten agar into Petri dishes were done close to the Bunsen burner flame in order to avoid contamination and maintain sterility.

3.5.3 Disinfection

The work tops were swabbed with 70% ethanol before any activities to provide a sterile condition and also after analysis in order to prevent contamination.

3.5.4 Hot Air Oven

Glassware's used in this study were thoroughly washed with detergent, rinsed with clean water and set to drain out before sterilization in the hot air oven at a temperature of 160^0C for two hours.

3.6 Methodology for Proximate Composition Analysis

Proximate composition analysis was carried out on the blended wet and dry samples of fermented *Citrullus colocynthis* according to standard methods of AOAC (1990);

3.6.1 Determining the moisture content

The moisture content of the ground sample was determined by weighing 2g of the blended sample, placed in the oven for a period of 24h at a temperature of 105°C, the drying continued until constant weight was obtained.

3.6.2 Determining the fat content

The fat content was determined through intermittent soxhlet extraction apparatus. Approximately 1g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced into the extraction tube. The cleaned, weighed and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and the heater was turned on to start extraction. After 4-6 siphoning, ether was allowed to evaporate and beaker was disconnected before last siphoning. Extract was transferred into clean glass dish with ether washing and ether was evaporated in water bath. Then it was placed in a dish in an oven at 105°C for 2hrs and cooled it in a desiccator.

3.6.3 Determining the ash content

The ash content of the ground sample was determined by weighing 5gm of the sample into a porcelain dish this was transferred into muffle furnace at 550°C for 7h, this was removed from the furnace, cooled and then moisten with dilute HNO3, the mixture was returned back to the furnace for about 3h to ensure complete ashing.

3.6.4 Determining the crude fibre

The crude fibre was determined by digesting the blended samples with 0.25N H2SO4, the mixture was refluxed for 1hour, the hot mixture was filtered and the residue was weighed (w1) then transferred into a crucible dish and placed inside the furnace for ashing at 550°C for 4h, the ash was weighed (w2), the difference between w1 and w2 gave the weight of crude fiber.

3.6.5 Determining the crude protein

Total nitrogen and protein content of each melon fruits was determined by Kjeldahl. Approximately 0.2g of the meal was weighed into a Kjeldahl digestion flask, into which 1g of copper sulphate, 1 tablet of Kjeldahl, 25ml of concentrated sulphuric acid and a few glass beads were added. The mixture was digested under a fume cupboard until a clear solution was obtained. All the digests were carefully transferred into a 100ml volumetric flask and made up to mark using distilled water. A 50ml portion of the digest was mixed with equal volume of 40% NaOH solution in a micro Kjeldahl (Markham distillation apparatus) unit and distilled. The distillate was collected into 10ml of 4% boric acid solution containing 3drops of mixed indicator (bromocresol green-methyl red). A total of 50ml distillate was collected and titrated against 0.02N H_2SO_4 solution to a color change from initial bluish-green color to pink (end point). Percentage total nitrogen and percentage crude proteins were calculated.

3.6.6 Determination of Carbohydrate content.

The carbohydrate content of the fruit was obtained by difference as below:

% carbohydrate = 100 – (% moisture content – % fat + % protein + %fibre + %crude ash)

CHAPTER FOUR

4.0 Results

Table 4.1 shows the temperature readings for fermenting mixture of *Citrullus colocynthis, Adansonia digitata,* and *Syzigium aromaticum.* The variables CP and CPA were recorded to be highest on day0 with the value 28°C, the temperature for all variables were recorded to have steady increase every 24 hours.

Ttable 4.2 shows the pH readings for fermenting mixture of *Citrullus colocynthis, Adansonia digitata,* and *Syzigium aromaticum.* On day0 the values for variables CW and CWA were recorded highest with the values 5.8 and 6.1 respectively and there was steady increase across all variables most especially for CW and CWA every 24 hours.

Table 4.3 shows the total titrable acidity (TTA) readings for fermenting mixture of *Citrullus colocynthis, Adansonia digitata,* and *Syzigium aromaticum.* The value 6.1 recorded for variable CWA was the highest on day0, and there was increase in values reported for other variables especially for the variable CP which has the highest value of 10.5 on day5 and variable CW was recorded the lowest with the value 1.8.

Table 4.4 shows the proximate composition of wet fermented mixyure of *Citrullus colocynthis, Adansonia digitata,* and *Syzigium aromaticum.* The variable WCON has the highest value of 90.5% for moisture content. WCW has the highest value of 2.56%, 6.76%, and 17.46% for proteins, fat and crude fiber respectively, while WCWA has the highest values of 1.04% and 5.88% for ash content and carbohydrate.

Table 4.5 shows the proximate composition of dry fermented mixyure of *Citrullus colocynthis, Adansonia digitata,* and *Syzigium aromaticum* The variable DCWA has the highest of 9.40% for

moisture content, while the variable DCW has the values 14.47% and 17.17% recorded highest for proteins and fat. The variable DCWA has the highest value of 47.85% recorded for crude fiber, the variable DCON has the highest value of 7.88% for ash content and the variable DCPA has the highest value of 28.49% for carbohydrate.

TABLE 4.1: Temperature readings for fermenting mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum*.

	DAY 0 Temp (^{0}C)	Day1 Temp (^{0}C) 8:am/3pm	Day2 temp(^{0}C) 8am/3pm	DAY3 temp(^{0}C) 8am/3pm	DAY 4 temp(^{0}C) 8am/3pm	DAY 5 temp(^{0}C)
CW	27	25/27	26/27.2	27/28	26/27.5	27.5
CP	28	26.5/27.2	26.7/27.6	26.3/28	27/28	28
CPA	28	26.3/27.3	26/28	26.8/27.7	26/28	28
CWA	27	27/27.5	26/27	26/27	26/27.8	28

Keys:

CPA: all ingredients and palmwine

CWA: all ingredients and distilled water

CP: fruits and palmwine only

CW: fruits and distilled water only

TABLE 4.2: pH readings for fermenting mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum*.

	DAY O	DAY 1 8am/3pm	DAY 2 8am/3pm	DAY3 8am/3pm	DAY4 8am/3pm	DAY 5
CW	5.8	4.2/4.5	4.1/4.5	4.3/4.3	4.5/4.6	4.6
CP	3.2	3.5/3.3	3.4/3.5	3.4/3.4	3.3/3.3	3.3
CWA	6.1	4.5/4.6	4.6/4.7	4.6/4/5	4.6/4.7	4.8
CPA	3.3	3.5/3.4	3.5/3.6	3.5/4.5	3.4/3.4	3.4

Keys:

CPA: all ingredients and palmwine

CWA: all ingredients and distilled water

CP: fruits and palmwine only

CW: fruits and distilled water only

TABLE 4.3: TTA readings for fermenting mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum*.

	DAY 0	DAY 1 8am/3pm	DAY 2 8am/3pm	DAY3 8am/3pm	DAY 4 8am/3pm	DAY 5
CW	1.2	2.2/1.2	1.7/2.0	2.0/2.3	1.2/1.6	1.8
CP	3.8	7.1/10.2	6.7/6.9	8.3/9.3	10.3/10.5	10.5
CWA	6.1	2.5/2.1	2.3/2.5	2.4/2.0	1.8/1.7	2.0
CPA	3.6	6.2/7.9	6.0/7.9	8.0/7.2	7.9/6.6	7.0

Keys:

CPA: all ingredients and palmwine

CWA: all ingredients and distilled water

CP: fruits and palmwine only

CW: fruits and distilled water only

TABLE 4.4: Proximate composition of wet fermented mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum*.

PARAMETER %	WCPA	WCW	WCP	WCWA	WCON
MOISTURE	85.3	70.0	88.0	77.8	90.5
PROTEIN	1.25	2.56	1.30	2.08	1.45
FAT	0.66	6.76	0.48	0.37	1.43
FIBER	6.61	17.46	4.65	10.86	3.74
TOTAL ASH	0.30	0.33	0.36	1.04	0.51
CHO	5.88	2.89	5.21	7.85	2.37

Keys:

WCPA: wet all ingredients and palmwine

WCWA: wet all ingredients and distilled water

WCP: wet fruits and palmwine only

WCW: wet fruits and distilled water only

WCON: wet fruits only (control)

TABLE 4.5: Proximate composition of dry fermented mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum*.

PARAMETER %	DCPA	DCON	DCP	DCW	DCWA
MOISTURE	9.25	7.50	8.75	7.75	9.40
PROTEIN	6.80	9.93	9.81	14.47	13.52
FAT	4.51	10.90	11.66	17.17	3.48
FIBER	43.80	38.76	33.81	42.88	47.85
TOTAL ASH	7.17	7.88	7.81	4.11	5.32
CHO	28.47	25.03	28.16	13.62	20.43

Keys:

DCPA: dry all ingredients and palmwine

DCWA: dry all ingredients and distilled water

DCP: dry fruits and palmwine only

DCW: dry fruits and distilled water only

DCON: dry fruits only (control)

CHAPTER FIVE

5.1 DISCUSSION

Legumes are important foods in addressing protein – energy malnutrition concerns in developing countries. They contain the essential amino acid lysine which is deficient in cereals (Fasoyiro *et al.*, 2012). The process of fermentation increases the digestibility of plant proteins and also reduces the anti-nutritional factors. Fermentation enhances flavour, colour and texture of legumes. Changes in these attributes are major stimuli in development of legume fermented products (Fasoyiro *et al.*, 2012).

The results obtained on the proximate composition of fermented mixture of *Citrullus colocynthis*, *Adansonia digitata*, and *Syzigium aromaticum* indicates that the fruit has high values in the following proximate components: proteins, fat, crude fiber and carbohydrate and this is in accordance with results obtained on the proximate composition of *Citrullus colocynthis* by Ojieh *et al.*, (2008) and with the results obtained by Taiwo *et al.*, (2016) on the comparative study carried out on *L. brevitlora* and *Citrullus colocynthis* which indicates that the *Citrullus colocynthis* fruit is richin proteins, fat, crude fiber and carbohydrate.

5.2 RECOMMENDATION

The research work shows that *C. colocynthis* is rich in both essential and non essential fatty acids which are essential in the metabolic build-up in humans, most especially the presence of proteins which are important in the skeletal make up of amino acids compared to other members of the *Cucurbitaceae* family. It is recommended that *Citrullus colocynthis* should be encouraged as a medicinal plant as is of high benefits in medicinal uses and the seeds contains high nutritional values in comparison with other members of the *Cucurbitaceae* family.

Moreover, *Citrullus colocynthis* fruit parts, especially seeds, are good protein source as they contain essential amino acids and non essential amino acids and thus could be used as a food supplement.

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