MICROBIOLOGICAL ANALYSIS OF MELON (*Citrullus lanatus*) SEEDS VENDED IN BENIN CITY, EDO STATE, NIGERIA

BY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

APRIL, 2024.

CERTIFICATION

This is to certify that this project work was carried out by Favour Chioma EZEUNARA in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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PROF. B.A. OMOGBAI **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by Favour Chioma EZEUNARA in partial fulfillment of the award of a Bachelor of Science, B.Sc. (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for His strength and grace during this academic journey and also to my parents, family members and loved ones for their continuous support and encouragements.

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I would like to express my profound gratitude and appreciation to God Almighty and also to my supervisor Prof. B.A. Omogbai for his invaluable aid during my project. I am also grateful to Prof.(MRS) F.I AKINNIBOSUN the Head of Department, and all the staff members of the Department of Microbiology for their assistance.

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ABSTRACT

This study accessed the microbial analysis of melon seeds purchased from different vendors in New Benin market, Ogida market, Oba market and Uselu market in Benin City, Edo state, Nigeria. Samples were collected in sterile plastic containers and taken to the laboratory for microbiological assessment following standard procedures. The microbiological assessment was carried out using cultural techniques. Identification of the microbial isolates was done using biochemical methods, phenotypic virulence properties were evaluated for the isolates and antimicrobial sensitivity was carried out using agar well diffusion method. The results showed that the bacterial counts ranged from 1.96±0.67 to 3.45±1.15x10^5 CFU/g. The total coliform counts ranged from 4.73±0.67 to 9.66±0.67 x10^3 CFU/g. The fungal counts ranged from 1.00±0.00 to 6.00±0.58x10^3 CFU/g. The bacterial isolated from the melon seed samples were *Bacillus* sp, *Escherichia coli*, *Pseudomonas* sp and *Staphylococcus aureus*. The fungal isolates identified were *Aspergillus flavus, Aspergillus niger, Rhizopus* sp, *Penicillium chrysogenum*, *Fusarium* sp and *Trichoderma* sp respectively. The antibacterial susceptibility testing showed that all isolates were susceptible to ciprofloxacin, erythromycin and levofloxacin but were also resistant to pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin and rocephin. It was also evident that all isolates were found to have an MAR index greater than 0.2 which means that the isolates were all pathogens of public health importance. The study therefore suggests regular surveillance and checks to monitor local vended foods on sale to ensure effective food safety.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Plant protein is the most sought-after food in the world today because of its capacity to substitute costly meat and animal products. Melon seed (*Citrullus lanatus* Thunb) of plant origin made available to customers will satisfy the nutritious function of conventional meals derived from meat, fish, and dairy. Melon seed, a wild member of the gourd family (Abaelu *et al*., 1990), has enormous potential for utilization in the global food industry. *Citrillus* is a member of the Benincaseae family, specifically the *Cucurbitoideae* subfamily. It consists of four species, two of which (*rehmii* and *ecrihosus*) originated in Namibia, while the other two (*colocynthis* and *lanatus*) are unique to West Africa and may be cultivated anywhere in the world (Bankole, 2005).

Melon seed is mostly used as a thickening in a traditional soup called "pistachio soup" in Côte d'Ivoire or "egusi soup" in West Africa. Despite its important social, nutritional, and economic function, this cucurbit is categorized as a secondary crop (Koffi *et al*. 2008). Melon seed is a useful diet due to its high nutritional density, which includes about 50% edible oil and 30% pure protein (Iwuoha and Eke, 1996). It is also a possible natural oil seed that might be examined as a new nutritionally acceptable meal. The oil includes four important fatty acids: linoleic, oleic, palmitic, and stearic acid.

Linoleic acid is the most prevalent, at 71.9 mg per 100 g of oil. The fat in egusi is unsaturated, with 14.5% monounsaturated fatty acids and 57.4% polyunsaturated fatty acids (Jarret and Levy, 2012). Despite these benefits, melon seed is underused, with indigenes from diverse melon-growing areas mostly using it to make egusi soup.

Melon seeds are a desirable export product to offer to African emigrants (Van der Vossen *et al*. 2004). Melon seed output in Nigeria was 347,000 tons from 361,000 hectares, whereas Cameroon produced 57,000 tons, Sudan 46,000 tons, Congo 40,000 tons, Central African Republic 23,000 tons, and Chad 20,000 tons (WHO/FAO 2003). Outside of Africa, China is a key producer, producing 25,000 tons. Nigeria trades an estimated 5000–7000 tons with other West African nations. Sudan exports around 27,000 tons, primarily to Arab nations (WHO/FAO, 2003; Vander Vossen *et al*., 2004). West and Central Africa are the main producers of melon seed, and their climates are susceptible to aflatoxin contamination.

A variety of significant illnesses affecting melon have been identified. These include bacterial, viral, and fungal infections as Fusarium wilt anthracnose, gummy stem blight, and powdery mildew. Field fungus grow before harvest and can be found by routine examination. Field illnesses do not continue to spread during storage provided grain is maintained at the right moisture content and temperature. Their numbers decline as storage time increases; they progressively die during storage, and by six months, they will be totally gone (Bankole, 2005). Storage fungi are rarely present in significant numbers prior to harvest. Small numbers of storage fungus spores may be present on grain being stored or on spilled grain in harvest, handling, and storage equipment or buildings. This modest amount of inoculum can rapidly multiply, resulting in considerable grain infection under inappropriate storage conditions.

Many fungi are known to infect egusi/melon seeds during storage, reducing seed storability and quality while also depositing poisonous compounds (mycotoxins) in the seeds. Seed-borne fungus are also responsible for the breakdown of food stores in seeds. Fungal degradation of seeds manifests as seed rot, seed sclerotization, and seed discoloration. The most frequent storage fungus are *Aspergillus* and *Penicillium*. These fungi are widespread and nearly always present.

Most storage fungi and bacteria are also known to create a wide range of compounds in seeds, some of which are harmful to humans. Several investigations have found that egusi becomes infected with a variety of fungal infections during storage. Fungi such as *Aspergillus*, *Rhizopus*, and *Penicillium* have been identified as seed pathogens in Nigerian stored egusi (Chiejina, 2006).

In Nigeria, *Aspergillus* spp. has not been documented to cause melon field disease. As a result, Aspergillus infection in melon, as well as aflatoxin synthesis, typically occurs after harvest. Aflatoxins are naturally occurring mycotoxins generated mostly by the *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are created as secondary metabolites by these fungi in circumstances that promote their proliferation (Othman and Al-Delamiy, 2012). Aflatoxins are among the most carcinogenic chemicals known, with several negative economic, biological, and health consequences. Almost all food processing and cooking procedures do not remove aflatoxins.

Aflatoxin contamination and other toxins produced by bacteria species such as *Bacillus cereus*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni*, and *Streptococcus pyogenes* result in lower crop value and poorer health for humans and domestic animals who consume contaminated crops (Wu and Khlangwiset, 2010). Aflatoxin has been categorized as a class one human carcinogen and is now well recognized as a public health risk. As a result, monitoring aflatoxin contamination locations in agricultural products should remain a top concern (Strosnider *et al*. 2006; Reddy *et al*. 2010). Furthermore, a higher percentage of melon seeds produced in Nigeria are not allowed outside Nigerian borders owing to aflatoxin contamination.

There is therefore the need to determine the critical stage in melon value chain when aflatoxin

contamination occurs as one of the key steps for proper management of aflatoxin production in melon seeds.

1.2 Aim and Objectives of Study

The aim of this research is to study the microbial contaminants of melon seeds vended in Benin City, Edo state.

The specific objectives of this research were to:

1. enumerate, isolate and identify the bacterial and fungal contaminants.
2. evaluate the susceptibility of the isolates to antibiotics and chitosan.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 Melon Plant

Citrullus lanatus subsp. *mucosospermus* is a melon plant of the Benincaseae family, which is a subfamily of *Cucurbitoideae* (Gusmini *et al*. 2004). The plant's delicacy lies in its seed. This indigenous plant species is mostly utilized as food in Asia, Central Africa, and West Africa, where it is known as egusi melon or bitter melon seed. *Citrullus lanatus* (Thunberg), Matsum, and Nakai are all referred to as bitter melon in certain regions of South Africa. *Citrillus* is a genus of seven desert vine species, with *Citrullus lanatus* (Thunberg), Matsum, and Nakai being major crops (Gusmini *et al*., 2004). Thunberg initially described *Momordica lanata* in 1794, and Matsum and Nakai refined it in 1916 before placing it in the *Citrullus* family (Dane and Liu, 2007). The cucurbit family has about 95 genera and 750 species (Mgidi *et al*., 2007).

Melons are grown as weak stem plants crawling over a surface and are members of the Cucurbitaceae family, having eatable and delicious flesh similar to that of watermelon and cucumber. *Citrullus lanatus* consists of four species: *colocynthis*, *lanatus*, *rehmii*, and *ecrihosus* (Levi *et al*., 2001). *Rehmii* and *ecrihosus* are popular in Namibia, but *colocynthis* and *lanatus* are more common in West and Central Africa. *Citrullus lanatus* subsp. *mucosospermus* may be recognized in Africa by two seed types: those with or without a black edge (Guo *et al*., 2013). These seed types are classified as separate cultivars and are more common in Africa (Guo *et al*., 2013).

In certain regions of South Africa, citron melon (Makaatan) provides water during the dry season, but egusi melon leaves and unripe fruit are used as green vegetables (Dane and Liu, 2007). Roasted egusi seeds have a nutty flavor and scent. *Citrillus* species similar to melon, known as *karkoer* or *bitterboela*, can be found in sandy, drier soils in Southern Africa, particularly in the Kgalagadi region of the Northern Cape (Henderson, 2007). It is cultivated in

the Western Cape, Northern Cape, North West, and Eastern Cape provinces. There is no official data on bitter watermelon production levels in South Africa because the country has not undergone industrialization.

Melon seed is mostly used as a thickening in a traditional soup called "pistachio soup" in Côte d'Ivoire or "egusi soup" in West Africa. Despite its important social, nutritional, and economic function, this cucurbit is categorized as a secondary crop (Koffi *et al*. 2008). In Côte d'Ivoire, melon seed is distinguished by its physical characteristics. Some *Citrillus* cultivars have a seed coat at the edge of the connection and are known locally as Bebu; other seed types are smooth with no black borders at the site of attachment. Melon produces good yields in a variety of intercropping systems, which are often typified by low inputs and ensure food security and long-term earnings for farmers (Bankole *et al*., 2005).

2.1.1 Propagation and Planting of Melon

Melon may be produced in tropical climates at heights of up to 1000 meters. It produces a better yield in the savannah zone than in places with consistent heavy rainfall. Melon seeds require an annual rainfall of 700-1000 mm and a temperature of 28-35ºC (Compton and Gray, 1993). In Southern Africa, bitter melon receives more than 400 mm of rainfall. High rainfall enhances melon plant development, resulting in a high yield (Ikeorgu and Ezumah, 1991). Dry cropping might produce higher yields, but most African farms lack irrigation systems, so farmers sow melon seeds during the rainy season. Melon may, however, be grown in Southern African soil during the spring, summer, and fall seasons. A loamy soil with a pH of 6-7 can be employed during cultivation on tropical land to provide a successful harvest (Ogbonna, 2009).

Nigeria has the largest production and yield of melon seeds in Africa. Melon's complete maturity is indicated by the collapse of its stalk and tendrils. The melon fruit is mature enough to be split in two with a sharp axe. The seeds are scraped out, covered with dry grass, and left to

ferment for approximately 14 days. Following fermentation, the seeds were removed from the pulp, cleaned, and sun-dried on dry mats. To achieve equal drying, the seeds are rolled over multiple times.

Melon seed drying is influenced by environmental temperature and hot wind conditions, and it takes 5-7 days to dry. Melon seed yields range from 225 kg/ha in West Africa to 1100 kg/ha in Senegal and 6000 kg/ha in Nigeria (Ntui *et al*., 2009). Melon seed yields in Namibia range from 550 to more than 3000 kg/ha, depending on the variety utilized. The average seed output of C. lanatus in China is 1500 kg per hectare. In Nigeria, melon seeds are extracted and dried in baskets or perforated bowls (20-25 kg). After drying, the seeds are dehulled with a machine or peeled by hand.

2.1.2 Structure of Melon

Citrullus lanatus is the most significant melon species in Africa (Gusmini *et al*. 2004). Melon plant is a kind of desert vine with a stem that climbs up its track. Members have tiny sepals, single staminate blooms, and a 5-parted corolla from the base.

The leaves are not or seldom split beyond the center, and the fruits are smooth, green-lined, or hairy, with a ground trailer (Mao *et al*. 2004). The leaves are velvety to the touch, with a large sinus and distant lobes at the base. Melon is composed of a fleshy mesocarp that forms during the third week of growing. Melon seed is encased in the mesocarp and must be extracted, dried, and dehulled before use (Ogbonna 2009). The excellent seeded melon (*Citrullus lanatus*), which is tiny, flat, and oval (Appendix II, fig. 2.1), has a soft golden brown seed coat that must be dehulled to retrieve the tinny white seed.

Dehulling can be done manually by twisting the flexible shell or mechanically with a dehulling equipment (Ogbonna, 2009).

The melon plant can tolerate pests and illnesses because it covers the ground as it develops, preventing the establishment of undesired plants (Gusmini *et al*., 2004). This feature of melon makes it the top choice for farmers when it comes to intercropping with sorghum, cassava, coffee, cotton, maize, and bananas. To retrieve the interior white seed of a melon, it must be dehulled. Researchers discovered that melon pods had a nearly spheroidal exterior form and an ellipsoidal seed cavity (Appendix II, Fig 2.2). Mature melons may also remain in the field for an extended period of time without decaying, reducing crop loss and plant nutrient loss owing to soil leaching and erosion, while plant waste after harvest is minimal. Rot and spoiling are uncommon after seed harvesting (Bankole *et al*., 2010).

2.1.3 Cultivation of Melon Seed

Melon grows best in warm to hot, sunny climates with healthy, well-drained soil. They grow on vines along the ground, trailing and scrambling. If plants are trained over a trellis, the fruits need to be supported as they grow to avoid damaging the plant. Warm and, in non-arid places, humid circumstances conducive to melon development can also promote the survival and growth of human infections, as well as the presence of mammals and pests. Melons are sensitive to a variety of insect pests and microbiological diseases. Soil amendments and agricultural chemicals can both be employed in traditional primary production, as well as in organic systems (Boughalleb *et al*., 2007).

Melon vines grow horizontally, and when the fruit grows in size and weight, it rests on the ground. Melons will be subjected to soil pollution directly or by water splashing, such as during heavy rain, spray irrigation, or flooding. Plastic mulch is frequently used to cover the ground, providing producers with a variety of production benefits such as boosting the quality and quantity of fruit harvests and shortening growing time as compared to bare ground. Plastic

mulch can also limit direct soil exposure. The phrase "ground spot" refers to the specific place on the rind where the melon is in touch with the ground or mulch. This region is thin and underdeveloped in melons, making them more prone to fungal and bacterial development. Muskmelons can be raised on plastic cups to avoid direct soil contact, and melons can be flipped throughout growth to reduce the incidence of ground spot and sunburn, both of which induce rind discoloration (Boughalleb *et al*., 2007).

Melons' ripeness may be determined using a variety of visual or sensory markers, as well as sugar content. When cantaloupes are mature, the stem readily separates from the fruit, forming an abscission zone or "slips" off the vine, leaving a dish-shaped scar. Industry employs terminology to indicate the stage of maturity and slip development, such as "half slip" or "¾ slip". Honeydew melons do not slip from the vine, and softness at the fruit's bloom end indicates maturity. Watermelons feature a little curled tendril on the end that grows brown and dies when the fruit is mature; moreover, the ground spot and the skin change hue. Harvesting cantaloupes between ¾ and full slip, and watermelons destined for distant markets when mature but not fully ripe, is crucial for preventing damage and loss of quality attributes (Boughalleb *et al*., 2007).

Melons have a variable storage life after harvest, with some cantaloupe cultivars lasting only a few weeks in unmanaged storage circumstances (Krarup *et al*., 2009). Soon after harvest, it is required to remove the "field" heat and manage temperature and relative humidity until the product reaches the market, or the quality deteriorates and the shelf life decreases. At exceptionally low temperatures, melons can sustain chilling harm for longer periods of time. Melons are chilled using cold water, cold air, or ice, and the chilling process varies depending on the melon variety and available facilities.

2.2 Nutritional Composition of Melon Seed

C. lanatus is a significant crop in impoverished nations; melon seeds are dehulled to produce a white kernel containing 50-60% w/w oil, 28-30% w/w protein, 20-10% w/w carbohydrate, 2-3% w/w ash, and 3-4% w/w fiber. According to Akubor and Ogbadu (2003), melon seed milk has 3.6% w/w protein, 4.0% w/w fat, and 2.5% w/w carbs, which is equivalent to soy milk. Thus, melon seeds may be used to enrich both modern and traditional foods. Most African nations use *C. lanatus* seed for its nutritional advantages, with research stating that it contains 60% w/w fat (defatted flour) and 30% w/w protein.

2.2.1 Melon Protein

The nutritional content of raw and defatted melon seeds is around 26-28% w/w protein and 56-60% w/w in defatted flour (El-Adawy and Taha, 2001). Raw melon has more protein than cowpeas, but somewhat less than soybeans (36%). Melon's main protein source is storage salt-soluble globulins, which are made up of three components with molecular weights of 570,000 - 590,000 gm-1, 310,000 gm-1, and 160,000 - 200,000 gm-1. A large group of protein molecules frequently has a molecular weight of over 60 kDa and contains more than 30% w/w hydrophobic amino acids. This category includes hemoglobin and egg white albumin. Globular proteins with molecular weights less than 23 kDa do not form gels under normal circumstances. Protein from cucurbit species has nutraceutical benefits such as antifungal, antibacterial, and anti-inflammatory effects (Ntui *et al*., 2010).

Melon seed contains a high concentration of important amino acids, which can help a youngster with an amino acid shortage. Melon is rich in glutamine, which is an important building ingredient for protein in the body (Hlatky *et al*., 2002). Glutamine, an α-amino acid, is required for protein production and must be acquired from food.

Glutamine reduces medicine adverse effects and increases pharmaceutical efficacy in treating diarrhea, discomfort, and swelling in the mouth (mucositis) (Calder and Yaqoob, 1999). Glutamine boosts the immune system and helps chemotherapy patients live a healthy lifestyle (Calder and Yaqoob, 1999). Glutamine (a protein building ingredient) is naturally occurring in the body but can also be obtained from foods such as melon, soy flour, and most oil seeds (Akobundu, 1989). Glutamine alleviates some of the negative effects of medicinal therapy. It is used to treat chemotherapy side effects such as diarrhea, discomfort and swelling in the mouth (mucositis), nerve pain (neuropathy), and muscle and joint pain induced by the cancer medicine.

Glutamine is also utilized to support the immunological and digestive systems in persons receiving radiochemotherapy for esophageal cancer. Soya bean has 36.0% protein, rapeseed 22.0%, sunflower 19.8%, and peanut 25.6%. Despite the variable percentages, melon seed contains a significant protein content and amino acid storage (Giwa *et al*., 2010). Melon seed's high protein content can assist address malnutrition issues caused by protein deficits in modern communities. Protein-energy malnutrition (PEM), also known as calorie malnutrition, occurs when children consume insufficient amounts of protein and energy to satisfy their nutritional requirements. In most situations, there will be a shortfall in both total calorie and protein consumption.

Melon has arginine in its composition, which ranges from 12-13.3% w/w. Arginine is primarily used in protein production and is considered a semi-essential or conditionally essential amino acid. The utilization of arginine is determined by the individual's health status or developmental stage. A preterm newborn cannot manufacture arginine on their own; thus, arginine-rich foods should be included in their diet to enable such youngsters naturally synthesize protein.

2.2.4 Melon Fat

Melon oil includes important fatty acids, with linoleic, palmitic, stearic, and oleic acid being the most abundant. Linoleic is the most prevalent in melon seed; about 59% linoleic, 16-17.1% w/w oleic (Akobundu, 1989), palmitic 12.4% w/w, and stearic acid 8.1% w/w (Akobundu, 1989), with only trace levels of linoleinic acid (LA) (Bankole *et al*., 2005). Roasting melon seed at 133.1oC for 20.2 minutes was shown to provide the highest output of high-quality oil from melon seed (Ntui *et al*. 2009). Experimental rats fed melon oil showed a substantial reduction in total cholesterol and atherosclerosis, indicating that melon can lower blood pressure (Ziyada *et al*., 2008).

Melon has the potential to prevent heart disease and aid in weight loss by burning adipose cells in the body, commonly known as brown fat, due to the presence of linoleic acid. Linoleic acid is a polyunsaturated omega-6 fatty acid. It is a colorless liquid at ambient temperature. In the physiological literature, it is described as a carboxylic acid with an 18-carbon chain and two cis double bonds, the first of which is positioned at the sixth carbon from the methyl end. Linoleic acid is one of two types of essential fatty acids, which indicates that the human body cannot produce it but may be found in dietary components (Kelly, 2001). A recent study by Harvard School of Public Health claims that conjugated linoleic acid (CLA) help reduces internal body fat (Khanal and Dhiman, 2004).

Conjugated linoleic acid raises the body metabolism, allowing the body burn visceral fat. In overweight people adipose tissue is inactive when food high in CLA is consumed, their fatty tissue is made active, and weight loss will be achieved (Kamphuis *et al*., 2003). Conjugated linoleic acid can also help to suppress appetite if it is incorporated into a weight loss diet (Dhiman *et al*., 2000). Furthermore, CLA has external benefit for the body. It produces prostaglandin which functions as anti-inflammatory and diuretic, helping the skin to maintain tone and moisturised after weight loss (Bassaganya-Riera *et al*., 2004). When the fat in the

belly and around the heart is removed, coronary heart disease is prevented (Kamphuis *et al*., 2003). Melon seed is high in polyunsaturated fatty acid 71.9 g/100 mg, making melon a nutraceutical food option (Akobundu, 1989).

2.2.5 Melon Carbohydrate

Melon carbohydrate, at 10.6% w/w, contributes to the production of functionally stable and nutritious foods. Amylose and amylopectin make up the majority of the carbohydrate component of plant meals. Amylose and amylopectin are both components of starch (Ponka *et al*. 2006). The two starches differ in their water absorption and solubility, as well as their tendency to swell in hot water. Melon carbohydrate is thought to be composed mostly of amylopectin and less of amylose due to its propensity to dissolve in water, swell, and create a stable emulsion. Amylose's job is to supply energy to plants (Blazek 2008). Amylopectin is increasingly employed in the production of food items as an emulsion stabilizer.

Melon which has been traditionally used as a thickener for ages proffers a hydrocolloid to the food industry in the area of emulsifying and stabilising food water balance (Wang *et al*., 2011). A hydrocolloid is defined as a colloid system wherein the colloid particles are hydrophilic polymers dispersed in water (Blazek, 2008). Melon starch globule forms irreversible light gels because they easily interact with their hydrophobic fragments (Giwa *et al*., 2014).

2.2.4 Mineral Composition of Melon Seed

Melon's minerals suggest that it is abundant in calcium and potassium. Calcium is present in bones and teeth, which aids in the development of healthy bones and teeth (Gillooly *et al*. 2005). Phosphorus also aids the body's ability to regulate glucose and protein metabolism (Cahill *et al*., 2013). It is required for the maintenance of protein globules, which are necessary for cell and tissue development and repair (Akobundu 1989).

Melon seed is a great source of magnesium. According to the literature, the typical body has 25 grams of magnesium (Saris *et al*., 2000). Magnesium catalyzes around 300 chemical processes that help the body operate effectively (Mordike and Ebert, 2001). Magnesium can be obtained from a magnesium-rich diet, however supplements can be used in severe cases of deficiency. Women consume far less magnesium than males. Calcium is utilized to avoid bone-related deficiencies that cause weak bones or poor bone density, softening of bone, and painful softening of bone known as porous bone or osteoporosis (Saris *et al*. 2000). Melon contains 28.2% calcium by weight (Ijoyah *et al*., 2012).

2.3 Anti-nutrient in Melon Seed

A significant level of antinutrients and toxins can be found in all raw foods. The amount of antinutrients in a fresh agricultural product influences its toxicity (Enujiugha and Ayodele-Oni, 2003). Melon contains trace amounts of oxalate, phytate, tannin, and nitrate. Enujiugha and Ayodele-Oni (2003) found antinutritional factors in melon seed at values of 1.8, 1.77, 0.50, and 0.11 g/kg dry matter for oxalates, phytic acid, phytate-P, and tannins. These results are within acceptable limits, and processing procedures are likely to significantly lower the quantities of these antinutrients. Melon seed's low anti-nutrient content will further improve its functioning in cuisine.

2.4 Functional Properties of Melon Seeds

The functional features of food from a processing standpoint show the desired characteristics of food. At the optimal component concentration and under the right conditions, it offers the product with desirable rheological and sensory attributes such as mouth feel, aeration, juiciness, and bulking properties (Kennedy and Mistry, 2003).

Functional properties of a food can be classified into four major categories including:

1. Functional properties that relates to structure of protein and rheological features, which

will results in the interaction of macromolecules and water;

2. Properties related with addition of water leading to thickening, wettability and solubility
3. Properties related to the protein surface as it related to whip-ability and foaming properties: and
4. Properties related to it sensory properties (Boye *et al*., 2010).

2.5 Prospects and Uses of Melon Seed

Citrullus lanatus seeds are increasingly being utilized locally for oil and meal by small populations in semi-arid places (Achigan-Dako *et al*. 2008). However, its oil can also be employed in the culinary, cosmetics, and pharmaceutical industries. Melon seeds are traditionally processed in Western Africa by manually kneading the grain to extract the oil from the seeds. Melon dough from the procedure is manually cut into pieces and shaped into little ball shapes. This tedious procedure frequently inhibits the use of this seed as a food or thickening. Melon seed, which is strong in protein and healthful fat, has not been used in any industrial applications.

Introducing melon plant into cropping system will assist in suppressing weed as melon blanket itself on the ground allowing minimum possibilities of weed interruption in a plantation, reducing production/maintenance cost (Ikeorgu *et al*., 1989). Further research must be made to improve agronomic practices, labour saving by upgrading the dehulling and defatting equipment and a more cost-effective method of defatting melon seed (Giwa *et al*., 2010).

2.5.1 Melon as a Functional Food Ingredient

Hydrocolloids, which are composed of protein and polysaccharides in food, are the key constituents that contribute to the structure and texture of food items (Bemiller 2011). Melon starch and protein have the ability to produce a thick gel that can serve as a bulking agent.

Melon's gelling properties will stimulate its use in composite flour-based pastries. Melon gelling and pasting qualities can be affected by pH, ionic content or strength, urea concentration, temperature, and the physical force applied during processing (Bemiller, 2011). A good gel's qualities are defined by its ability to react extensively with water, resulting in a coagulated gel of a clear gel.

Proteins with non-polar residues generate coagulated gels, whereas amino acids that dissolve in water make translucent gels (Rojas *et al*., 1999).

Defatted melon flour and concentrates are likely to create solid, rigid, and robust gels, whereas melon protein isolates will form semi-liquid gels. Acceptable texture and water retention are difficult to achieve, especially in low-fat and low-salt protein systems. Hence, melon meal/protein is a viable element in these systems. Melon hydrocolloid can be used as a foaming and emulsifying agent in multiphase systems such as emulsions and foams, where stability is controlled not only by the interface between the immiscible components but also by the viscosity of the continuous phase (Kundu, 2005). It not only improves the stability of the constant phase but also strengthens the interface. *Citrullus lanatus* has the potential to fortify cereals and staple food by serving as a rich protein source (Uruakpa, 2004).

However, before this can happen, future perspectives on the full use of melon seeds as food components will focus on publicity and their usefulness in malnutrition programs. More study on its functional qualities for thickening, stabilizing, and strengthening processed foods is needed. Hydrocolloid from plants offers an advantage over hydrocolloid from animals and microbes (Burey *et al*., 2008).

According to Akubor and Ogbadu (2003), defatted melon flour has a water-holding capacity of 0.7 ml/g and an oil-holding capacity of 2.6 ml/g. As a result, melon's capacity to form a gel

provides thickening properties. However, there has been no published research on the hydrocolloid characteristics of melon seeds, despite their use as a soup thickening in some regions of Africa (Achigan-Dako *et al*., 2008). Exploring melon's hydrocolloid qualities, namely its capacity to create gel, will promote its application in both the food and non-food industries. Furthermore, melon's nutritious makeup will improve its use in a food system.

They have greater user-friendly potential and provide more nutritional advantages that are health-promoting. A polysaccharide's hydrophobicity increases as its contact with water decreases. The hydroxyl group in carbohydrate preferentially interacts with two water molecules, resulting in less interference or contact with other hydroxyl groups. Hydrocolloids are significant in the food industry and can also be used in non-food areas (Armstrong and Barringer, 2013). Some foods naturally contain hydrocolloid, while others require the addition of hydrocolloid from another source to influence their functional behavior (Armstrong and Barringer, 2013).

2.5.2 Cooking Oil from Melon Seed

Oilseeds are widely utilized in diet preparation across the world. Castor oil and flaxseed oil are used in the preparation of meals and might occasionally be utilized for nutraceutical purposes. When cooking at high temperatures, stable oils are desirable since they will not grow rancid due to heat (Innawong *et al*., 2004). During oil oxidation, oxygen combines with free profound compounds, resulting in off-flavors in food that can be detrimental to health if ingested.

The degree of saturation of free fatty acids present in food components at both high and low heat processing is an important element when considering oxidation and rancidity (Quintal, 2012). Saturated, monounsaturated, and polyunsaturated fatty acids (PUFAs) contain single bonds, one double bond, and two or more double bonds, respectively (Smith *et al*., 2007). Saturated and monounsaturated fatty acids are heat-sensitive and should be avoided during

cooking. This is due to the creation of radicals, which causes toxin generation and off-flavor in the food generated from such oil. Melon oil is claimed to have a high polyunsaturated fatty acid content and a low saturated fatty acid content, making it heat stable.

Melon oil is cholesterol-free, making it a heart-healthy oil for all ages. The world production of sunflower (*Helianthus annuus* L.) is projected to be 26.5 × 106 t, with a harvested area of around 21.7 × 106 hectares. Sunflower oil's oil capacity allows for large-scale manufacturing as a cooking oil. It is light and tasteless, with a high content of polyunsaturated fatty acids and linoleic acid (Perakis *et al*. 2005).

The new breakthrough of using a natural, non-toxic supercritical CO_2 technology to extract oil from seed improves the quality of the oil produced by such processing (Perakis *et al*., 2005). When melon seed meal is extracted using the solvent technique, it darkens with increasing temperature and constant pressure. At 75°C, the meal at the reactor entrance for most major seeds solvent extraction will become off-white to light brown, then dark brown when the temperature reaches 95°C (Mbah *et al*., 2014). Melon meal will appear highly red and less yellow after extraction; the meal is discarded, while the oil must be purified before use (Machida *et al*., 2011).

2.6 Health Benefits of Melon

Melon seeds are recognized to provide several nutritional and therapeutic advantages to humans. Melon seeds have been proven to help manage blood pressure. Watermelon seeds contain arginine, which helps regulate blood pressure (Enujiugha *et al*., 2003).

Melon seeds can also help avoid coronary heart disease. Melon seed oil can help relieve itchy scalp and dandruff (Nyam *et al*., 2012). Melon seeds contain critical vitamins and elements that can be used as dietary supplements. Melon seeds (*Citrullus lanatus*) are rich in mineral

concentration (mg/100g). Melon seeds contain 21.05mg of zinc and up to 20.46mg of magnesium per 100g. The mineral analysis revealed that iron has the highest value at 144.7mg/100g, followed by zinc at 21.05mg/100g and manganese at 22.73mg/100g. Calcium has the lowest concentration of any mineral, at 0.10mg/100g. The sodium to potassium ratio is 0.043 and the calcium to phosphorus ratio is 0.002 (Akubor *et al*., 2004).

Melon seeds include 7.16% moisture, 27.41% protein, 30.65% oil, 29.96% carbs, 4.83% ash, 25.32% fiber, and a high concentration of antioxidants such as phenolic compounds. Melon has significant amounts of potassium, calcium, and magnesium (Kennedy and Mistry 2003). The amino acid composition of seeds reveals that children require more lysine, threonine, and leucine than adults do. Overall, the investigations demonstrate that melon seeds may be used as a nutritional source (Jaret and Levy, 2012).

2.7 Microbial Compositions and Micro-Organisms Associated with Melon Plant and Seeds

Microbial species found in spontaneously fermented melon seed shells included *Bacillus*, *Micrococcus*, *Aerobacter*, *Rhizopus*, *Mucor*, *Aspergillus*, *Penicillium*, and *Candida*. These microorganisms can benefit melon seeds by enhancing their nutritional, probiotic, and preservative contents (Khalid *et al*., 2021). Melon seeds are prone to microbial infection, which might offer a major health risk when consumed. Poor planting, harvesting, storage, and handling techniques can contaminate melon seeds and expose them to bacterial and fungal illness.

2.8 Health Hazards and Effects of Effects of Contamination of Living Cells

Melon seeds are extremely susceptible to fungal infection in tropical warehouses due to high ambient temperatures and relative humidity levels (Bankole, 2005). Some of these fungal pollutants have been shown to create hazardous metabolites (mycotoxins), specifically

aflatoxins. According to reports, about 98% of persons in West Africa tested positive for aflatoxin biomarkers. Aflatoxins generated by *A. flavus* and *A. parasiticus* strains are hepatocarcinogenic, teratogenic, and mutagenic. They have also been linked to growth stunting, underweight, and immune function changes in West African children. At now, nothing is known about the prevalence of aflatoxin in melon seeds. A. flavus was discovered to be one of the most common molds that infect post-harvest melon seeds (Bankole *et al*. 2010).

2.9 Good Manufacturing Practices and Preservation Techniques of Melon during Planting, Harvesting and Storage

It is critical to maintain strong manufacturing processes during the planting, post-planting, harvesting, processing, handling, and storage phases of melon seeds to avoid contamination by microorganisms or plant diseases that are economically significant. Melons are typically collected every other day for the first two or three harvests, then every day for the next 20 to 25 days. The length of harvest in a planting is determined by the vine condition, amount of melons, season of year, and market (Brandenberger *et al*., 2021).

Cultural activities can help restrict the development, spread, and survival of viruses that cause cantaloupe disease. Many fungal, bacterial, and nematode diseases live in old crop debris and soil. Fields should be cycled with non-cucurbit crops for at least three years to limit disease populations. Grass crops are suitable for rotations in which nematodes are an issue. Fields with the appropriate soil qualities should be chosen. Avoid acid soils and fields having a history of Fusarium wilt, root rot, or vine decline. Melons are quite perishable. Even if picked, handled, and stored under ideal conditions, they will only be of acceptable grade two weeks following harvest.

Melons that are half-slip to three-fourths slip should be kept at temperatures ranging from 35 to 45 degrees Fahrenheit. Ripe melons (equal to full slip) may be kept from 32 F to 35 F. Certain

types of melons should not be kept or stored below 45°F. They are susceptible to chilling harm at lower temperatures (Brandenberger *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection

Four different samples of melon seed were obtained from New Benin market, Ogida market, Oba market and Uselu market, Benin City, Nigeria. The samples were taken to the laboratory for analysis before spoilage began.

3.2 Preparation of Culture Media

The media used were prepared according to the manufacturer's instructions. The media used were MacConkey agar and Nutrient Agar.

3.2.1 Preparation of MacConkey agar

MacConkey agar (MCA) was prepared by dissolving 51.55 g of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{o}C for 15 min. The medium was allowed to cool to 45^{o}C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.2.2 Preparation of Mueller Hinton Agar

To prepare, 38g of Mueller Hinton agar powder was suspended in 1L of distilled water and dissolved completely. Sterilize by autoclaving at 121°C for 15 minutes. Pour the liquid into the petri dish and wait for the medium to solidify. Be sure to prepare the agar in the clean environment to prevent any contamination.

3.2.3 Preparation of Nutrient Agar

Twenty eight (28 g) grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed

thoroughly and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.2.4 Preparation of Potato Dextrose Agar

Thirty nine (39 g) grams of Potato Dextrose Agar powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C and before dispensing aseptically into Petri dishes. Plates were then incubated for 72 hr at 25°C (room temperature).

3.3 Isolation of Bacteria

One (1g) gram of the sample was weighed and placed in 9ml sterile water. The aliquot was then transferred aseptically to sterile Petri plates. The prepared agar (for bacteria growth) was poured in aseptically and incubated at 37°C for 24 hr. After successful growth of microorganisms, the colonies were counted with a colony counter and the results per dilution count were recorded.

3.4 Preparation of Pure Cultures

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate medium. Pure cultures were checked from nutrient agar plates. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant. These cultures were incubated at 37°C for 24 hr.

3.5 Bacterial Identification

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial isolates. The fungal isolates were identified using colonial morphological characteristics such

as size, texture colour and reverse colour. These parameters were evaluated by physical examination. Microscopy was also carried out usingn lactophenol cotton blue staining and a bright field microscope.

3.5.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

3.5.2 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.5.3 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.5.4 Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hr. Urease positive cultures produced a red-pink color due to changes in the color of the indicator.

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3.5.5 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result.

3.5.6 Hydrogen Sulphide (H_2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}) or lead (Pb^{2+}) ion as H_2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate.

3.5.7 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethylaminobenzaldehyde and amyl alcohol is used.

The broth was inoculated with the test organism and incubated for 18 hr at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red

colour at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.5.8 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. The growth medium comprised of peptone water, sugar (1%) and the indicator (bromocresol purple). The mixture was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 min. After sterilizing, tubes were allowed to cool and then inoculated with the isolates and incubated at 37°C for 24hr. Acid and gas production or acid only were observed after about 24 hr of incubation. Acid production was indicated by the change of the medium from purple to yellow colour indicated a positive test.

Sugars used were: lactose, sucrose, glucose, fructose, maltose, starch and sorbitol

3.6 Antibiotic Susceptibility Test

The test isolates were subjected to antibiotic sensitivity test using the Kirby Bauer disc diffusion technique. Antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule after a 24 hr incubation period.

3.7 Identification of Fungal Isolates

3.7.1 Cultural Characteristics

Each colony morphology e.g., size, texture, color, reverse color was determined by physical examination.

3.7.2 Preparation of Pure Cultures

One single colony was identified and re-streaked as a primary inoculum on the surface of a potato dextrose agar plate medium to make a pure culture. After achieving a pure culture, the

same colony was streaked onto potato dextrose agar slant. These cultures were left at room temperature (25°C) for 72 hr.

3.7.3 Lactophenol Cotton Blue Staining

Lactophenol cotton blue is a stain commonly used for making semi-permanent microscopic preparation of fungi. It stains the fungi cytoplasm and provides a light blue background, against which the wall of hyphae can readily be seen. It contains four constituents: phenol, which serves as fungicide; lactic acid, which act as a clearing agent; cotton blue, which stains the cytoplasm of the fungus; and glycerine, which gives semi- permanent preparation. Firstly, a drop of lactophenol cotton blue stain was placed on a clean slide. Then using a sterile wire loop, a small tuft of the fungus was smeared on the drop on the slide. A cover-glass was then placed on the slode and viewed under a bright field microscope.

3.8 Preparation of Chitosan

To prepare chitosan for the susceptibility testing, 5g of Chitosan was weighed and dissolved in 100 ml of sterile distilled water to produce a concentration of 50 mg/ml. 50ml of the stock was taken and diluted with 50ml of sterile distilled water to yield a 25 mg/ml concentration. Then 50 ml of the 25mg/ml solution was taken and further diluted with 50 ml of sterile distilled water to yield a concentration of 12.5 mg/ml. Then 50 ml of the 12.5 mg/ml solution was taken and further diluted with 50 ml of sterile distilled water to yield a concentration 6.25 mg/ml.

3.9 Antibacterial Assay

The test was carried out using the agar well diffusion technique, in which wells were dug into freshly prepared nutrient agar medium using a sterile cork borer and the different concentrations of the chitosan were introduced into the wells. The zones of inhibition were measured in millimeters after 24 hrs of incubation.

CHAPTER FOUR

4.0 RESULTS

The results for the Total heterotrophic bacteria counts (CFU/g) in melon seed samples from various markets in Benin City is shown in Table 4.1. The Total heterotrophic bacterial counts in melon seed samples ranged from $1.96\pm0.67x10^5$ to $3.45\pm1.15x10^5$ CFU/g. Highest Total heterotrophic bacteria count occurred in melon seed samples from vendor 1 in New Benin market while the lowest Total heterotrophic bacteria count was observed in melon samples from vendor 2 in Uselu market.

Table 4.2 reveals the result obtained for the Total heterotrophic fungal counts (CFU/g) in Melon seed samples from various markets in Benin City. The Total heterotrophic fungal counts ranged from $1.00\pm0.00x10^3$ to $6.00\pm0.58x10^3$ CFU/g. Highest Total heterotrophic fungal count was observed in melon samples from vendor 2 in Uselu market while the lowest Total heterotrophic fungal count was observed in melon samples from vendor 1 in Oba market.

The results for the Total Coliform counts (CFU/g) in Melon samples on MacConkey agar is shown in table 4.3. The Total Coliform counts ranged from $4.73\pm0.67x10^3$ to $9.66\pm0.67x10^3$ CFU/g. Highest Total Coliform count was observed in melon samples from vendor 1 in New Benin market while the lowest Total Coliform count was observed in melon samples from vendor 2 in Uselu market.

Table 4.4 shows the results for Total bacterial counts (CFU/g) in Melon samples on Salmonella-Shigella agar. The Total bacterial counts in melon seed samples on SSA ranged from $0.80\pm0.00x10^2$ to $5.26\pm0.33x10^2$ CFU/g. Highest Total bacteria count occurred in melon seed samples from vendor 1 in Oba market while the lowest Total bacteria count was observed in melon samples from vendor 1 in Ogida market.

Table 4.5 reveals the results for the cultural, morphological and biochemical characteristics of the bacterial isolates in melon samples from various markets in Benin City. Morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation were taken into account. Biochemical tests were also carried out to further identify the bacterial isolates. Among the biochemical tests used were catalase, oxidase, indole and citrate tests. The bacteria isolated from the melon seed samples include, *Bacillus* spp., *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus* and *Salmonella* spp.

Table 4.1: Total heterotrophic bacteria counts (CFU/g) in Melon samples from various markets in Benin City

Sample	Total heterotrophic bacteria count ($x10^5$ CFU/g)
New Benin (I)	3.45±1.15
New Benin (II)	2.67±1.20
Ogida (I)	2.48± 0.33
Ogida (II)	3.10 ±1.73
Oba (I)	2.58±0.33
Oba (II)	2.61±0.88
Uselu (I)	2.35±1.15
Uselu (II)	1.96±0.67

Values are represented as mean ± standard error,

I = Vendor 1, II = Vendor 2

Table 4.2: Total heterotrophic fungal counts (CFU/g) in Melon samples from various markets in Benin City

Sample	Total heterotrophic fungal count ($x10^3$ CFU/g)
New Benin (I)	4.27±0.33
New Benin (II)	1.66±0.33
Ogida (I)	3.20± 0.00
Ogida (II)	2.87 ±0.33
Oba (I)	1.00±0.00
Oba (II)	1.87±0.33
Uselu (I)	2.47±0.33
Uselu (II)	6.00±0.58

Values are represented as mean ± standard error

I = Vendor 1, II = Vendor 2

Table 4.3: Total Coliform counts (CFU/g) in Melon samples on MacConkey agar

Sample	Total Coliform Count ($x10^3$ CFU/g)
New Benin (I)	9.66±0.67
New Benin (II)	6.26±0.67
Ogida (I)	6.87± 0.33
Ogida (II)	5.93±1.20
Oba (I)	8.13±0.67
Oba (II)	7.73±0.33
Uselu (I)	8.20±0.57
Uselu (II)	4.73±0.67

Values are represented as mean ± standard error

I = Vendor 1, II = Vendor 2

Table 4.4: Total bacterial counts (CFU/g) in Melon samples on *Salmonella-Shigella* agar

Sample	Total bacteria count ($x10^2$ CFU/g)
New Benin (I)	0.00±0.00
New Benin (II)	0.00±0.00
Ogida (I)	0.80± 0.00
Ogida (II)	0.00±0.00
Oba (I)	5.26±0.33
Oba (II)	1.80±0.00
Uselu (I)	3.06±0.33
Uselu (II)	2.80±0.00

Values are represented as mean ± standard error

I = Vendor 1, II = Vendor 2

Table 4.5: Cultural, Morphological and Biochemical characteristics of the bacterial isolates in Melon samples from various markets in Benin City

Characteristics	B1	B2	B3	B4	B5	B6	B7	B8	B9
Cultural									
Shape	Irregular	Irregular	Irregular	Rhizoid	Irregular	Circular	Irregular	Irregular	Circular
Size	Large	Small	Small	Small	Small	Small	Small	Medium	Medium
Elevation	Raised	Flat	Flat	Flat	Raised	Flat	Flat	Flat	Flat
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Translucent
Margin	Undulate	Entire	Entire	Undulate	Entire	Lobate	Entire	Entire	Entire
Color									
Agar 1 (NA)	Cream	Cream	Cream	Cream	-	-	-	-	-
Agar 2 (MCA)	-	-	-	-	Cream	Off white	Cream	Off white	Cream
Agar 3 (MCA)	-	-	-	-	-	-			
Morphological									
Gram stain	+	+	-	+	-	-	-	-	-
Cell type	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Clusters	Single	Chains	Single	Pairs	Clusters	Chains	Chains	Pairs
Biochemical									
Urease	-	+	-	+	-	-	-	-	-
Indole	-	-	+	-	+	-	+	-	-
Citrate	+	+	-	+	-	+	-	+	-
Catalase	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	+
Coagulase	-	-	-	+	+	-	-	-	-
Oxidase	-	-	-	-	-	+	-	+	-

Table 4.5 (contd.): Cultural, Morphological and Biochemical characteristics of the bacterial isolates in Melon samples from various markets in Benin City

Lactose	-	-	+	+	+	-	+	-	-
Sucrose	+	+	+	+	+	-	+	-	-
Glucose	+	+	+	+	+	-	+	-	+
Fructose	+	+	+	+	+	-	+	-	-
Maltose	+	+	+	+	+	-	+	-	+
Starch	+	+	-	-	-	-	-	-	-
Sorbitol	+	-	+	-	+	-	+	-	+
Isolates	***Bacillus* spp.[1]**	***Bacillus* spp.[2]**	***Escherichia coli*[1]**	***Staphylococcus aureus***	***Escherichia coli*[2]**	***Pseudomonas* spp.[1]**	***Escherichia coli*[3]**	***Pseudomonas* spp.[2]**	***Salmonella* spp.**

KEY: +: Positive to test, -: Negative to test

Table 4.6 indicates the results obtained for the fungal cultural characteristics of isolates in melon samples from various markets in Benin City. The identified fungal isolates were *Aspergillus flavus Aspergillus niger, Rhizopus* spp*., Penicillium chrysogenum*, *Fusarium* spp. and *Trichoderma* spp.

Table 4.7 reveals the results for the frequency of occurrence and percentage frequency of bacterial isolates in melon samples from various markets in Benin City. Highest frequency of occurrence (117) and percentage frequency (31%) of bacteria isolates was observed to occur for *Salmonella* sp. while lowest frequency of occurrence (11) and percentage frequency (3%) of bacterial isolates was observed to occur for *Pseudomonas* spp.

Table 4.8 reveals the results for the frequency of occurrence and percentage frequency of fungal isolates in melon samples from various markets in Benin City. Highest frequency of occurrence (72) and percentage frequency (69%) of fungal isolates was observed to occur for *Penicillum chrysogenum* while lowest frequency of occurrence (3) and percentage frequency (3%) of fungal isolates was observed to occur for *Trichoderma* spp.

Table 4.9 shows the result of the antibiotic sensitivity test on Gram positive and Gram negative bacterial isolates in Melon samples from various markets in Benin City. The *in vitro* antimicrobial assay was carried out using the Kirby Bauer disk diffusion technique. The antibiotics utilized for the assay were; Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxacillin, Rocephin, Ciprofloxacin, Streptomycin, Septrin, Erythromycin, Saprifloxacin, Chloramphenicol, Tarivid and Augmentin. For Gram positive bacteria isolates, *Bacillus* sp.[1] was observed to exhibit the highest bacterial resistance with a MAR index of 0.7 while *Bacillus* sp.[2] exhibited the lowest bacterial resistance with a MAR index of 0.5. For Gram negative bacteria isolates, *Pseudomonas* sp.[1] was observed to exhibit the highest bacterial resistance with a MAR index of 0.7 while *Escherichia coli*[2] and *Salmonella* sp. exhibited the lowest bacterial resistance with a MAR index of 0.1 respectively.

The antimicrobial activity of chitosan on the bacterial isolates is shown in table 4.10. Highest activity of chitosan was revealed on *Bacillus* sp.[1] and *Escherichia coli*[1] treated with 50mg/ml of chitosan extract producing zones of inhibition of 35mm respectively. Lowest activity of chitosan was revealed on *Escherichia coli*[2] treated with 6.25mg/ml of chitosan extract producing zones of inhibition of 1mm.

Table 4.6: Cultural characteristics of fungal isolates in Melon samples from various markets in Benin City

Characteristics	F1	F2	F3	F4	F5	F6
Cultural						
Nature of colony	Fluffy mass with light green spores/ yellow areas and a pale reverse side	Fluffy colonies with black spores and a pale reverse side.	Fluffy white/grey colonies with black spores at the air interface.	Green dome shaped colonies with dirty white colouration.	Wooly white colonies with orange spores	Green colonies with pale reverse side and powdery texture
Morphological						
Nature of hyphae	Septate	Septate	Non-septate	Septate	Septate	Septate
Spore type	Conidiospore	Chlamydospore	Sporangiospore	Conidiospore	Chlamydospore	Conidiospore
Organism	***Aspergillus flavus***	***Aspergillus niger***	***Rhizopus* spp.**	***Penicillum chrysogenum***	***Fusarium* spp.**	***Trichoderma* spp.**

Table 4.7: Frequency of occurrence and percentage frequency of bacterial isolates in Melon samples from various markets in Benin City

Isolates	Frequency of occurrence	% Frequency
***Bacillus* spp.**	91	24
Escherichia coli	103	28
Staphylococcus aureus	51	14
***Pseudomonas* spp.**	11	3
***Salmonella* spp.**	117	31
Total	373	100

Values are represented as mean ± standard error

Table 4.8: Frequency of occurrence and percentage frequency of fungal isolates in Melon samples from various markets in Benin City

Isolates	Frequency of occurrence	% Frequency
Aspergillus flavus	15	14
Aspergillus niger	7	6
***Rhizopus* spp.**	4	4
Penicillum chrysogenum	72	69
***Fusarium* spp.**	4	4
***Trichoderma* spp.**	3	3
Total	105	100

Table 4.9: Antibiotic sensitivity test on Gram positive bacterial isolates in Melon samples from various markets in Benin City

Bacteria isolates	Antibiotics (Zone of Inhibition [mm])										
	PEF	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**AZ**	**LEV**	**E**	**MAR Index**
Gram positive											
Staphylococcus aureus	14(I)	0(R)	2(R)	0(R)	0(R)	0(R)	20(S)	10(R)	16(S)	16(S)	0.6
Bacillus sp.[1]	0(R)	0(R)	2(R)	4(R)	0(R)	14(I)	20(S)	0(R)	18(S)	6(R)	0.7
Bacillus sp.[2]	16(S)	12(I)	0(R)	0(R)	4(R)	16(S)	16(S)	0(R)	20(S)	6(R)	0.5
Gram negative	**LEV**	**CF**	**SP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**AZ**	**R.I**
Escherichia coli[1]	16(S)	0(R)	14(I)	10(R)	0(R)	0(R)	4(R)	20(S)	14(I)	0(R)	0.6
Escherichia coli[2]	0(R)	0(R)	16(S)	14(I)	0(R)	0(R)	18(S)	20(S)	20(S)	0(R)	0.5
Pseudomonas sp.[1]	0(R)	0(R)	10(R)	20(S)	2(R)	10(R)	10(R)	8(R)	12(I)	0(R)	0.7
Escherichia coli[2]	16(S)	14(I)	16(S)	18(S)	14(I)	6(R)	20(S)	24(S)	24(S)	12(I)	0.1
Pseudomonas sp.[2]	14(I)	16(S)	16(S)	16(S)	18(S)	10(R)	18(S)	18(S)	16(S)	0(R)	0.2
Salmonella sp.	0(R)	12(I)	16(S)	16(S)	14(I)	18(S)	18(S)	20(S)	20(S)	14(I)	0.1

KEY: Resistance (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) = 17mm and above, PEF: Pefloxacin, CN: Gentamycin, APX: Ampiclox, Z: Zinnacef, AM: Amoxacillin, R: Rocephin, CPX: Ciprofloxacin, E: Erythromycin, LEV: Levofloxacin, AZ: Azithromycin, CF: Cefotaxim, SP: Saprifloxacin, OFX: Tarivid, SP: Sparifloxacin, AZ: Azithromycin

Table 4.10: Antimicrobial activity of chitosan on the bacterial isolates

Isolates	Concentration (mg/ml)	Zones of Inhibition (mm)
***Bacillus* sp.[1]**	50	35
	25	30
	12.5	-
	6.25	-
***Bacillus* sp.[2]**	50	30
	25	27
	12.5	23
	6.25	15
***Escherichia coli*[1]**	50	35
	25	33
	12.5	13
	6.25	7
Staphylococcus aureus	50	31
	25	25
	12.5	-
	6.25	-
***Escherichia coli*[2]**	50	15
	25	10
	12.5	4
	6.25	1
***Pseudomonas* sp.[1]**	50	25
	25	-
	12.5	-
	6.25	-
***Escherichia coli*[3]**	50	18
	25	9
	12.5	-
	6.25	-
***Pseudomonas* sp.[2]**	50	23
	25	20
	12.5	-
	6.25	-
Salmonella sp.	50	24
	25	11
	12.5	-
	6.25	-

N.B: The results were recorded after an incubation period of 24 hr and the zones of inhibition are represented in millimeters

CHAPTER FIVE

5.0 DISCUSSION

According to the findings of this inquiry, melon seeds from the New Benin market, Ogida market, Oba market, and Uselu market were contaminated with foodborne human pathogenic bacteria. This investigation identified five (5) different bacterial genera *Bacillus* sp, *Staphylococcus aureus*, *Pseudomonas* sp., *Escherichia coli* and *Salmonella* sp. The prevalence of *Escherichia coli* and *Staphylococcus aureus* is consistent with the findings of Dike-Ndudim *et al*. (2016), who investigated the public health implications of fermented melon seed intake as a culinary condiment in South-eastern Nigeria and elsewhere. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were among the isolates found in fermented melon seeds.

The bacterial counts ranged from 1.96±0.67 to 3.45±1.15x10^5 CFU/g. The fungal counts ranged from 1.00±0.00 to 6.00±0.58x10^3 CFU/g. These results are in corroboration with a study by Iwu Irenus and Duru, (2019). In their study, microorganisms were isolated from fermented castor oil seeds and melon seeds. The total bacteria load in fermented castor oil seed and melon seeds was in average of 195.0±9.6 x 10^7 cfu/g and 145.7±12.5 x 10^7 cfu/g respectively, while the total viable count of fungi cells show that castor oil seed contained 3.3±1.5 x 10^2 cfu/g and 2.7±0.6 x 10^2 cfu/g for melon seed. The bacteria flora genera obtained include *Lactobacillus*, *Bacillus*, *Enterobacter*, *Streptococcus*, *Pseudomonas* and *Escherichia*. Fungi flora include *Rhizopus*, *Penicillium*, *Aspergillus* and *Saccharomyces* species.

According to Croxen *et al*. (2013), pathogenic variants of Escherichia coli cause severe morbidity and death in susceptible people; some of these pathotypes pose a serious public health threat due to low infectious doses and extensive transmission. The presence of Pseudomonas sp. may indicate contamination caused by seed exposure to air.

Although *Escherichia coli* is not hazardous, its presence in any quantity can be interpreted as evidence of contamination with feces, which, if not of human origin, is a major source of food poisoning. Pseudomonas sp. causes a variety of illnesses, including gastroenteritis, septicemia, and typhoid, which are spread by food or water and were found in kitchen utensils in accordance with Croxen *et al*. (2013). In fact, the presence of Staphylococcus aureus in food condiment samples raises a number of concerns. In comparison to coliforms, it can live outside of water for a longer amount of time.

Staphylococcus aureus is also responsible for food poisoning, which occurs when toxins are produced in food and consumed. Its incubation time ranges from one to six hours, and the disease itself can last from 30 minutes to three days. To help avoid the transmission of the disease, wash your hands thoroughly with soap and water before preparing meals. Rasmussen *et al*. (2011) also report that it is a prominent cause of bloodstream infections in much of the industrialized world. Once in the circulation, the bacteria can infect several organs, resulting in infective endocarditis, septic arthritis, and osteomyelitis.

The significant fungal contamination of melon seeds is due to the direct transmission of germs from infected food handlers and fungal spores. Furthermore, these spores have the potential to proliferate in conditions of low water activity. If fungal spores are not thoroughly rinsed away, they may contaminate other foods. Because food products such as melon seeds are particularly sensitive, they should be stored in very clean and low-humid settings because they have the capacity to transfer various microbiological agents to and from humans, hence having a significant influence on food consumer health.

Compared to the other fungal isolates found, *Aspergillus flavus* had the highest frequency of occurrence (14%). *Aspergillus flavus* is a saprotrophic, pathogenic fungus. It is well recognized for colonizing cereal grains, legumes, and tree nuts (Masayuki and Iwai, 2019). The presence of *Aspergillus flavus* and *Aspergillus niger* in foods raises concerns about mycotoxin contamination. In humans, *Aspergillus flavus* aflatoxin synthesis can cause acute hepatitis, immunosuppression, hepatocellular cancer, and neutropenia. The lack of control of fungal screening in countries with a high prevalence of viral hepatitis significantly raises the risk of hepatocellular cancer (Crawford, 2005).

It was also discovered that the majority of the bacteria isolated in this investigation were resistant to the antibiotics utilized. This raises the possibility of multidrug-resistant strains among the isolated bacteria. Bacteria that have acquired resistance mechanisms to conventional antibiotics have spread. Microorganisms use resistance mechanisms such as drug inactivation and medicine absorption into their cells (Kunwar *et al*., 2019). Chitosan's antibacterial activity is closely connected to its structure and physicochemical properties, the type of bacteria being targeted, and the surrounding environmental factors (De Gaetano *et al*., 2021).

In this investigation, chitosan inhibited the isolates better at 50 mg/ml and 25 mg/ml, with the exception of *E. coli*, *Staphylococcus aureus*, *Samonella* sp., and *Pseudomonas* sp., which were completely resistant to chitosan at 12.5 mg/ml and 6.25 mg/ml. Future research should focus on improving the sustainability and efficiency of chitosan manufacturing while also promoting the development of innovative chitosan-based materials and applications (Lopez-Moya *et al*., 2019).

5.1 Conclusion

This analysis found that melon seeds sold in marketplaces around the Benin City were infected with dangerous bacteria. There was also an alarming amount of resistance among the bacterial isolates discovered. Antibiotic resistance among microorganisms is becoming more common. Individuals who use antibiotics excessively should avoid them, and alternative antimicrobial drugs to treat infections should be developed.

REFERENCES

Abaelu, A.M., Olukoya, D.K., Okochi, V.I. and Akinrimisi, E.O. (1990). Biochemical changes in fermented melon (egusi) seeds (*Citrullis vulgaris*). *Journal of Industrial Microbiology* **6**: 211-214.

Achigan-Dako, E.G., Fagbemissi, R., Avohou, H.T., Vodouhe, R.S., Coulibaly, O. and Ahanchede, A. (2008). Importance and practices of melon crops (*Citrullus lanatus* (Thunb.) Matsum. and Nakai, *Cucumeropsis mannii Naudin* and *Lagenaria siceraria* (Molina) Standl. cv. "Aklamkpa") in sociolinguistic areas in Benin. *Biotechnologie, Agronomie, Société et Environnement* **12**: 393–403.

Adelberg, J.W., Rhodes, B.B., Skorupska, H.T. and Bridges, W.C. (1994). Explant origin affects the frequency of tetraploid plants from tissue cultures of melon. *HortScience* **29**: 689–692.

Akanda, M.J.H., Sarker, M.Z.I., Ferdosh, S., Manap, M.Y.A., Rahman, N.N.N. and Ab Kadir, M.O. (2012). Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. *Molecules* **17:**1764–94.

Akobundu, E. (1989). Effect of full-fat melon (melon seed) meal on quality of cooked Nigerian sausage. *Food Quality and Preference* **3**(3): 44-49.

Akubor, P.I. (2004). Influence of processing methods on the chemical and functional properties of melon (*Colocynthis citrullus*) seeds. *Journal of Food Science and Technology-Mysore* **41**: 181–185.

Akubor, P.I. and Ogbadu, R.L. (2003). Effects of processing methods on the quality and acceptability of melon milk. *Plant Foods for Human Nutrition* **58**: 1–6.

Aladedunye, F. and Przybylski, R. (2013). Frying stability of high oleic sunflower oils as affected by the composition of tocopherol isomers and linoleic acid content. *Food Chemistry* **141**: 2373–8.

Armstrong, M.E. and Barringer, S. a. (2013). Improving adhesion of seasonings to crackers with hydrocolloid solutions. *Journal of Food Science* **78**: 1704–1712.

Bande, Y.M., Adam, N.M., Jamarei, B.O., Azmi, Y. and Razali, S.Z. (2013). Examining the physicochemical properties and engine performance of biodiesel from whole seed and extracted kernels of melon (*Citrullus lanatus*). *Scienceasia* **39**: 492–499.

Bankole, S.A. (2006). Fungal contamination and aflatoxin B1 of 'Egusi' melon seeds in Nigeria. *Food Control* **17**(10): 814-818.

Bankole, S.A., Adenusi, A.A., Lawal, O.S. and Adesanya, O.O. (2010). Occurrence of aflatoxin B1 in food products derivable from "melon" seeds consumed in southwestern Nigeria. *Food Control* **21**:974–976.

Bankole, S.A., Osho, A., Joda, A.O. and Enikuomehin, O.A. (2005). Effect of drying method on the quality and storability of "melon" melon seeds (*Colocynthis citrullus L.*). *African Journal of Biotechnology* **4**:799–80.

Bassaganya-Riera, J., Reynolds, K., Martino-Catt, S., Cui, Y., Hennighausen, L., Gonzalez, F., Rohrer, J., Benninghoff, A.U. and Hontecillas, R. (2004). Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology* **127**: 777–791.

Bemiller, J.N. (2011). Pasting, paste, and gel properties of starch-hydrocolloid combinations. *Carbohydrate Polymers* **86**, 386–423.

Berghout, J. A. M., Boom, R.M. and Goot, A.J. V. (2014). Understanding the differences in gelling properties between lupin protein isolate and soy protein isolate. *Food Hydrocolloids* **43**: 465–472.

Blazek, J. (2008). Role of amylose in the structure-function relationship in starches from Australian wheat varieties. *Dissertation* **8**(4): 1-12.

Boughalleb, N., Tarchoun, N., El-Mbarki, A. and El-Mahjor, M. (2007). Resistance evaluation of nine cucurbit rootstocks and grafted watermelon (*Citrullus lanatus* L.) varieties against Fusarium wilt and Fusarium crown and root rot. *Journal of Plant Sciences* **2**(1): 102-107.

Boye, J., Zare, F. and Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Research International* **43**:414–431.

Brandenberger, L., Pokjpshrefler, E.R. and Damicone, J. (2021). Melon production. *Scientific Research*. **5**(2): 30-32.

Burey, P., Bhandari, B.R., Howes, T. and Gidley, M.J. (2008). Hydrocolloid gel particles: formation, characterisation, and application. *Critical reviews in food science and nutrition* **48**: 361–377.

Cahill, F., Shahidi, M., Shea, J., Wadden, D., Gulliver, W., Randell, E., Vasdev, S. and Sun. G. (2013). High dietary magnesium intake is associated with low insulin resistance in the newfound land population. *PLoS ONE* **8**: 2–9.

Calder, P.C. and Yaqoob, P. (1999). Glutamine and the immune system. *Amino acids* **17**: 227–241.

Chiejina, N.V. (2006). Studies on seed-borne pathogens of some Nigerian melons. *Journal of Agriculture, Food, Environment, and Extension* **5**(1): 13-16.

Compton, M.E. and Gray, D.J. (1993). Shoot organogenesis and plant-regeneration from cotyledons of diploid, triploid, and tetraploid watermelon. *Journal of the American Society for Horticultural Science* **118**: 151–157.

Crawford, J. M. (2005). The liver and the biliary tract. In V. Kumar, A. K. Abbas and N. Fausto (Eds.), *Robbins and Cotran Pathologic Basis of Disease* (7th ed.). Elsevier Saunders Publications, Philadelphia. pp. 892

Croxen, M. A., Robyn, J. L., Roland, S. and Kristie, M. K. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews* **26**(4): 822-880.

Dane, F. and Liu, J. (2007). Diversity and origin of cultivated and citron type watermelon (*Citrullus lanatus*). *Genetic Resources and Crop Evolution* **54**: 1255–1265.

De Gaetano F., Marino A., Marchetta A., Bongiorno C., Zagami R., Cristiano M.C., Paolino, D., Pistarà, V., Ventura C.A. (2021). Development of Chitosan/Cyclodextrin Nanospheres for Levofloxacin Ocular Delivery. *Pharmaceutics* **13**:1293.

Dhiman, T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K. and Tolosa, M.X. (2000). Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *Journal of dairy science* **83**: 1016–1027.

Eckert, C.A., Pouillot, F.L.L., Gurdial, G.S., Knutson, B.L., Scott, L.S., Nemours, E.I.D.P. De and Station, E. (2005). Selective separation of valerie acid from dibasic acids using supercritical carbon dioxide. *Energies* **45**:1–5.

El-Adawy, T.A. and Taha, K.M. (2001). Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. *Journal of Agricultural and Food Chemistry* **49**: 1253–1259.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A. A and Abu-salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranean*). *African Journal of Biotechnology* **5**: 82– 90.

Enujiugha, V.N. and Ayodele-Oni, O. (2003). Evaluation of nutrients and some anti-nutrients in lesser-known, underutilized oilseeds. *International Journal of Food Science and Technology* **38**: 525–528.

Ford, E.S. and Mokdad, A.H. (2003). Dietary magnesium intake in a national sample of US adults. *The Journal of nutrition* **133**: 2879–2882.

Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V.M., Hénaff, E., Câmara, F., Cozzuto, L., Lowy, E., Alioto, T., Capella-Gutiérrez, S., Blanca,J., Cañizares, J., Ziarsolo, P., Gonzalez-Ibeas, D., Rodríguez-Moreno, L., Droege, M.,Du, L., Alvarez-Tejado, M., Lorente-Galdos, B., Melé, M., Yang, L., Weng, Y., Navarro, A., Marques-Bonet, T., Aranda, M. a, Nuez, F., Picó, B., Gabaldón, T., Roma, G., Guigó, R., Casacuberta, J.M., Arús, P. and Puigdomènech, P. (2012). The genome of melon (*Cucumis melo L.*). *Proceedings of the National Academy of Sciences of the UnitedStates of America* **109**: 11872–11877.

Gillooly, J.F., Allen, A.P., Brown, J.H., Elser, J.J., Martinez del Rio, C., Savage, V.M., West, G.B., Woodruff, W.H. and Woods, H.A. (2005). The metabolic basis of whole-organism RNA and phosphorus content. *Proceedings of the National Academy of Sciences USA* **102**: 11923–11927.

Giwa, S., Abdullah, L.C. and Adam, N.M. (2010). Investigating "melon" (*Citrullus colocynthis L.*) seed oil as potential biodiesel feedstock. *Energies* **3**: 607–618.

Giwa, S.O., Chuah, L.A. and Adam, N.M. (2014). Fuel properties and rheological behaviour of biodiesel from melon (*Colocynthis citrullus L.*) seed kernel oil. *Fuel Processing Technology* **122**:42–48.

Goto, M. (2009). Chemical recycling of plastics using sub- and supercritical fluids. *Journal of Supercritical Fluids* **35**: 165–375.

Gouda, N., Singh, R.K., Meher, S.N. and Panda, A.K. (2017). Production and characterization of

bio-oil and biochar from flaxe seed residue obtained from supercritical fluid extraction industry. *Journal of the Energy Institute* **90**: 265–275.

Guo, S., Zhang, J., Sun, H., Salse, J. and Lucas, W. (2013). The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nature* **45**: 51–8.

Gusmini, G., Wehner, T.C. and Jarret, R.L. (2004). Inheritance of melon seed type in watermelon. *Journal of Heredity* **95**:268–270.

Henderson, L. (2007). Invasive, naturalised and casual alien plants in southern Africa: A summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia* **37**: 215–248.

Henzler-Wildman, K. and Kern, D. (2007). Dynamic personalities of proteins. *Nature* **450**: 964–972.

Hlatky, R., Furuya, Y., Valadka, A.B., Goodman, J.C. and Robertson, C.S. (2002). Comparison of microdialysate arginine and glutamate levels in a severely head-injured patient. *Acta neurochirurgica Supplement* **81**: 347–349.

Igwenyi, I.O. and Akubugwo, E.I. (2010). Analysis of four seeds used as soup thickeners in the southeastern part of Nigeria. In: *ICCCE 2010 - 2010 International Conference on Chemistry and Chemical Engineering, Proceedings*. Pp. 426–430.

Ijoyah, M.O., Bwala, R.I. and Iheadindueme, C.A. (2012). Response of cassava, maize and melon in a three crop intercropping system at Makurdi, Nigeria. *International Journal of Development and Sustainability* **1**:1–10.

Ikeorgu, J.E.G. and Ezumah, H.C. (1991). Some analytical aspects of cassava, maize, okra, and melon-melon complex mixture: Soil temperature in relation to leaf-area variation. *Field Crops Research* **53**: 78–84.

Ikeorgu, J.E.G., Ezumah, H.C. and Wahua, T.A.T. (1989). Productivity of species in cassava, maize, okra, melon and melon complex mixtures in Nigeria. *Field Crops Research* **21**: 1–7.

Innawong, B., Mallikarjunan, P. and Marcy, J.E. (2004). The determination of frying oil quality using a chemosensory system. *Food Science and Technology* **37**: 35–41.

Ishihara, S., Nakauma, M., Funami, T., Nakaura, Y., Inouchi, N. and Nishinari, K. (2010). Functions of Gum Arabic and Soybean Soluble Polysaccharide in Cooked Rice as a Texture Modifier. *Bioscience, Biotechnology, and Biochemistry* **74**:101–107.

Jarret, R.L. and Levy, I.J. (2012). Oil and fatty acid contents in the seed of *Citrullus lanatus Schrad*. *Journal of Agricultural and Food Chemistry* **60**: 5199–5204.

Kamphuis, M.M.J.W., Lejeune, M.P.G.M., Saris, W.H.M. and Westerterp-Plantenga, M.S. (2003). Effect of conjugated linoleic acid supplementation after weight loss on appetite and food intake in overweight subjects. *European journal of clinical nutrition* **4**: 623–665.

Kelly, G.S. (2001). Conjugated linoleic acid: a review. *Alternative medicine review : a journal of clinical therapeutic* **6**: 367–382.

Kennedy, J.F. and Mistry, J. (2003). Chemical and Functional Properties of Food Components.

Carbohydrate Polymers **54**:532.

Khalid, W., Ikram, A., Rehan, M., Adeem, F., Ambreen, S., Ahmad, M., Aziz, A., and Sadiq, A. (2021). Chemical composition and health benefits of melon seeds. *Pakistan Journal of Agricultural Research* **34**(2): 309-317.

Khanal, R.C. and Dhiman, T.R. (2004). Biosynthesis of conjugated linoleic acid (CLA): a review. *Pakistan Journal of Nutrition* **3**: 72–81.

Koffi, K.K., Gbotto, A.A., Malice, M., Djè, Y., Bertin, P., Baudoin, J.P. and Zoro Bi, I.A. (2008). Morphological and allozyme variation in a collection of *Cucumeropsis mannii Naudin* (Cucurbitaceae) from Côte d'Ivoire. *Biochemical Systematics and Ecology* **36**: 777– 789.

Kundu, S. (2005). Amino acid network within the protein. *Physica* **346**: 104–109.

Kunwar, A., Samyukta, T., Shyaron, P., Sony, S. and Pramila, P. (2019). Bacteriological assessment of the indoor air of different hospitals of Kathmandu District. *International Journal of Microbiology* **10**: 11-23.

Levi, A., Thomas, C.E., Keinath, A.P. and Wehner, T.C. (2001). Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genetic Resources and Crop Evolution* **48**: 559–566.

Linke, D. and Berger, R.G. (2011). Foaming of proteins: New prospects for enzyme purification processes. *Journal of Biotechnology* **152**: 125–131.

Lopez-Moya, F., Suarez-Fernandez, M. and Lopez-Llorca, L.V. (2019). Molecular mechanisms of chitosan interactions with fungi and plants. *International Journal of Molecular Science* **20**:332.

Loukou, A.L., Gnakri, D., Djè, Y., Kippré, A. V, Baudoin, M.M.J. and Bi, I.A.Z. (2007). Macronutrient composition of three cucurbit species cultivated for seed consumption in Côte d' Ivoire. *African Journal of Biotechnology* **6**: 529–533.

Machida, H., Takesue, M. and Smith, R.L. (2011). Green chemical processes with supercritical fluids: Properties, materials, separations and energy. *Journal of Supercritical Fluids* Pp. 2–15.

Mao, L., Karakurt, Y. and Huber, D.J. (2004). Incidence of water-soaking and phospholipid catabolism in ripe watermelon (*Citrullus lanatus*) fruit: Induction by ethylene and prophylactic effects of 1-methylcyclopropene. *Postharvest Biology and Technology* **33**: 1–9.

Martin, A.H., Grolle, K., Bos, M. a, Cohen Stuart, M. a, and Vliet, T. van. (2002). Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. *Journal of Colloid and Interface Science* **254**: 175–183.

Masayuki, N. and Iwai, H. (2019). Functions and mechanisms: Polygalacturonases from plant pathogenic fungi as pathogenicity and virulence factors. *Journal of General Plant Pathology* **85**(4): 14-23.

Mbah, G.O., Ph, D., Amulu, N.F., Eng, M., Onyiah, M.I. and Eng, M. (2014). Effects of process parameters on the yield of oil from melon seed (*Colocynthis Citrullus*). *Journal of Agricultural and Food Chemistry* **15**: 43–49.

Mgidi, T.N., Maitre, D.C. Le, Schonegevel, L., Nel, J.L., Rouget, M. and Richardson, D.M. (2007). Alien plant invasions-incorporating emerging invaders in regional prioritization: A pragmatic approach for Southern Africa. *Journal of Environmental Management* **84**:173–187.

Mordike, B.L. and Ebert, T. (2001). Magnesium Properties, applications and potential. *Materials Science and Engineering* **302**: 37–45.

Ntui, V.O., Thirukkumaran, G., Azadi, P., Khan, R.S., Nakamura, I. and Mii, M. (2010). *SolocynFusarium* wilt and *Alternaria* leaf spot in *Citrullus L.* Resistance to table integration and expression of wasabi defensin gene in "Melon" melon. *Plant Cell Reports* **54**:163–187.

Ntui, V.O., Thirukkumaran, G., Iioka, S. and Mii, M. (2009). Efficient plant regeneration via organogenesis in "Melon" melon (*Colocynthis citrullus L.*). *Scientia Horticulturae* **119**: 397–402.

Nyam, K.L., Tan, C.P., Karim, R., Lai, O.M., Long, K. and Man, Y.B.C. (2010). Extraction of tocopherol-enriched oils from Kalahari melon and roselle seeds by supercritical fluid extraction (SFE-CO2). *Food Chemistry* **119**: 1278–1283.

Ogbonna, P.E. (2009). Yield responses of "Melon" Melon (*Colocynthis citrullus*) to rates of NPK 15:15:15 fertiliser. *American-Eurasian Journal of Sustainable Agriculture* **3**:764–770.

Olivares, A., Navarro, J.L., Salvador, A. and Flores, M. (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Science* **86**:251– 257.

Othman, G.A. and Al-Delamiy, K.S. (2012). Aflatoxin B1 production by *Aspergillus flavus* in different media and containers and the antifungal activity of garlic and black cumin. *Research Journal in Engineering and Applied Sciences* **1**(2): 117-121.

Pascua, Y., Koç, H. and Foegeding, E.A. (2013). Food structure: Roles of mechanicalproperties and oral processing in determining the sensory texture of soft materials. *Current Opinion in Colloid and Interface Science* **18**: 324–333.

Perakis, C., Louli, V. and Magoulas, K. (2005). Supercritical fluid extraction of black pepper oil. *Journal of Food Engineering* **71**: 386–393.

Piorkowski, D.T. and McClements, D.J. (2013). Beverage emulsions: Recent developments in the formulation, production, and applications. *Food Hydrocolloids* **42**:5–41.

Ponka, R., Fokou, E., Fotso, M., Tchouanguep, F.M., Leke, R., Souopgui, J. and Bih, M.A. (2006). Composition of dishes consumed in Cameroon. *International Journal of Food Science and Technology* **41**: 361–365.

Pourmortazavi, S.M. and Hajimirsadeghi, S.S. (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. *Journal of Chromatography* **14**:133–137.

Quintal, B. (2012). Frequency-dependent attenuation as a potential indicator of oil saturation. *Journal of Applied Geophysics* **82**: 119–128.

Raghavendra, S.N., Rastogi, N.K., Raghavarao, K.S.M.S. and Tharanathan, R.N. (2004). Dietary fibre from coconut residue: Effects of different treatments and particle size on the hydration properties. *European Food Research and Technology* **218**: 563–567.

Rasmussen, R. V. (2011). Future challenges and treatment of *Staphylococcus aureus* bacteremia with emphasis on MRSA. *Future Microbiology* **6**(1): 43–56.

Reddy, K.R.N., Salleh, B., Saad, B., Abbas, H.K. and Abel, C.A. and Shier, W.T. (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews* **29**: 3–26.

Rojas, J. a., Rosell, C.M. and Benedito de Barber, C. (1999). Pasting properties of different wheat flour-hydrocolloid systems. *Food Hydrocolloids* **13**:27–33.

Saris, N.E., Mervaala, E., Karppanen, H., Khawaja, J.A. and Lewenstam, A. (2000). Magnesium. *Clinical Chimica Acta* **294**: 1–26.

Smith, S. a., King, R.E. and Min, D.B. (2007). Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. *Food Chemistry* **102**: 1208–1213.

Song, Y. and Zheng, Q. (2007). Dynamic rheological properties of wheat flour dough and proteins. *Trends in Food Science and Technology* **24**:93–117.

Sorgentini, D.A. and Wagner, J.R. (2002). Comparative study of foaming properties of whey and isolate soybean proteins. *Food Research International* **35**: 721–729.

Strosnider, H., Azziz-Baumgartner, E. and Banziger, M. (2006). Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives,* **114**: 1898–1903.

Tenllado, D., Reglero, G. and Torres, C.F. (2011). A combined procedure of supercritical fluid extraction and molecular distillation for the purification of alkylglycerols from shark liver oil. *Separation and Purification Technology* **83**:74–81.

Uruakpa, F. (2004). Heat-induced gelation of whole melon (*Colocynthis citrullus L.*) seeds. *Food Chemistry* **87**: 349–354.

van der Vossen, H.A.M., Denton, O.A. and El Tahir, I.M. (2004). *Citrullus lanatus* (Thunb.) Matsum. and Nakai. In: G. J. H. Grubben and O. A. Denton (Eds.), Plant Resources of Tropical Africa 2. Vegetables Wageningen, Netherlands: PROTA Foundation/Backhuys Publishers/CTA. pp. 185-191.

Vignola, M.B., Bustos, M.C. and Pérez, G.T. (2018). Comparison of quality attributes of refined and whole wheat extruded pasta. *Food Science and Technology* **89** 329–335.

Wang, J.P., Yu, B., Xu, X., Yang, N., Jin, Z. and Kim, J.M. (2011). Orthogonal-function spectrophotometry for the measurement of amylose and amylopectin contents. *Food Chemistry* **127**: 102–108.

World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). (2003). *Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation*. Geneva: WHO. 75p.

Wu, F. and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. *Food Additives and Contaminants* **27**: 496–509.

Ziyada, A.K., Elhussien, S.A. and Medani, W. (2008). Physical and Chemical Characteristicsof *Citrullus lanatus* Var. Colocynthoide Seed Oil. *Journal of Physical Science* **19**: 69–75.

Zushi, K. and Matsuzoe, N. (2011). Utilization of correlation network analysis to identify differences in sensory attributes and organoleptic compositions of tomato cultivars grown under salt stress. *Scientia Horticulturae* **129**:18–26.

APPENDIX I

Table 2.1: Nutritional Profile of Some Major Oilseeds and Melon Seed

Major oilseeds Contents % w/w	Cotton seed	Peanut	Soybean	Sunflower	Olive	Melon
Protein	32.6	25.6	36.0	19.8	0.9	28
Fat	36.3	46.0	7.3	47.5	11	50
Saturated fatty Acid	9.7	8.7	0.9	4.5	1.7	28.10
Monounsaturated fatty acid	6.7	22.0	1.4	9.8	5.7	14.50
Polyunsaturated fatty acid	18.1	13.1	3.5	31.0	1.3	57.40
Carbohydrate	21.9	12.5	5.1	18.6	_	10.6
Fibre	5.5	6.2	6.1	6.6	2.9	12.0

(Jarret and Levy, 2012)

Table 2.2: pH of the melon samples from different locations

Samples	Replicate 1	Replicate 2	Replicate 3
New Benin (I)	7.41	7.39	7.40
New Benin (II)	7.08	7.14	7.12
Ogida (I)	7.12	7.12	7.13
Ogida (II)	7.04	7.02	7.03
Uselu (I)	7.11	7.13	7.12
Uselu (II)	7.17	7.16	7.16
Oba (I)	7.14	7.13	7.16
Oba (II)	7.05	7.07	7.07

Table 2.3: Concentration of Positive and Negative Antibiotics Disc

KEY: POSITIVE DISC			**KEY: NEGATIVE DISC**		
Abbreviation	Antibiotics	Concentration	Abbreviation	Antibiotics	Concentration
PEF	Pefloxacin	10µg	LEV	Levofloxacin	20µg
CN	Gentamycin	10µg	CF	Cefotaxim	10µg
APX	Ampliclox	30µg	SP	Sparifloxacin	10µg
Z	Zinnacef	20µg	CPX	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	AM	Amoxacillin	30µg
R	Rocephin	25µg	AU	Augmentin	10µg
CPX	Ciprofloxacin	10µg	CN	Gentamycin	30µg
AZ	Azithromycin	12µg	PEF	Pefloxacin	30µg
LEV	Levofloxacin	20µg	OFX	Tarivid	10µg
E	Erthromycin	10µg	AZ	Azithromycin	12µg

APPENDIX II

Plate 2.1: (a) Dehulled melon: (b) Hard shelled melon seed(Bankole *et al.* 2005)

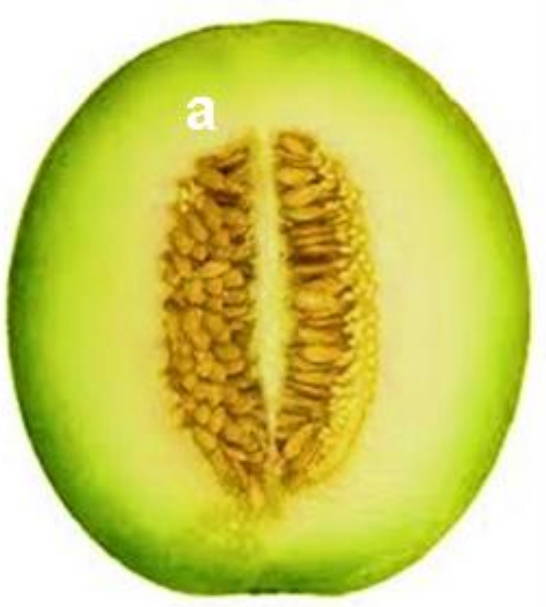

Plate 2.2: Melon plant (Garcia-Mas *et al.* 2012)

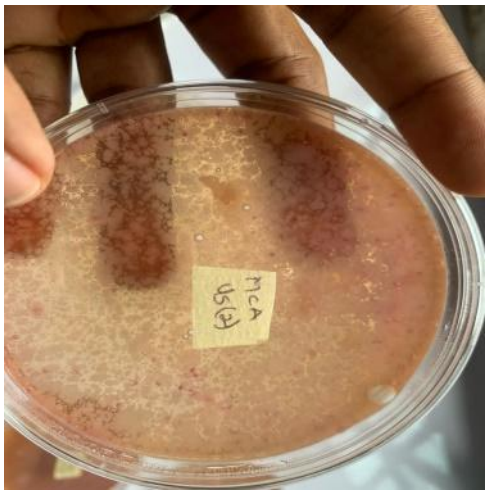

Plate 3.1: Bacterial isolates on MacConkey agar 1

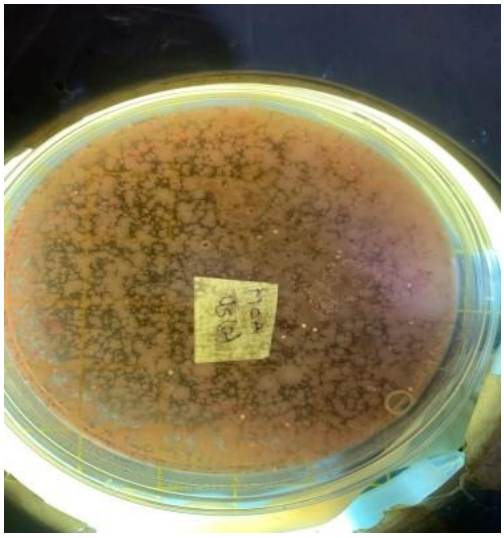

Plate 3.2: Bacterial isolates on MacConkey agar 2

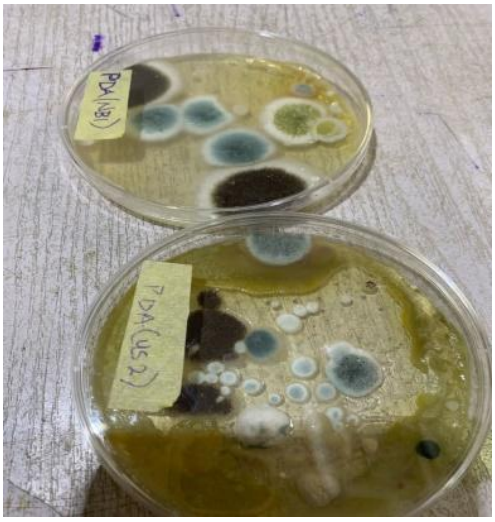

Plate 3.3: Fungal isolates on Potato Dextrose Agar