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Evaluation of the chemical characteristics and microbiological status of Awara sold in Dutsinma town, Dutsinma Katsina state

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Abstract

Awara is hawked at public places such as motor parks, streets, school gate and market areas which are unhygienic spots and hence predisposes it to toxic metals and microbial contamination. Also, poor handling methods such as the use of contaminated water and un-sanitized processing equipment are traditionally apparent in ready to eat foods of which Awara is inclusive. The objective of this work is to evaluate the chemical characteristic and microbiological status of Awara sold in Dutsin-ma town, Dutsin-ma local government area Katsina state. Results indicates that all the samples collected from the five different locations were highly contaminated, Wednesday market (Commercial Area) which is the third location had the highest bacterial count 9.9×10^2 and Hayin gada had the least bacteria count 5.8×10^2. Fudma gate had the highest fungi count 6.1×10^2 and Hayin gada had the least fungi count 4.6×10^2. There was presence of toxic some chemicals with Wednesday market having the highest amount of lead 0.41mg/kg. The Awara samples from Isa kaita junction had the lowest lead content 0.11mg/kg. The Awara samples from Darawa had the least moisture content (16.19%) while the Awara sample from Fudma gate had the highest moisture content (18.95%). Awara from Isa kaita junction and Fudma gate had the highest pH value (6.8 and 6.8 respectively). Awara samples from Wednesday market had the lowest pH values. A total of two fungi species were isolated from the food (Awara) samples and they were identified as Aspergillus species and Sacchraromy cescerevisiae. Although all the Awara samples showed growth on various culture media with varying counts but the population was not high enough to produce effective dose. However, the need for processors of Awara to adopt strict hygiene practices cannot be overemphasized.

Keywords: Awara; Chemical Characteristics; Microbial Status; Dutsinma

1. Introduction

The Awara is an unfermented soybean product (also known as soybean curd). It is a soft-cheese -like food produced by curding fresh hot soy milk with either a salt or an acid (Egbo,2012). Traditionally, the curdling agent used to make tofu/Awara is calcium sulphate. The coagulant produces a soy protein gel, which traps water, soy lipids, and other constituents in the matrix, forming curds. The curds are then generally pressed to remove the excess water and then cut into cubes (Ganiyu and Ekperigin, 2007).

Awara also known as Tofu is an important dietary snack food throughout Asia. It is the most important and popular food product from soybean in Eastern and Southern Asian countries. It is also gaining an increasing popularity in Western countries. Awara was developed some 2000 years ago and has become the world's most popular soy food product due to its high protein (Ganiyu and Ekperigin, 2007).

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Soya beans or Soy beans cake, popularly called 'Awara' in northern Nigeria is a cheap source of protein especially in the menu of resource-poor classes of the northerners. It can also be taken at any time of the day as a snack, usually made from soya beans. Awara is easy to digest and is substituted for meat, cheese and certain dairy products such as yoghurt, in the diets for dairy-sensitive individuals, vegans and elderly (Ganiyu and Ekperigin, 2007). Based on the coagulant type, Awara can be a good source of calcium added to its inherent B-complex vitamins, isoflavones, minerals fibre and unsaturated fatty acid content (Ganiyu and Ekperigin, 2007). It has a very low levels of saturated fat and no cholesterol. It acts like sponge and has the miraculous ability to absorb any flavor that is added to it. Tamarind fruit extracts, vinegar, lemon juice, alum and citric acid are the main coagulants used to precipitate or curdle the casein-like protein, making it easy and possible to separate it from the whey-like protein. There is a wide variety of ready-to-eat foods, these include, but are not limited to Awara,gurasa, sandwiches, doughnut, takeaway foods and bakery products. Ready-to-eat foods usually include a number of ingredients which may or may not be cooked (Odedeji and Oyeleke, 2011).

The word Heath Organization (WHO) indicated that food-borne diseases most of which are of microbial origin are perhaps the most widespread problems in the contemporary world and this is responsible for about one third of death worldwide, through infectious conditions, with adverse effects that can reduce economic productivity. Poor sanitary condition in most of the local markets and the environment, being highly polluted and charged with spoilage and pathogenic flora is likely the source of contamination of food items sold by such vendors. It is known that poor hygienic conditions in a food environment may encourage the multiplication of pathogenic organisms in food (Nyenjeet al., 2012). It has been observed that Bacillus cereus and Staphylococcus Aureusgrow to toxigenic levels in food (Nyenjeet al., 2012). Therefore, microbiological examination of foods may provide information concerning the quality of the raw food, and the sanitary conditions under which the food is processed (Michael et al., 2004).

Bacteria may pass from equipment to food which has not been properly cleaned and sanitized before being used to prepare another food. This implies that the food to be consumed by humans should be pure and free from contamination especially by pathogenic and spoilage microorganisms. Failure to ensure the safety and wholesomeness of the food consumed by the public might lead to some illness. To reduce contamination by microorganisms to a minimum level, and obtain good keeping quality of the products, the raw materials should regularly be monitored and examined.

In Africa, Mensah, et al., (2002) reported the presence of Bacillus cereus, Staphylococcus aureus, Shigellasonnei, Escherichia coli, and Salmonella Arizonae on different foods sold on streets of Accra. El-Shenawyet al., (2011) reported the contamination of Street-vended ready-to-eat food sold in Egypt, with Listeria species which include Listeria monocytogenes and Listeria innocua. (Nyenjeet al., 2012) investigated the microbiological quality of ready to eat foods sold in Alice, South Africa and reported the contamination of these foods by Listeria species., Enterobacter species, Aeromonashydrophila, Klebsiellaoxytoca, Proteus mirabilis, Staphylococcus aureusand Pseudomonas luteola.

Currently, researches into food-borne human pathogens have identified ready to eat foods as a veritable source of food borne pathogens (Akinyemiet al., 2012; Isiboret al., 2013). In Nigeria, the upsurge of ready to eat products poses a managerial challenge to the food safety authority for continuous surveillance of the quality of this category of food. This is predicated on the lack of good manufacturing practices among most of the processors during the production and distribution system. Commonly, these categories of foods are hawked at public places such as motor parks, schools, on street and market areas with glaring deviation from sanitary practices. Therefore, to provide current knowledge and possible future developments in increasingly important area of food safety, this study will investigate the chemical characteristic and bacteriological status of Awara sold in Dutsin-ma, Dutsin-ma local government area of Katsina state.

1.1. Statement of problem

The world Health organization (WHO) Indicated that food-borne diseases most of which are of microbial origin are perhaps the most widespread problems in the contemporary world and this is responsible for about one third of death worldwide. Poor handling methods such as the use of contaminated water and un-sanitized processing equipment are traditionally apparent in ready to eat foods (Shamsudden and Ameh, 2012). Inappropriate holding temperature that characterized the itinerant handling of ready to eat foods could also be a major factor in the increased microbial load (Barroet al., 2014). Awara in addition to the afore mentioned processing/handling problems is hawked at public places such as motor parks, streets, school gate and market areas which are unhygienic spots and hence predisposes it to toxic metals and microbial contamination.

1.2. Objectives of the study

The objective of this research work is to evaluate the chemical characteristic and microbiological status of Awara sold in Dutsin-ma town, Dutsin-ma local government area Katsina state.

The specific objectives of this work are

- To determine the microbiological status of Awara sold in in Dutsin-ma
- To Characterize the microorganism, present in Awara sold in Dutsin-ma town
- To determine the toxic chemicals, present in Awara sold in Dutsin-ma town
- To evaluate the moisture and pH of Awara sold in Dutsin-ma town.

2. Literature review

2.1. Awara and its Origin

Awara (Soya bean curd) is a low-fat, high-protein soy food that is often offered in blocks. In northern Nigeria, it is a popular healthful and tasty food (Jennifer,2022). It's high in protein and a convenient meat substitute for those who want to reduce their meat consumption. That's because it's prepared in such a way that it has a fantastic meaty texture and flavor, and it can be eaten on its own or added to soups and stews. It is mostly found in northern Nigeria and is consumed as a snack by the Hausa people. It's manufactured from soya beans, an Asian plant that's high in protein. Its plant is indigenous to China, where it has been grown for 13,000 years (Jennifer, 2022). It was only centuries later that it was brought to other parts of Asia, and it wasn't until the early twentieth century that it was employed as animal feed in the west. Soybeans are now the most frequently farmed and used legume in the planet. Soya beans, like other beans, grow in pods with edible seeds that are normally green, but can also be yellow, brown, or black.

Soya beans is a common legume in northern Nigeria. It is an indigenous tropical legume which serves as a dietary protein source for a large number of people especially children (Aletor and Ojelabi, 2007). People who follow a vegetarian diet use soya bean to replace meat as a meat- analogue recipes in the form of soya flour, soya protein concentrate, and soya protein isolate. Soya ingredients are the most commonly used in meat analogues because of their characteristic functional properties, such as water-holding, gelling, fat-absorbing, and emulsifying capacities. Soya flour is the least-processed of soybean protein products. In the market, there are several types of flours available such as full-fat, defatted, toasted flour, etc. (Geertset al., 2018).

Soya bean is an excellent source of protein (35-40%). The soya bean seed is the richest in food value of all plant foods consumed in the world (Kure et al., 1998). It is used in the production of bread as composite flour (Dhingraet al., 2002). Soya bean is used by leading infant food manufacturers in the country because of its high nutritional value. Soya bean is also processed into flour and its oil is used in local paint, cosmetics and soap making industries (Basmanet al., 2003). Soya bean is consumed in Nigeria as soya milk; the cake is used for livestock feeding and the flour is added to corn pudding as food for infant and children. Soya bean is a widely used, inexpensive and nutritional source of dietary protein (Basmanet al., 2003).

Soybean (Glycine max) has been proven suitable for the production of Awara (Egbo and Seidu 2012). It is a legume of an exceptionally high protein content ranging between 38% and 42% with lysine constituting a substantial proportion (Egbo and Seidu., 2012) Soybean is considered as a good source of plant protein to man. It is also cheaper and could serve as an alternative to animal protein sources. It contains up to 40% protein compared with 1.0% to 5.6% protein content of most animal milk (Farindeet al., 2008). Soybean is one of the most important legumes of the tropics. It has gained an increase in its utilization as a stable crop due to its high nutritional and excellent functional properties. It is also rich in carbohydrates (27.1%) and oil (20.6%) as reported by Osundahunsiet al. (2007). Soybeans contain Omega 3 fatty acids, devoid of cholesterol and easily digestible if properly processed.

2.2. Nutritional Composition of Soya Beans

Soybeans are mainly composed of protein but also contain good amounts of carbohydrate and fat.

2.2.1. Protein

Soybeans are among the best sources of plant-based protein. The protein content of soybeans is 36–56% of the dry weight (Christine et al., 2003). One cup (172 grams) of boiled soybeans boasts around 31 grams of protein (FDA 2018).

The nutritional value of soy protein is very good, although the quality is not quite as high as some animal proteins (Insafet al., 2019). The main types of protein in soybeans are glycinin and conglycinin, which make up approximately 80% of the total protein content. These proteins may trigger allergic reactions in some people (M.C Garcia et al., 2000) Consumption of soy protein has been linked with a modest decrease in cholesterol levels (Ogawa et al., 2000).

2.2.2. Fat

Soybeans are classified as oilseeds and used to make soybean oil. The fat content is approximately 18% of the dry weight — mainly polyunsaturated and monounsaturated fatty acids, with small amounts of saturated fat (Jan Van 2009). The predominant type of fat in soybeans is linoleic acid, accounting for approximately 50% of the total fat content.

2.2.3. Carbohydrate

Being low in carbohydrate, whole soybeans are very low on the glycemic index (GI), which is a measure of how foods affect the rise in blood sugar after a meal. This low GI makes soybeans suitable for people with diabetes.

2.2.4. Fiber

Soybeans contain a fair amount of both soluble and insoluble fiber. The insoluble fibers are mainly alpha-galactosides, which may cause flatulence and diarrhea in sensitive individuals (Cristina et al., 2008). Alpha-galactosides belong to a class of fibers called FODMAPs, which may exacerbate the symptoms of irritable bowel syndrome (IBS) (Derrick et al., 2010). Despite causing unpleasant side effects in some people, soluble fibers in soybeans are generally considered healthy.

2.2.5. Vitamins and minerals

Soybeans are a good source of various vitamins and minerals, including

- Molybdenum. Soybeans are rich in molybdenum, an essential trace element primarily found in seeds, grains, and legumes (Hiroyuki et al., 2004).
- Copper. Dietary intake of copper is often low in Western populations. Deficiency may have adverse effects on heart health.
- Manganese. A trace element found in most foods and drinking water. Manganese is poorly absorbed from soybeans due to their high phytic acid content.
- Phosphorus: Soybeans are a good source of phosphorus, an essential mineral abundant in the Western diet.
- Thiamine: Also known as vitamin B1, thiamine plays an important role in other plant compounds

Soybeans are rich in various bioactive plant compounds, including (Patricia et al., 2002).

2.2.6. Anti-vitamins

- Isoflavones: A family of antioxidant polyphenols, isoflavones have a variety of health effects.
- Phytic acid: Found in all plant seeds, phytic acid (phytate) impairs the absorption of minerals like zinc and iron. Levels of this acid can be reduced by boiling, sprouting, or fermenting the beans.

Saponins: One of the main classes of plant compounds in soybeans, saponins have been found to reduce cholesterol in animals.

2.3. Health benefits of Soybeans

Like most whole foods, soybeans have a number of beneficial health effects. May reduce cancer risk. Cancer is one of the leading causes of death in modern society. Eating soy products is linked to increased breast tissue in women, hypothetically increasing the risk of breast cancer (Amnonet al., 2008). However, most observational studies indicate that consumption of soy products may reduce breast cancer risk. Studies also indicate a protective effect against prostate cancer in men (Catherine et al., 2018). A number of soybean compounds, including isoflavones and lunasin may be responsible for the potential cancer-preventive effects (Elvira et al., 2003). Exposure to isoflavones early in life may be particularly protective against breast cancer later in life.

2.4. Microorganism associated with Foodborne Illness

Most food borne illness are classified as acute i.e., they are usually self-limiting and of short duration will symptoms including wild gastro-enteritis. However, some illnesses progress to life threatening neurological or renal syndromes called sequelae. Harmful microorganisms may contaminate food during receiving, during preparation and serving, during preparation techniques such as cooking and cooling by cross contamination of raw meat poultry or eggs with other foods from employees to food by unwashed hands, coughing or sneezing, from unsanitized facilities and equipment, from disease spreading pest such as cockroaches, flies and mice.

In these developing countries a major source of ready to eat food are prepare or sold at public places such as markets places, schools, canteens and along the streets, all together termed street foods. In addition, the vendors practice poor personal hygiene and reports of food vendor being carry and therefore could serve as potential sources of transmission of enteric fevers are many. At the same time, most of the people who patronize these foods are more interested in its convenience than question of its bacteriological quality and hygiene. The bacteriological quality of food indicates the number of bacterial contaminants it has; a high level of contamination indicates low quality and more likely to transmit infection. Although epidemiological data on the incidence of foodborne diseases are inadequate, and the outbreaks often not investigated, the recurrent episodes of food borne illness with symptoms of gastro intestinal distress like diarrhea, vomiting, abdominal cramp and nausea has remained a major cause of mortality and morbidity in Nigeria (Nwezeet al., 2010).

Chemicals, heavy metals parasites, fungi, viruses and bacteria can cause foodborne illness; bacteria related food poisoning is the most common, but fewer than 20 of the culprits. More than 90% of the cases of food poisoning each year are caused by Staphylococcus aureus, Salmonella, Clostridium perfringes, Clostridium botulinum, campylobacter, vibroparahaemolyticu, Bacillus cereus and Enteropathogenic Escherichia coli. These bacteria are commonly found on many raw foods. Normally a large number of food poisoning bacteria must be presented to cause illness, therefore illness can be prevented by controlling number of bacteria present, by preventing the small number from growing, destroying the bacteria by proper cooking and avoiding recontamination (Chaicumpar, 2006).

In Nigeria, consumption of street food has witnessed a phenomenal growth over the years as rapid population growth over the years, Urbanization, unemployment and poverty; occupational pressures and lifestyles changes has created a poll of mobile and transient population who depend almost entirely on these relatively low cost foods for their nutrition (Martin et al., 2006), cause illness, therefore illness can be prevented controlling number of bacteria present .by preventing the small number from growing, destroying the bacteria by proper cooking and avoiding recontamination (Chaicumpar, 2006).

Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensil could cause contamination of raw and cooked foods. Mishandling of raw and cooked foods allows bacteria to grow. Raw and cooked foods should not be kept in this danger zone any longer than absolutely necessary.

Analyzing foods for the presence of both pathogenic and spoilage bacteria is a standard way of ensuring food safety and quality. If microorganisms are able to survive and grow on food which are sold and consumed by people, then the risk of food borne illness is increased in the society. The presence of microorganisms on food can be important; because the essential nutrients of the food are ingested by some organisms to stimulate growth, while some organisms are known to be pathogenic to man as long as their growth conditions are favorable.

Bacteria may pass from equipment to food when the equipment that has touched the food has not been properly cleaned and sanitized before being used to prepare another food (Maduekeet al., 2014). Food eaten has direct influence on health; it is the duty of the manufactures and food handlers to keep food safe from pathogenic microorganisms, especially when such foods are to be consumed without Further processing (Munide and Kuria, 2005), when a food with harmful bacteria is ingested, there is a period of time before symptoms of the food-borne illness begin. The number of times varies with the different bacteria, how many consumed and the individual's physical condition.

2.5. Pre-Disposing Factors to Food-Borne Illnesses

The Center of disease control has identified improper hand washing; sanitizing are some of the major contributing factors to the spread of food-borne illness. Therefore, it is necessary to take the proper steps to ensure that these improper practices are avoided at all times (CDC 2004).

2.5.1. Improper Hand Washing

Proper Hand Washing has long been known to be beneficial public health practice for preventing the spread of infectious diseases According to the Center for Disease Control, hand washing is the single most important procedure for preventing the spread of infection Bacteria, such as the food-borne pathogen Staphylococcus aureus are found naturally on the human body and apparently healthy people may host food-borne pathogens, such as Salmonella. These people may be "carriers" and are capable of infecting others, yet they may not be aware that they are carriers because they may not show symptoms or become ill themselves. Therefore, it is necessary to utilized proper hand washing techniques after coughing sneezing and blowing the nose. Failure to use proper hand washing techniques increases the risk of transmission of food-borne illness. The Association for professionals in infection control and Epidemiology (APIC) states that hand-washing" causes a significant reduction in the carriage of potential pathogens on the hands and

recommends several steps for proper hand washing to prevent the spread of pathogens. During the hand-washing procedure, failure to cover all surfaces on the hands because of poor techniques or use of insufficient cleaning agents may lead to subsequent contamination of surfaces (CDC, 2004).

2.5.2. Prevention of Food-Borne Illnesses

Most cases of food-borne illnesses can be prevented through proper cooking or processing of food, which kills bacteria in addition, because bacteria multiply rapidly between 40°F and 140°F, food must be kept out of this temperature range.

Refrigerate foods promptly. If prepared food stands at room temperature for more than 2 hours, it may not be safe to eat. Set your refrigerator at 40°F or lower and your freezer at 0°c

Cook food to the approximate interval temperature 145°F for roasts, steaks, and chops of beef, veal and lamb, 160°F for pork, ground veal, and ground beef, 160°F for ground poultry; and chops of beef, veal and ground beef, 165°F for ground; and 180°F for whole poultry. Use a meat thermometer to be sure foods are properly cooked only when they are heated long enough and at a high enough temperature to kill the harmful bacteria that cause illnesses. Handle food properly. Always wash your hands for at least 20 seconds with warm, soapy water before and after handling raw meat, poultry, fish shellfish, produce or changing diapers, or touching animals.

Wash utensils and surfaces before and after use with hot, soapy water. Better still; sanitize them with diluted bleach-teaspoons with hot water (CDC, 2004).

2.6. Food Hygiene

Food hygiene is defined as a sanitary science which aims at producing food which is safe for human consumption and of good keeping quality and this includes any sanitation measures designed to prevent bacteria and other microorganisms of human origin from reaching food stuff (Umoh and VJ, 2006). Food hygiene is a subject of wide scope, it aims at studying methods for production and preparation of food, which is safe and of good-quality. It covers not only the proper handling of every variety of food stuff and drinks, but also food contact surfaces such as utensils, and apparatus used in the preparation, services and consumption of the food and also the care to prevent contamination with food poisoning bacteria which may originate from the animal or part plant host supplying the food (Umoh, 2006)

2.7. Factors that Contribute to Food-Borne Illness

Factors that contribute to food — borne illnesses include improper cooling of foods, time between preparing and serving, poor personal hygiene, not cooking food properly, abuse of the time temperature relationship, cross contaminating raw and cooked foods

2.7.1. Poor Personal Hygiene

Poor personal hygiene can result in food contamination for example when a food personnel, fails to wash hands properly after using the restroom, toilet, is a serious risk of fecal contamination (FDA, 2004). Everyone has bacteria on the skin, mouth, hands and so many other organisms on various parts of the body like hair. Food service personnel can contaminate food and cause food borne illness. Food workers may transmit pathogens to food from a contaminated surface, from one food to another food or from hands contaminated with organisms from the gastrointestinal tracts (Munide and Kuria, 2005). Therefore, hand contact with ready to eat food i.e., food that is edible without washing, cooking or additional preparation by the consumer or by the food establishment and that is expected to be consumed in that manner, represents a potentially important mechanisms by which pathogens may enter the food supply (Munide and Kuria, 2005).

2.7.2. Abuse of the Time -Temperature Relationship

Abuse of time temperature relationship is also another factor that can cause food-borne illnesses. To prevent food-borne illness, it is important to control the time that food is in the temperature danger zone. This means hot foods should be kept at 140°F or above and cold foods at 41°F or below (FDA, 2004).

2.7.3. Cross-Contaminating Raw and Cooked Food

Cross-contaminating raw and cooked food is transferring of harmful microorganisms from a surface to food or from one food to another food. Cross contamination can occur when food contact surfaces is not cleaned or sanitized as necessary for food safety (FDA, 2004). To prevent cross contamination, it is important to wash hands with soap and warm water before you start preparing food, before you handle a different food (for example, if you just handled raw chicken, wash

hands before preparing a salad), and after using the bathroom. Don't sneeze or cough on food. Organisms can "travel" from raw to cooked food, so never let raw food touch cooked food (FAD, 2004).

2.7.4. Features of some common Food -Borne Bacteria Pathogens

Salmonella is a generic name applied to a group of nearly 2,000 biochemical related serotypes responsible for food borne illness. The disease is grossly underreported because it is generally self-limiting gastroenteritis which may be misdiagnosed as intestinal influenza by patient or the physician. As a consequence, estimates of the true incidence of disease are based as assumptions derived from epidemiological evidence. Clearly, salmonellosis continues to be an important cause of food-borne disease worldwide.

Two clinical manifestations caused by Salmonella are recognized. Enteric fever (a severe, life-threatening illness) and the more common food-borne illness syndrome: In both cased, the oral route. Enteric fever, commonly referred to a typhoid fever, is primarily caused by one species, Salmonella typhi. But other Salmonellae such as Salmonella paratyphi, are potentially capable of producing this syndrome. The illness is commonly associated with foreign travel and affects an estimated 800 people annually (Although the route of entry of the pathogen into the body is primarily oral, the symptoms of enteric fever are generally not elicited through the intestinal tract. However, a short episode of vomiting and diarrhoea sometimes occurs in the first day or two in typhoid fever. The onset times vary considerably between typhoid and paratyphoid enteric fevers. Onset time for typhoid is usually 8-15 days, seldom as short as five days but sometimes as long as 30-35 days; while onset time for paratyphoid fever tends to be shorter. Salmonella is destroyed at cooking contamination of cooked foods occurs from contact with utensils that were not properly washed after use with raw products. If salmonella is presented in raw, or cook food, it can be grown by refrigeration below 40°F. There are various environmental sources that include water, soil, kitchen surfaces and animal faeces that helps in the transmission, Salmonella are transmitted through the fecal matter of people or animals and are usually transmitted to humans by eating foods that have been contaminated with fecal matter via cross-contaminations. As few as 15 to 20 cells depending on the age and health of the host and strain of bacteria are necessary to cause illness (FDA, 2004). It is estimated that approximately 40,000 cases of Salmonelosis are reported each year in the U.S.A (FDA, 2004).

Man's respiratory passage, skins and superficial wounds are common sources of Staphylococcus aureus. When Staphylococcus aureus is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, the toxin produce is heat stable and may not be destroyed. Staphylococcal food poisoning occurs most often in foods that require hand preparation. Sometimes these types of foods are left at room temperature for periods of time, allowing the bacteria to grow and produce toxin. Good personal hygiene when handling foods will keep Staphylococcus aureus out of foods and refrigeration of raw and cooked foods will prevent the growth of these bacteria if any is present.

Shigellosis, or bacillary dysentery, as it is commonly known, is caused by bacteria of the genus Shigella, which include Shigella dysenteriae, Shigella flexneri, Shigella boydil and Shigella sonnei. The normal habitat for Shigella is the intestinal tract of humans and other primates. Primarily mode of transmission appears to be person to-person by the fecal oral-route. Shigella is mostly associated with chicken, raw vegetables, dairy products and poultry. Contamination of these foods is usually through the Fecal-Oral route and is most commonly due to fecal contaminated water and unsanitary handling by food handlers (Todar et al., 2008). As few as 10 cells, depending on the age and body condition of the host are necessary to cause diseases as with Escherichia. coil, Shigella, are present in the diarrhea stool of infected person and can be transmitted during infection as well as one to two weeks after symptom subsides, most infections that occur are the result of the bacterium passing from stools or of soiled fingers of one person to the mouth or finger of another person. Shigella dysenteriae type cause deadly epidemics in developing countries (CDC, 2004). Shigella is transmitted through the fecal matter of people or animals and is usually transmitted to humans by eating foods that have been contaminated with fecal matter through cross contamination. As few as 15- 20 cells, depending on the age and health of the host and strain of bacteria are necessary to cause food-borne illness. Generally, food-borne shigellosis is characterized by a high attack rate, common-source epidemiology, and short incubation periods of 12-50 hours (FDA, 2004).

A lactose-fermenting species is usually not harmful but some strains cause gastrointestinal infections. Ingestion of the pathogenic serotype Escherichia coli 0157 derived from infected meat causes colitis with bloody diarrhea, which may give rise to the complications of hemolytic uraemic syndrome (Elizabeth and Martin, 2003). Escherichia coli is a significant cause of diarrhea in developing countries and localities of poor sanitation. Indeed, it has been associated with 'traveler diarrhea'. However, the latest outbreak in North America occurred in a nursing home in Ontario. There are at least four sub-group of Escherichia coli, namely Enterbinvasive, Enterohemorrhagic, Entertoxogenic, and Enteropathogenic. Each strain has different characteristics, the major source of the bacteria in the environment is probably the faces of infected human but there may also be animal reservoirs and untreated water are the most likely

sources for contamination of food. Escherichia coli 0157: H7 and its link to food become well known to the public as a result of the 1993 Escherichia coli 0157: H 7 outbreak caused by contaminated hamburgers. Over 700 people become ill from this outbreak and four children died (Buzby, 2001). Escherichia coli 0157: H7 may be acquired through consumption of meat that has not been sufficiently cooked, and person to person transmission can occur via the fecal oral route Escherichia coli 0157: H7 can be found in the diarrhoea stool of infected persons.

3. Methods

3.1. Sample Collection and Processing

Five samples of Awara were collected from five different vendors in Dutsin-ma local government. The sampling site included Hayingada, Darawa, Fudma gate, Isa Keita junction, and Dutsin-ma market. These samples were transported to the laboratory for microbiological analysis. The reagents and equipment used were of analytical grade and were gotten from Food Science and Technology (FST) laboratory of Federal University Dutsin-ma, Kastina state.

3.2. Microbiological Load Evaluation of Awara samples.

3.2.1. Media preparation

Nutrient agar, Eosin methylene blue agar, Salmonella shigella agar, Mannitol salt agar, Methyl Red-VogesProskauer, Simmon's Citrate agar, Nutrient agar slant, Saboaurd dextrose agar, and normal saline were prepared according to the manufacturer's instructions. Sterilization were be done by autoclaving at 121°C for 15 minutes.

3.2.2. Serial dilution

1.0 gram of Awara sample was be weighed aseptically using a weighing balance and homogenized in a clean and sterile mortar with 9ml of distilled water. Using a sterile syringe, 1ml of the homogenate was transferred into a sterile test tube containing 9ml normal saline labeled 10-1 and swirled to mix; another 1.0ml was withdrawn from 10-1 and was transferred to test tube containing 9ml normal saline labeled 10-2 and so on to make tenfold serial dilution of samples up to 10-5

3.2.3. Pour plate method

Samples were plated in duplicate using pour plate technique. 1 ml of the diluted samples was delivered into sterile nutrient agar, Eosin methylene blue, Mannitol salt agar, Salmonella shigella agar and Sabroaud dextrose agar. The plate was swirled carefully to mix the diluted samples with the sterilized ager and the plates was incubated inverted in at 37°C for 24hours for bacteria and at 26±1°C for 1-7 days for fungi isolates. Total viable counts and fungi counts was carried out on nutrient agar plates and Sabroaud dextrose agar using colony counter. The numbers of colony forming units (CFU) per gram was counted and recorded after 24 hours.

Pure colonies were aseptically picked from Nutrient agar plates and purified on prepared sterile nutrient agar slant by streaking on the slant surface using a sterilized wire loop.

3.2.4. Identification of bacteria from Awara samples

Distinct morphological properties of colonies were observed on Mannitol salt agar (MSA) for isolation of Staphylococcus aureus, Eosin Methylene Blue agar for Escherichai Coli and Salmonella Shigella Agar for Salmonella species present in the Awara sample. On Mannitol salt agar, Colonies that appear yellowish were presumptively identified as Staphylococci while the colonies that produced greenish metallic sheen on Eosin Methylene Blue agar were presumptively regarded as Escherichai Coli while colorless colonies with black spot on the Salmonella Shigella were identified as Salmonella species. The plates were incubated at 37°C for 24hours. The isolates were subjected to Gram staining and biochemical tests (Cheesebrough, 2003).

3.2.5. Gram staining and Biochemical tests

Gram staining procedure

A smear was prepared using two-three pure colonies of Awara isolate, heat fixed and each slide was covered with crystal violet and was allowed to stay for 60 seconds and washed with water. Iodine was applied for 60 seconds, and waswashed with water. Alcohol was applied and washed immediately, finally safranin was applied and allowed to stay for 60 seconds and washed with water.

Microscopic examination

The stained slides were viewed under X100 objectives lens using oil immersion (Cheesbrough, 2010).

Biochemical tests for identification of Awara sample isolates

Most of the methods was done according to the microbiology laboratory manual (Cappuccino and Sherman, 2005).

Catalase test

Using a sterile inoculating loop, two-three isolates from 24-hour pure culture was placed onto microscopic slide and 1 drop of 3% H_2O_2 was placed onto the organism on the microscopic slide using a dropper and immediate bubble formation indicated a positive result. (Cappuccino and Sherman, 2005).

Citrate utilization test

Using sterile technique, isolate from 24-hours old pure culture was inoculated into vials by means of a streak inoculation method with an inoculating needle and the vials was incubated for 48 hours at 37°C. Color change of media to blue indicated positive for citrate. (Cappuccino and Sherman, 2005).

Indole test

Tryptophan broths were inoculated with colony of the isolate and was incubate at 37°C for 24-48 hours in a burnt air then 0.5ml of kovac's reagent was added to the both cultures. Positive result showed pink color (Cappuccino and Sherman, 2005).

Methyl red test

Using sterile technique, purified bacteria isolate from 24-hours old pure culture was inoculated into the tubes by means of an inoculating loop and the tubes was incubated for 24 hours at 37°C. After 24 hours 3.5 ml from the culture tubes was transferred to clean test tubes for Voges- Proskauer test and the remaining broth was re-incubated for additional 24 hours. After 48-hour of incubation 5 drops of methyl red indicator was added directly into the remaining aliquot of the culture tubes to observe the immediate development of a red color which indicates a positive result (Cappuccino and Sherman, 2005)

Voges Proskauer test

To the aliquot of MR-VP broth after 24-hour incubation, 0.6 ml (12 drops) of 5% alpha naphthol (reagent A) was added followed by 0.2 ml (4 drops) of 40% KOH (reagent B). The tube was shaken gently and the medium was allowed to remain undisturbed for 10-15 minutes. The test was read, but not beyond, one hour following the addition of the reagents and development of a pink-red color on the surface of the medium indicated positive (Cappuccino and Sherman, 2005).

3.3. Determination of Heavy Metals

3.3.1. Sample Preparation

Five samples of Awarawas collected from the different locations. The foods were cut into small pieces and kept in tightly sealed glass Petri dish in the refrigerator for analysis.

3.3.2. Digestion of Samples

One gram of the powdered samples was quantitatively transferred into a well-glazed porcelain crucible and placed in a muffle furnace at 500ºC for 5 h. The resultant ash samples were cooled at room temperature and dissolved with 5 mL of concentrated nitric acid. The solution was then filtered into a calibrated 50 mL volumetric flask using Whatman No. 42 filter paper and made up to mark with deionized water (Lanre-Iyandaet al., 2012).

3.3.3. Analysis.

Flame atomic absorption spectrophotometer (Buck Scientific Model 210) was used to determine the concentrations of lead, Cadmium, Copper, and Nickel. Reagent blanks was used in all analyses to check impurities in reagent and other environmental contaminations during analyses. Samples were analyzed in duplicates to check for precision of the chosen method and the measuring instrument (Mohammed et al., 2016)

3.4. Chemical Analysis

Moisture content was determined as described by A.O.A.C., 2010. The pH was measured using a digital pH meter (Hannah model: HP 2211 pH/ORP meter)

3.4.1. Moisture

Moisture content was determined by drying 1 g (W1) of the sample in a hot air-oven (Uniscope, SM9053, England) at 105 ± 1 ºC until constant weight (W2) was obtained, the samples were removed from the oven, cooled in a desiccatorand weighed. The results were calculated as follows;

M.C= ((W_1) -(W_2))/W_1 X100 ……Eqn. i

Were

- M.C= Moisture content (%)
- W1 = $massof$gurasa$before$ (g),
- $W2$ = $massof$gurasa$afterdrying$ (g)

3.4.2. pH

The pH was determined by weighing 5 grams of Awara sample into clean mortar and pestle, 5ml of distilled water was added into the mortar and it was homogenized. The pH meter was inserted into the homogenate and the reading was taken.

3.5. Statistical Analysis

All the results obtained were subjected to one – way Analysis of variance (ANOVA) and the mean suspected by Duncans Multiple Range Test (DMRT) using the Stastitical Package for Social Sciences (SPSS) version 20.0 and significance was taken at 5% probability level.

4. Results

4.1. pH and Moisture content of Awara sold in Dutsin-ma town

Table 4.1 shows the results of the pH and moisture content of the Awara samples sold in Dutsin-ma town. The Awara samples from Darawa, Hayingada and Wednesday market had the lowest pH values (6.6, 6.6 and 6.5 respectively) while Awara from Isa kaita junction and Fudma gate had the highest pH value (6.8 and 6.8 respectively). There was no significant difference among the samples at 5% probability level. The pH values from all the samples obtained indicated that the food was slightly acidic to neutral, this favors the proliferation and survival of bacteria Maduekeet al., (2014).

The moisture content of the Awara obtained from Darawa, Fudma gate, Wednesday market, Isa kaita junction and Hayingada were, 16.19%, 18.95%, 17.78%, 17.89% and 17.76% respectively. The Awara samples from Darawa had the lowest moisture content (16.19%) while theAwara sample from Fudma gate had the highest moisture content (18.95%). There was a significant difference among the samples at 5% probability level.High moisture content accelerates food spoilage and generally provides a good media for the growth and proliferation of microorganisms especially bacteria (Prescott et al., 2008).

4.2. Toxic chemical present in Awara samples

Table 4.2 shows some toxic chemicals present in the Awara samples. The lead content of theAwara obtained from Darawa, Fudma gate, Wednesday market, Isa kaita junction and hayingada were 0.24mg/kg, 0.21mg/kg, 0.41mg/kg, 0.11mg/g and 0.30.mg/kg respectively. The Awara samples from Isa kaita junction had the lowest lead content (0.11mg/kg).

Table 1 pH and moisture content of Awara

Samples	pH	Moisture
A	6.6±0.070[a]	16.19±0.007[d]
B	6.8±0.070[a]	18.95±0.007[a]
C	6.5±0.070[a]	17.89±0.156[c]
D	6.8±0.141[a]	18.70±0.014[b]
E	6.6±0.070[a]	17.76±0.014[c]

Values in the same column with different superscripts are significantly different from each other (P ≤ 0.05); Key Codes (Sample location); A = Darawa; B = Fudma gate; C = Wednesday market; D = Isa kaita junction; E = Hayingada

Table 2 Toxic chemicals concentration in Awara samples

Samples	Lead (mg/kg)	Cadmium (mg/kg)	Copper (mg/kg)	Nickel (mg/kg)
A	0.24±0.28[c]	0.041±0.000[c]	7.41±0.014[c]	0.16±0.007[c]
B	0.21±0.00[c]	0.05±0.000[a]	8.13±0.000[b]	0.12±0.001[d]
C	0.41±0.007[a]	0.040±0.0014[d]	9.23±0.021[a]	0.49±0.007[a]
D	0.11±0.007[d]	0.044±0.0007[b]	6.67±0.007[d]	0.39±0.007[b]
E	0.302±0.007[b]	0.044±0.0007[b]	6.41±0.007[e]	0.15±0.000[c]
WHO/FAO (2009) Permissible limit	>0.3	>0.2	>73.3	>67.9

Wednesday market had the highest lead count (0.41mg/kg). There was a significant difference among the samples at 5% probability level.

The Awara samples from Wednesday market in our study was above the permissible limit of lead (0.3mg/kg) was regarded not safe retarding lead content. The toxic effects of lead have been principally established in studies on people exposed to lead in the course of their work. Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anemia and gastrointestinal symptoms. Longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system FSAI (2009). Lead levels in Awara samples of our study were higher than those reported by Khanikiet al., (2005).

The Awara samples from Fudma gate had the highest cadmium content (0.05mg/kg) while Awara from Wednesday market had the lowest cadmium content (0.040mg/kg). The cadmium of Awara samples obtained from Hayingada, Darawa and Isa kaita junction were (0.044mg/kg), (0.041mg/kg), (0.044mg/kg) respectively. There was a significant difference among the samples at 5% probability level.

The principal toxic effect of cadmium is its toxicity to the kidney, although it has been found to be associated with lung (including induction of lung tumors) and skeletal changes in occupationally exposed populations Yasaminet al., (2017). This may bring credence to the fact that vehicular and industrial emission may be a veritable source of exposure heavy metals in road side food.

The copper content of the Awara obtained from Darawa, Fudma gate, Wednesday market, Isa kaita junction and hayingada were 7.41mg/kg, 8.13mg/kg, 9.23 mg/kg, 6.67mg/g and 6.41mg/kg respectively. The Awara samples from Hayingada had the lowest copper content (6.40mg/kg) while the Awara sample from Wednesday market had the highest copper content (9.23mg/kg). There was a significant difference among the samples at 5% probability level.

These concentrations of copper as shown in table 4.2 were below the permissible level of copper in food (73.3mg/kg) (Yasaminet al., 2017). The result of our study was higher comparing with (Demirözüet al., 2011) (1.6-3.0mg/kg). Copper is present in various food. Copper has many important roles to play in maintaining a healthy body. The health benefits of copper relate to its anti-inflammatory actions to assist reducing the symptoms of arthritis. Copper is highly essential for normal growth and health. Thus, it is definitely important to include this element in regular diet. It is helpful in protecting skeletal, nervous and cardiovascular systems. Copper is either an element or a cofactor of as many as 50 different enzymes that take part in various biological reactions in the body. Also, it helps in producing of red blood cells, hemoglobin, and bone.

The Awara samples from Fudma gate had the lowest nickel content (0.12mg/kg) while Awara from Wednesday market had the highest nickel content (0.49mg/kg). The nickel content of Awara samples obtained from Hayingada, Darawa and Isa kaita junction were (0.16mg/kg), (0.15mg/kg), (0.39mg/kg) respectively. There was a significant difference among the samples at 5% probability level. The World Health Organization recommends 0.1– 0.3mg/kg of nickel for daily intake as described by (Yasaminet al., 2017). The highest nickel content was observed in Awara sample from Wednesday market which was below the WHO/FAO (2009) permissible limit of nickel (67.9mg/kg).

4.3. Microbial counts of the Awara samples

The microbial count of Awara samples from five different locations in Dutsin-ma as shown in table 4.3revealed that the total bacterial aerobic count (TBAC) of Awaraobtained from Darawa, Fudma gate, Wednesday market, Isa kaita junction, were 5.2 × 102, 8.6 × 102, 9.9 × 102, 8.1 × 102, 5.8 × 102 respectively. The yeast and moulds count (YMC) of Awara samples obtained were 4.9 × 102, 6.1 × 102, 5.4 × 102, 6.1 × 102 and 4.6 × 102 respectively.

From the result obtained in these analyses, Wednesday market (Commercial Area) had the highest bacterial count. Since the processing of these foods normally involve a form of heat treatment, it is obvious that considerable number of Bacteria associated with raw materials would have been killed. The reason for high microbial load might be linked to presence of heat resistance and post handling contamination (Zumbes et al., 2014). Microbial guideline for cook food stipulated that "the plate count must be less than 107cfu/g, for meat, less than 1.0 x 104cfu/g for plant products, less than 10 x 105cfu/g for ready to eat frozen meals, less than 1.0 x 104cfu/g and less than >105 for ready to eat meal (Gilbert et al., 2000). This agrees with the fact that immense microbial contamination of food is linked to poor post processing handling practices. Therefore, the microbial load on the foods is an indication of poor sanitary conditions during preparation storage and personal hygiene of the food by handlers (Zumbes et al., 2014).

Yeast count was indicated to be high in Fudma gate (Commercial Area) and low in Hayingada (Dorm area) which is due to direct exposure to air. Suleet al (2015), reported that, dusty, unhygienic environment in addition to poor handling by vendors are factors contributing to the high,, microbial load.

Table 3 Microbial counts of the Awara samples

Samples	Total bacterial aerobic count	Fungi
A	5.2 × 10	4.9×10^2
B	8.6×10^2	6.1×10^2
C	9.9×10^2	5.4×10^2
D	8.1×10^2	6.1×10^2
E	5.8×10^2	4.6×10^2

4.4. Morphological Characteristics and Biochemical Characteristics of the Bacterial Isolates

The cultural, morphological, gram reaction and biochemical characterization of the microbial isolates isolated from Awara samples analyzed in this study as shown in Table 4.4 indicated the presence of Staphylococcus aureus, Bacillus spp, pseudomonas aeruginosa, Escherichia coli and Salmonella species as the predominant contaminants. The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling as described by various researchers (Aboloma, 2008, Shamsuddeen and Ameh, 2008; Shamsuddeenet al., 2008).

The occurrence and percentage of individual bacteria isolated from Awara sold in Dutsin-ma local government area. Of the total of 10 samples examined, 16.67% were positive for Escherichia. coli. The occurrence of 16.67 % of Escherichiacoli recorded in this study is less than the 24.7% for ready-to-eat salad reported by (Udoet al., 2009) in Calabar Nigeria. Oyeyi et al., (2008) reported E coli incidence of 27.7% in street vended foods in Bayero University campuses, Kano, Nigeria.

The presence of E. coli is an indicator of faecal contamination which could be attributed to the method used in the preparation, unhygienic activities of the handlers. In a report by Bukar et al., (2009), on the investigation carried out on food handlers in three small scale food industries in Kano metropolis showed that 10.0% out of 50 food handlers carried Escherichia coli on their hands. This percentage could easily cross contaminate a whole production batch unnoticed.

Ironically good personal hygiene is not practice by most of the food handlers and they do not follow good manufacturing practices, which could minimize the presence of such organisms in foods (Bukaretal.,2009).

Table 4 Morphological Characteristics and Biochemical Characteristics of the Bacterial Isolates

Isolates ID	Gram staining	Catalase	Oxidase	Methyl Red	Indole	Urease	Citrate	Confirmed Bacteria
A	+ coccus	+	-	-	+	+	+	*Staphylococcus aureus*
B	- rods	+	-	-	-	-	+	*Pseudomonas aeruginosa*
C	-rods	+	-	+	+	-	-	*Escherichia coli*
D	+ bacilli	+	-	-	-	-	+	*Bacillus species*
E	+ rods	-	-	-	-	-	+	*salmonella species*

Keys Codes; A = Darawa; B = Fudma gate; C = Wednesday market; D = Isa kaita junction; E = Hayingada

Specie of pseudomonas spp organisms are widely distributed in water, soil and sewage. The presence of Salmonella and Shigella in Awara is an indicator of post-processing contamination which could cause typhoid fever and other food poisoning.

Staphylococcus aureus recorded the least number of positive samples with 8.33% out of the 10 samples examined. In Mexico, (Diaz-lopezet al., (2011) detected Staphylococcus aureus in (9.3%) street foods which is similar to the one examined in by this work. According to Guvenet al., (2010) meals prepared on the street provide a suitable culture medium for the emergence of Staphylococcus aureus.

The presence of Staphylococcus aureus in the samples is indicative of human contamination after production. This could be from direct human contact such as fingers or indirectly through additives or utensils. The organism is associated with endotoxin characterized by short incubation period (1-8 hours), violent nausea, vomiting and diarrhea (Maduekeet al., 2014).

4.5. Identification of fungi in Awara in Dutsin-Ma town

Table 5 Cultural morphological characteristics and identification of fungi

Samples	Macroscopic	Microscopic	Fungi Detected
A1	Pin like black growth	Non-Branched conidiophore with bulb end carries conidia like sun rays.	*Aspergillusniger*
A2	Flat, smooth, moist, glistening or dull, and cream to tannish cream in color.	Unicellular cocci or ovoid shape, larger than bacterial cells	*Saccharomyces cerevisiae*
B1	Flat, smooth, moist, glistening or dull, and cream to tannish cream in color.	Unicellular cocci or ovoid shape, larger than bacterial cells	*Saccharomyces cerevisiae*
B2	Pin like black growth.	Non-Branched conidiophore with bulb end carries conidia like sun rays.	*Aspergillusniger*
C1	Flat, smooth, moist, glistening or dull, and cream to tannish cream in color.	Unicellular cocci or ovoid shape, larger than bacterial cells	*Saccharomyces c Erevisiae*
C2	Pin like black growth.	Non-Branched conidiophore with bulb end carries conidia like sun rays.	*Aspergillusniger*

Keys Codes (sampling site); A = Darawa; B = Fudma gate; C = Wednesday market;D = Isa kaita junction

A total of two fungi species were isolated from the food (Awara) samples as shown in table 4.5, and they were identified as Aspergillus species and Sacchraromycescerevisiae. The presence of Aspergillus species and

Sacchraromycescerevisiae in the food sample is not surprising as they disperse in the form of spores which is abundant in the environment and can be introduce through dust and soil Maduekeet al., (2014). Their presence in these food samples is of serious public health concern as these fungi have all been implicated with the production of mycotoxin (Makunet al., 2009). Sacchraromycescerevisiaecan cause invasive infections (i.e., gets into the bloodstream or other normal sterile body fluid or into a deep site tissue such as lungs, liver or spleen) that can go systemic (involve multiple organs). Such conditions are life threatening (Murphy et al., 2005). The finding is in agreement with the work (Oranusi and Braide, (2012).

5. Conclusion

The results of this study revealed high bacterial loads in the Awara sold in Dustin-ma. Hence most are not fit for consumption since they have been contaminated by both pathogenic and spoilage microorganisms. The presence of the organisms and toxic chemicals thus renders the quality of the foods examined inadequate. Relevant regulatory authorities should educate food handlers on good personal hygiene and good manufacturing practices, which are possible ways of minimizing the likelihood of the food serving as routes for food-borne diseases or illnesses.

Appropriate handling and hygienic practices should be ensured by food vendors engaged in the production of ready-to- eat food. Furthermore, food handlers should be educated on appropriate storage temperature for cooked foods. This kind of study should also be conducted in other areas of Dustin-Ma metropolises so as to provide a comprehensive data for the local government public health section. Ready to eat foods should not be exposed to environmental contamination such as exhaust fumes and dust.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest/ Competing Interests in the publication of the manuscript or with any institution or product that is mentioned in the manuscript and/or is important to the outcome of the study presented.

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