Research Article

Microbiological quality of ready – to- eat soya bean curd (*Awara*) sold in Wudil town, Kano-Nigeria

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Abstract

Objective: This research was carried out to determined microbial counts and microbial profile of Soya beans curd (*Awara*) sold in the study area. **Material and methods:** total of fifty (50) samples of *Awara* were purchased from different locations of Wudil town within the period of eight weeks, in which ten (10) samples were purchased for the first week up to week eight with the interval of two weeks between each samples collections. The samples were blended, serially diluted and subjected to microbiological analyses using; aerobic plate counts, staphylococcal counts, the most probable number techniques for the detection of coliform, yeast counts, and mold counts. Discrete colonies were further subjected to Gram's staining and standard biochemical test for identification. **Results:** The results of aerobic mesophilic bacterial counts showed that the samples contained mean counts ranging from 9.48×10^3 to 5.07×10^5cfu/ml. The result of *staphylococcal* counts showed that the samples contained mean counts ranging from 1.7×10^3 to 1.57×10^4cfu/ml. The result of yeast counts showed that the samples contained mean counts ranging from $1.13\times10^2$to 6.64×10^2cfu/ml. The result of mold counts shows that the samples contained mean counts ranging from 3.54×10^2 to 1.25×10^3cfu/ml. The result of coliform counts using the most probable number shows that the samples contained mean coliform per g within the range of 4.36 to 35.92MPN/g. The overall results of this study indicated that, all the samples had mean aerobic mesophilic bacterial counts within the standard limits set by FAO, (2020), that is 10^5 cfu/g, also all the samples had total mean yeast and mold counts within the acceptable limits set by AOAC (2020) which is 10^4cfu/g. Biochemical characterization of the bacterial isolates obtained from the study revealed the presence of *Staphylococcus aureus* (26.5%), *Escherichia coli* (17.1%), *Salmonella* sp (16.2%), *Shigella* sp (9.4%), *Pseudomonas* sp (17.1%) and *Vibrio* sp (10.3%). Morphological characteristics, microscopy and fermentation of carbon source by the yeast isolates obtained from the study revealed the presence of *C. albicans* (32.8%), *C. cerevisiae* (39.7%) and *D. hansennii* (26.7%). Morphological characteristics and microscopy of mold isolates from the study also revealed the presence of *Aspergillus flavus* (21.8%), *Aspergillus fumigatus* (19.0%), *Aspergilus niger* (23.2%), *Mucor* sp (19.7%) and *Rhizopus* sp (16.2%). **Conclusion:** The presence of a host pathogenic bacteria and fungi (mold and yeast) in *Awara* samples examined poses a serious threat to consumers that can lead to the out break of infections. This calls for the need to regulate the activities of *Awara* sellers in Wudil town coupled with campaign for improved personal hygiene of the sellers so as to safe guard the healthy condition of general public in Wudil town Kano State.

Keywords: Microbiological, quality, counts, soya bean curd, Wudil town

Introduction

Awara is a Soybean product (also known as Soybean curd). It is soft-cheese-like food produced by curling fresh hot soy milk with either a salt or an acid (Egbo, 2012). *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. *Awara* was developed some 2000 years ago and has become the world's most popular soy food product due to its high protein content (Egbo, 2012).

Soybean 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 to 42% with lysine constituting a substantial proportion

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(Egbo and Seidu, 2012). It is also rich in carbohydrate (27.1%) and oil (20.6%) as reported by Osundahusi et al., 2007. Soy bean contain omega 3 fatty acid, devoid of cholesterol and easily digestible if properly processed. It contains phytoestrogens like isoflavones, genistein that have been reported in the prevention of cancer (Beatrice et al., 2012).

In Nigeria, *Awara* has been regarded as a cheap source of protein that is readily available and affordable for common man as compared to animal food product (Beatrice, 2012). This product due to it nutrients and high moisture content makes it suitable for the growth of microorganisms, especially if there is no good manufacturing practice and proper storage that would increase its shelf-life. *Awara* has been used as meat and cheese substitute in rural and urban areas of South West Nigeria. Local processing of soy-cheese is usually done at the home level and usually has no good processing methods (Beatrice, 2012).

Materials and methods

Site of Sampling

Wudil is a Local Government Area in Kano State, Nigeria. It's head quarter is in the town of Wudil on 237 highways. It is located at latitude 11.817°N and longititude 8.850°E at an altitude of 403 meters above the sea level. It has an area of 362km^2 and a population of 185, 189 at the 2006 census. The postal code of the area is 713101 (NIPOST, 2009). The area has an annual temperature of 850°C to 870°C with minimum and maximum temperature of 26°C to 36°C. The relative humidity of the region is always low and ranges between 40-51% (Olafin, 2008).

Collection of Samples

Awara samples were collected by method adopted by Thais et al. (2017), in which ten (10) *Awara* samples were collected aseptically in a sterile container from ten different locations in Wudil town for eight (8) weeks at an interval of 2 weeks making a total of 50 samples. Each of the samples collected was immediately labeled, placed in a sterile container and brought to the Laboratory of the Department of Food Science and Technology, Kano University of Science and Technology (KUST) Wudil, for the determination of organoleptic and nutritional composition. Some the samples were taken to the Microbiology laboratory KUST, Wudil for the microbiological analysis.

Sample preparation

This was carried out according to the method described by Food and Agricultural Organization (FAO, 2020). Twenty five grams (25g) of each sample was aseptically collected and placed in a sterile blender to which 225ml of buffered peptone water was added and homogenized gently by shaking. Serial dilution was carried out according to the procedure described by Madigan et al. (2012).

Analysis of bacterial viable cell counts

Bacterial count (aerobic plate count) was achieved by using pour plate method by pippetting 1ml of serially diluted sample into a sterile petridishes, this was followed by the addition of molten Nutrient Agar and mixing well by gently swirling the plates on the bench top. The molten agar was allowed to solidify and the plates were then incubated at 37°C for 24 hours. Colonies on the plate were counted and recorded (Madigan et al., 2012).

For yeast and mold count, the malt extract agar and potato dextrose agar were inoculated separately and incubated at room temperature for 3 to 5 days, the colonies formed were counted and recorded as Colony Forming Unit per milliliter (cfu/ml) (Nwachukwu, 2001).

Isolation and identification of bacterial isolates

Bacterial isolates were identified by their morphology on culture followed by biochemical characterization as described by Cheesbrough, (2006).

Gram's staining of bacterial isolates

Gram's staining was carried out as described by Cheesbrough, (2006). Thin smear of 200mm diameter was made on grease free slide which was fixed over a burning flame. A crystal violet solution was applied to cover the smear for 30 seconds and washed with distilled water. Secondly lugol`s iodine was applied to the surface for good 30 seconds and washed again. Acetone was used to decolorize the stain and lastly, the safranein solution was applied on the surface for one minute, which was washed and the slide was allowed to dry at room temperature. The stain was observed under microscope after oil immersion has been applied onto it. Consequently, red stain indicates Gram-negative and purple stain indicates Gram- positive bacteria (Cheesbrough, 2006).

Isolation of *salmonella* species

The homogenate was cultured onto MacConkey and *Salmonella-Shigella* Agar followed by incubation at 37°C for 24hours (Cloak et al., 1999), the colonies formed were Gram stained and tested for motility followed by biochemical tests (Cheesbrough, 2006).

Isolation of *staphylococcus aureus*

Plates of Mannitol Salt Agar were inoculated with the homogenate and incubated at 35°C for 24 hours in which *S. aureus* produced yellow zone surrounding their growth (Cappucino and Sherman, 2002), the organism was confirmed biochemically by catalase and coagulase tests (Cheesbrough, 2006).

Isolation of *vibrio* spp

This was achieved by culturing the homogenate in an Alkaline Peptone Water for 24 hours followed by streaking on

Thiosulphate Citrate Bile Salt Agar and incubated at 37°C for 24 hours. Yellow colonies indicating sucrose fermentation coupled with motility were exhibited by *vibrio* species, the organism was confirmed by biochemical tests (Cheesbrough, 2006).

Isolation of *shigella* species

The homogenate was cultured onto MacConkey and *Salmonella-Shigella* agar followed by incubation at 37°C for 24 hours (Cloak et al., 1999), the colonies formed were Gram stained and tested for motility followed by biochemical tests (Cheesbrough, 2006).

Coliform counts by using most probable number (MPN)

Coliform counts were carried out as described in http://www.fda.gov. Three (3) set of three test tubes were taken and lactose broth was used as liquid media for the test. Nine (9) ml of the lactose broth was placed into each of the test tube. Then, Durham's tubes were inserted in inverted position in each test tube of all set. All the test tubes were cotton plugged and sterilized using autoclave for 15 minutes at 121°C, after cooling Iml of 10^{-1} of diluted sample was placed in the 1st set, this was followed by placing 1ml of 10^{-2} of serially diluted sample into 2nd set of test tubes and lastly, 1ml of 10^{-3} diluted sample was placed into the last set of test tubes.

All the test tubes were incubated at 35°C for 24hours, after incubation gas production was observed in the Durham's tubes. Tubes in which gas production was 10% or more were recorded as positive tubes and tubes in which gas production is less than 10% were recorded as doubtful, the doubtful tubes were incubated again for another 24hours, if the gas is still less than 10%, then the tubes are recorded as negative and discarded.

All the positive tubes obtained from presumptive test were confirmed by inoculating one loop full of the sample from each positive tube into a test tube containing *E. coli* media followed by incubation at 44.5°C for 24hours. All test tubes with gas production of 10% and above were recorded as positive others were negative. The negative tubes were reincubated for 48hours and examined again. The results obtained were used to calculate total coliform MPN by comparing with standard MPN table.

Isolation and Identification of Yeasts and Molds

For isolation of yeasts, pour plate techniques was used in which a portion of 10g of the sample was added into a flask containing 90ml of Ringar's solution, which was blended and mixed carefully. A portion of 1ml of the homogenate was transferred into a test tube containing the 9ml of the diluents, it was mixed carefully with a fresh pipette by aspirating 10 times then from the second dilution with the same pipette another 1ml was transferred into the next tube containing 9ml of the sterile diluents, it was then mixed with a fresh pipette and repeated with the same steps using 3rd, 4th and 5th tubes of the diluent. A portion of 1ml of the sample diluted was pipetted from the 3rd, 4th and 5th tube into each of the appropriately marked sterile petridishes. A portion of 15ml of Malt Extract agar (kept at 44 to 46°C in water bath) was poured into each of the petridish, the diluted sample and agar medium were mixed thoroughly and uniformly by swirling gently 5 times clockwise and 5 times anticlockwise and five times back and forth on the level bench surface, this was allowed to set and solidify. The prepared petridishes was incubated in inverted form at 32-37°C (in the cupboard) for 3 to 5 days and the result was recorded as CFU/g (Shehu, 2018).

Biochemical tests for the identification of yeast

The biochemical test for identification of yeast isolates was carried out by the modified method of Olutiola et al. (2000). Isolates were sub-cultured on malt extract agar to check for purity and incubated at 28°C for 48hours. The purified culture was routinely maintained on MEA slant and kept at 4°C. The strain was stained using methylene blue and viewed under a high power microscope. Color, texture and other features were observed on the colonies. Biochemical tests of the isolates were carried out by means of fermentation of different carbon sources. The identities of the isolates were confirmed by comparing the characteristics with those of known texa using the schemes of Rhode and Hartmann, (1980) and Ellis et al. (2007).

Carbohydrate fermentation test for the identification of yeast

The carbon source, Sodium chloride, and Phenol red were weighted and dissolved in 100 ml distilled water and transferred into conical flasks. 0.5% to 1% of the desired carbohydrate was added into all flasks. Durham tubes were inserted in inverted position into all tubes; the Durham tubes were fully filled with broth and sterilized in an autoclave at 115°C for 15 minutes. The sugar was transferred into fermentation tubes and labeled properly (Tille, 2014). Each tube was inoculated with 1 drop of an 18 hours or 24-hours cultural broth in an aseptic condition (uninoculated tubes were kept as control tubes) and the tubes were incubated for 18-24 hours at 37°C. The tubes were examined for acid and gas production (Tille, 2014).

If the medium changed to yellow color indicates that, the organism ferments the given carbohydrate and produces organic acids thereby reducing the pH of the medium into acidic condition. When the medium changed to yellow color with production of gas in the Durham tube indicated that, the organism ferments the given carbohydrate and produces organic acids and gas. Gas production was detected by the presence of small bubbles in the inverted Durham tubes. But when there is no change in color (retains red color), the organism cannot

utilize the carbohydrate but the organism continues to grow in the medium using other energy sources in the medium (Tille, 2014).

Germ tube test for the identification of *Candida* species

Germ tube test was carried out as described in https://microbeonline.com. In which aliquot 0.5 ml (12 drops) of serum was placed in a test tube. A light suspension of the suspected yeast colonies was made (by touching 1-2 large colonies or 3-4 smaller colonies with a sterile wooden applicator stick or loop) on serum. The tube was incubated for 2-3 hours at 35– 37°C in an incubator. A drop of the suspension was placed on a slide using a Pasteur pipette and covered with a cover slip followed by examining the wet mount microscopically (at 40X) for production of germ tubes (long tube-like projections extending out from the yeast cells).

For positive result a short hyphal (filamentous) extension arising laterally from a yeast cell with no constriction at the point of origin was observed and was confirmed as *C. albicans.* For negative result there is no hyphal extension arising from a yeast cell or a short hyphal extension with constriction at the point of origin.

Isolation and identification of molds

The enumeration of molds colonies was carried out by pour plate method described by Shehu, 2018. A serially diluted *Awara* sample was inoculated onto Potato Dextrose Agar containing 250mg/l chloramphenicol antibacterial agent in a petridishes, the plates were incubated at room temperature for 3 to 5 days and colonies formed were observed and counted.

Wet mount microscopy of the mold isolates

A portion of 72 hours culture from the plates was picked and placed on a clean glass slide, a few drops of lactophenol were added and heated to steam while gently teasing the culture then a few drops of lactophenol cotton blue stain were added and the set up was heated to steam for few seconds and removed from heat. The glass slide was covered with a cover slip and the edge of the cover slip was gently pressed and finally it was observed under the microscope (Shehu, 2018).

Biochemical tests

Biochemical tests for the identification of the isolates was carried out to confirm the identity of the organisms obtained base on standard protocols described by Cheesbrough, 2006. These tests are; indole, methyl red, citrate utilization, oxidase, catalase and coagulase tests.

Results

The results of aerobic mesophilic bacterial count of the samples examined showed that location B had the highest average count of 1.9×10^6cfu/g while location E had the least average count of 1.0×10^4cfu/g and the results for staphylococcal count showed that location I had the highest average staphylococcal count of 7.2×10^4cfu/g and location C and D had the least count (Table 1).

Table 1. Total Mean Counts of Aerobic Mesophilic Bacteria and *Staphylococcus* Obtained from *Awara* Samples during the Study Period

SL		Cfu/g in week					
		0	2	4	6	8	MC
A	BC	1.3×10^4	1.5×10^4	3.6×10^4	2.8×10^4	3.2×10^4	2.48×10^4
	SC	1.4×10^3	1.0×10^3	1.2×10^3	-	3.2×10^3	1.7×10^3
B	BC	1.9×10^6	1.3×10^4	4.2×10^5	3.0×10^4	1.7×10^5	5.07×10^5
	SC	1.5×10^3	1.0×10^2	1.0×10^2	1.7×10^2	1.2×10^4	3.07×10^3
C	BC	1.4×10^5	2.3×10^4	2.6×10^4	1.8×10^4	8.0×10^3	4.3×10^4
	SC	-	2.3×10^3	1.7×10^3	1.6×10^3	3.2×10^3	2.2×10^3
D	BC	3.3×10^4	2.8×10^4	3.5×10^4	2.3×10^4	7.5×10^3	2.53×10^4
	SC	4.2×10^3	2.7×10^3	3.0×10^3	1.23×10^3	1.92×10^3	2.61×10^3
E	BC	1.0×10^4	2.2×10^3	1.6×10^4	7.2×10^3	1.2×10^4	9.48×10^3
	SC	3.6×10^3	3.01×10^3	1.01×10^3	5.0×10^3	1.34×10^3	2.79×10^3
F	BC	2.9×10^4	3.2×10^4	2.6×10^4	6.2×10^3	8.7×10^3	2.03×10^4
	SC	2.3×10^3	2.7×10^3	2.12×10^3	3.0×10^3	1.92×10^3	1.87×10^3
G	BC	2.4×10^4	3.1×10^4	3.4×10^4	2.7×10^4	9.2×10^3	2.5×10^4
	SC	1.6×10^3	2.1×10^3	1.9×10^3	1.01×10^3	3.02×10^3	1.93×10^3
H	BC	3.6×10^4	3.1×10^4	2.8×10^4	6.4×10^3	5.1×10^3	1.13×10^4
	SC	3.2×10^3	2.9×10^3	4.02×10^3	7.03×10^3	6.71×10^3	4.77×10^3
I	BC	1.0×10^5	4.4×10^4	3.3×10^3	2.9×10^4	3.1×10^3	3.59×10^4
	SC	7.2×10^4	1.7×10^4	9.6×10^3	8.4×10^3	9.1×10^3	2.32×10^4
J	BC	5.0×10^4	3.3×10^5	4.2×10^4	1.4×10^5	4.7×10^4	1.21×10^5
	SC	1.23×10^3	4.5×10^3	4.9×10^3	6.0×10^4	7.8×10^3	1.57×10^4

Key: S/N= Serial Number, SL= Sample Location, A= Wudil town cattle market, B= Wudil town main market, C= entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya, J= Quarters Wudil, SL= Sample Location, BC= Bacterial Counts, SC= *Staphylococcal* Counts and MC= mean counts.

Analysis of the samples for yeast and mold counts showed that location G had the highest yeast count of 4.0×10^2cfu/g while location F had the least count of 0 and location A had the highest average mold count of 2.7×10^3cfu/g while location D had the average mold count of 1.9×10^2cfu/g (Table 2). The total mean counts of aerobic mesophilic bacteria, *staphylococci*, yeast and mold counts were shown in table 3.

Analysis of the total coliform count using most probable number showed that location H had the highest mean coliform count of 36MPN/cfu/g while location j had the least mean coliform count of 4.36MPN/cfu/g (Table 4).

Table 5 shows the microscopic, cultural and biochemical characteristics for the identification of the bacterial species

Table 2. Total Mean Counts of Molds and Yeast for *Awara* Samples during the Study Period

SL		Cfu/g in week					
		0	2	4	6	8	MEC
A	MC	1.2×10^2	1.5×10^2	3.2×10^2	2.7×10^3	3.0×10^3	1.25×10^3
	YC	1.0×10^2	1.4×10^2	1.1×10^2	1.6×10^2	1.4×10^2	1.3×10^2
B	MC	4.6×10^2	5.7×10^2	3.2×10^2	7.2×10^2	2.8×10^3	9.74×10^2
	YC	1.4×10^2	2.1×10^2	1.0×10^2	2.0×10^2	2.0×10^2	1.7×10^2
C	MC	7.5×10^2	6.3×10^2	7.2×10^2	8.2×10^2	6.8×10^2	7.2×10^2
	YC	-	1.0×10^2	3.0×10^2	1.2×10^2	1.6×10^2	1.7×10^2
D	MC	5.2×10^2	6.0×10^2	3.4×10^2	1.2×10^2	1.9×10^2	3.54×10^2
	YC	1.2×10^2	1.6×10^2	-	-	1.0×10^2	1.27×10^2
E	MC	7.2×10^2	1.2×10^2	4.6×10^2	5.1×10^2	6.4×10^2	4.9×10^2
	YC	1.0×10^2	1.4×10^2	1.7×10^2	2.0×10^2	3.0×10^2	1.82×10^2
F	MC	6.2×10^2	4.8×10^2	7.2×10^2	5.7×10^2	4.0×10^2	5.58×10^2
	YC	1.3×10^2	-	-	-	-	1.3×10^2
G	MC	6.2×10^2	7.5×10^2	4.7×10^2	8.6×10^2	6.2×10^2	6.64×10^2
	YC	3.0×10^2	1.5×10^2	-	-	6.2×10^2	6.64×10^2
H	MC	4.3×10^2	6.7×10^2	4.1×10^2	8.9×10^2	6.1×10^2	6.02×10^2
	YC	1.02×10^2	1.5×10^2	3.0×10^2	-	-	1.84×10^2
I	MC	3.7×10^2	4.9×10^2	5.3×10^2	6.2×10^2	7.4×10^2	5.5×10^2
	YC	1.0×10^2	7.6×10^2	1.6×10^2	2.1×10^2	-	1.53×10^2
J	MC	8.9×10^2	7.6×10^2	5.1×10^2	7.3×10^2	6.8×10^2	7.14×10^2
	YC	1.1×10^2	1.3×10^2	1.0×10^2	-	-	1.13×10^2

Key: S/N= Serial Number, SL= Sample Location, A= Wudil town cattle market, B= Wudil town main market, C= entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya, J= Quarters Wudil, SL= Sample Location, MC = Mold Counts, YC = Yeast Counts and MEC = Mean counts

Table 3. Total Mean Counts of Bacteria, Staphylococcus, Mold and Yeast during the study period

Sites	Mean counts of the organisms			
	BC	SC	MC	YC
A	$2.48\times10^4\pm4.60\times10^3$a	$1.7\times10^3\pm5.06\times10^2$a	$1.25\times10^3\pm6.52\times10^2$a	$1.3\times10^2\pm1.0\times10^1$a
B	$5.07\times10^5\pm5.59\times10^3$b	$3.07\times10^3\pm2.25\times10^2$a	$9.74\times10^2\pm4.61\times10^2$a	$1.7\times10^2\pm2.1\times10^1$a
C	$4.3\times10^4\pm4.44\times10^3$a	$2.2\times10^3\pm3.67\times10^2$a	$7.2\times10^2\pm3.20\times10^1$a	$1.7\times10^2\pm4.5\times10^1$a
D	$2.53\times10^4\pm9.13\times10^2$a	$1.27\times10^2\pm5.03\times10^2$a	$3.54\times10^2\pm9.2\times10^1$a	$1.27\times10^2\pm1.7\times10^1$a
E	$9.48\times10^3\pm3.16\times10^2$a	$2.79\times10^3\pm7.4\times10^2$a	$4.9\times10^2\pm1.0\times10^2$a	$1.82\times10^2\pm3.3\times10^1$a
F	$2.03\times10^4\pm3.77\times10^2$a	$1.87\times10^3\pm4.12\times10^2$a	$5.58\times10^2\pm5.5\times10^1$a	$1.3\times10^2\pm1.5\times10^1$a
G	$2.5\times10^4\pm3.11\times10^2$a	$1.93\times10^3\pm3.29\times10^2$a	$6.64\times10^2\pm6.6\times10^1$a	$6.64\times10^2\pm6.7\times10^1$a
H	$1.13\times10^4\pm4.79\times10^2$a	$4.77\times10^3\pm8.77\times10^2$a	$6.02\times10^2\pm8.7\times10^1$a	$1.84\times10^2\pm5.9\times10^1$a
I	$3.59\times10^4\pm7.83\times10^3$a	$2.32\times10^4\pm1.23\times10^4$b	$5.5\times10^2\pm6.2\times10^1$a	$1.53\times10^2\pm2.2\times10^1$a
J	$1.21\times10^5\pm5.14\times10^3$a	$1.57\times10^4\pm1.11\times10^4$a	$7.14\times10^2\pm6.1\times10^1$	$1.13\times10^2\pm8.82$a

Values are in Mean±SEM. Values with same coefficient within the same column are considered not significantly fifference ($p\geq0.05$); Key: S/N= Serial Number, SL= Sample Location, A= Wudil town cattle market, B= Wudil town main market, C= entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya, J= Quarters Wudil, SL= Sample Location, MC = Mold Counts, YC = Yeast Counts, MEC = Mean counts and SEM= standard error of mean.

Table 4. Coliform Counts of *Awara* Sample during the Study Period

SL	MPN/cfu/g in week					
	0	**2**	**4**	**6**	**8**	**Mean**
A	23	15	3.6	3.6	3.6	9.76
B	150	15	3.6	3.6	7.4	35.92
C	3.6	3.6	15	3.6	15	8.16
D	3.6	3.6	3.6	15	3.6	5.88
E	43	21	3.6	93	3.6	32.82
F	23	3.6	3.6	3.6	28	10.2
G	150	28	28	3.6	93	30.52
H	21	3.6	150	3.6	3.6	36.36
I	3.6	3.6	3.6	3.6	15	5.88
J	3.6	3.6	3.6	3.6	7.4	4.36

Key: S/N= Serial Number, SL= Sample Location, A= Wudil town cattle market, B= Wudil town main market, C= Entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya, J= Quarters Wudil and SL = Sample Location

Table 5. Microscopic, Cultural and Biochemical Characteristics of Bacteria Isolated from *Awara* at Wudil Town- Kano Nigeria, during the study period

Isolates Cultural Characteristics	**Biochemical Characteristics**								
	GR	**CO**	**CA**	**OX**	**UR**	**IN**	**VP**	**MR**	**Isolates**
1. Light yellow, entire elevated colonies 0.1mm elevated colonies 0.1mm	+	+	+	+	-	-	-	+	*S. aureus*
2. Smooth pink circular colonies that ferment lactose.	-	-	-	+	+	+	-	-	*E. coli*
3. Small greenish raised smooth circular colonies	-	+	+	+	-	-	-	-	*Pseudomonas* Sp
4. Colourless colonies to opaque within the surrounding medium pink.	-	-	-	+	-	-	+	-	*Salmonella* sp
5. Non spore forming, rod shape bacteria.	-	+	+	-	-	-	-	+	*Shigella* sp
6. Yellow or Green sp Colonies on TCBS	-			+					*Vibrio* sp

Key: GR = Gram reaction, CO= coagulase, CA = Catalase, OX= Oxidase, UR= Urease, IN =Indole VP= Voges preskaur, MR= Methyl red and S. Oraginism= suspected organism.

Table 6. Percentage occurrence of bacteria isolated in the study area during the study period

Sites	**Isolates**			**Total**			
	Staph. aureaus	***E. coli***	***Pseudomonas* sp**	***Salmonella* sp**	***Shigella* sp**	***Vibrio* sp**	
A	4(33.33)	3(25)	3(25)	1(8.33)	Nil	1(8.33)	12(100)
B	3(23.08)	3(23.08)	3(23.08)	1(7.69)	2(15.38)	1(7.69)	13(100)
C	4(36.36)	1(7.69)	2(15.38)	4(36.36)	Nil	Nil	11(100)
D	4(30.77)	Nil	4(30.77)	1(7.69)	1(7.69)	3(23.08)	13(100)
E	3(27.27)	3(27.27)	1(9.09)	2(18.18)	2(18.18)	Nil	11(100)
F	3(27.27)	3(27.27)	2(18.18)	1(9.09)	Nil	2(18.18)	11(100)
G	3(21.43)	1(7.14)	1(7.14)	4(28.57)	4(28.57)	1(7.14)	14(100)
H	2(20)	2(20)	2(20)	2(20)	Nil	2(20)	10(100)
I	3(25)	3(25)	2(16.67)	2(16.67)	1(8.33)	1(8.33)	12(100)
J	3(25)	2(20)	3(30)	1(10)	1(10)	1(10)	10(100)

Key: *S. aureaus = Staphylococcal aureaus*, *E. coli= Escherichia* coli, A= Wudil town cattle market, B= Wudil town main market, C= entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya and J= Quarters Wudil.

Table 7. Cultural and Biochemical Characteristic of Yeast Isolated from *Awara* Samples in the Study Area

S/N	Cultural characteristics	Glu	Suc	Mal	Lac	Man	Cit	Gal	IO
1.	white creamy, flat, smooth and Ellipsoidal	+	+	+	-	+	+	+	*C. albicans*
2.	white creamy, flat, smooth, dull, Moist and produce ascospores	+	+	-	+	-	+	+	*S.cerevisiae*
3.	White creamy, round and oval	+	+	+	-	+	+	+	*D. hansenii*

Key: S/N = seria number, Glu = glucose, Suc = Sucrose, Mal = Maltose, Lac = lactose, Man = Mannitol, Cit = Citrate, Gal = Galactose, EO = Isolated Organism, + = positive, - = negative

Table 8. Percentage occurrence of the yeast isolated from *Awara* samples during the study period in the study area

Sites	Isolates			Total
	C. albicans	***S. cerevisiae***	***D. hansenii***	
A	4(4.44)	3(33.33)	2(22.22)	9(100)
B	1(14.29)	5(71.41)	1(14.29)	7(100)
C	1(16.67)	3(50)	2(33.33)	6(100)
D	3(60)	2(40)	Nil	5(100)
E	3(42.86)	2(28.57)	2(28.57)	7(100)
F	1(100)	Nil	Nil	1(100)
G	2(40)	1(20)	2(40)	5(100)
H	1(25)	Nil	3(75)	4(100)
I	2(25)	4(50)	2(25)	8(100)
J	5(50)	3(30)	2(20)	10(100)

Key: A= Wudil town cattle market, B= Wudil town main market, C= Entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya and J= Quarters Wudil.

Table 9. Colonial characteristics of mold isolated from Awara samples sold in the study area during the period of the study

S/N	Colonial appearance	Morphological characteristics	Isolate
1.	White to yellow	Septate hyphae	*Aspergillus flavus*
2.	Grey to green	Conidiophores borne laterally on hyphae	*Aspergillus fumigatus*
3.	Black	Conidia rise from the sterigmata and borne in chain	*Asprgillus niger*
4.	Initially white and later grey with black dot, smooth in appearance.	Hyphae–thick, reproductive structure- columella is present and bear the sporangia	*Mucor* sp
5.	Large colony, initially white and later turned brown and black.	Non septate hyphae and cottony mycelium produced clusters of root-like structure- rhizoid and stolon	*Rhizopus* sp

obtained from the samples examined. Table 6 shows the percentage occurrence of the bacterial species from the samples examined and indicated that *S. aureus* had the highest percentage of occurrence of 26.5% while *Shigella* sp had the least percentage of occurrences of 9.4%. Table 7 shows the Cultural and biochemical characteristics of the yeast isolated from the samples.

Percentage occurrence of yeast species from the sample examined were shown in table 4.8 and indicated that *C. albicans* had the highest percentage occurrence of 32.8% while *D. hansenii* had the least percentage of occurrence of 27.6%.

Colonial characteristics of mold isolates were shown in table 9. Percentage occurrence of mold species from the

Table 10. Percentage occurrence of molds isolated from *Awara* samples during the study period

Sites	Isolates					Total
	Aspergillus Flavus	***A. fumigatus***	***A. niger***	***Mucor* sp**	***Rhizopus* sp**	
A	4(23.53)	4(23.53)	2(11.76)	5(29.41)	2(11.76)	17(100)
B	3(20)	3(20)	3(20)	2(13.33)	4(26.67)	15(100)
C	3(21.43)	3(21.43)	4(28.57)	3(21.43)	1(7.14)	14(100)
D	4(28.57)	2(14.29)	3(21.43)	3(21.43)	2(14.29)	14(100)
E	2(14.29)	4(28.57)	3(21.43)	4(28.57)	1(7.14)	14(100)
F	3(21.43)	3(21.43)	4(28.57)	2(14.29)	2(14.29)	14(100)
G	5(33.33)	2(13.33)	2(13.33)	3(20)	3(20)	15(100)
H	3(23.08)	1(7.69)	4(30.77)	2(15.38)	3(23.08)	13(100)
I	1(9.09)	2(18.18)	4(36.36)	2(18.18)	2(18.18)	11(100)
J	3(20)	3(20)	4(26.67)	2(13.33)	3(20)	15(100)

Key: A= Wudil town cattle market, B= Wudil town main market, C= Entrance of Wudil General Hospital, D= Sabon Garin Wudil, E= Entrance of Wudil main station, F= Wudil main station, G= Kofar Yammma Wudil town, H= Hausawa Wudil town, I= Unguwar Danya and J= Quarters Wudil. percentage in parenthasis

samples examined were shown in the table 10 and indicated that *A. niger* had the highest percentage occurrence of 23.2% while *Rhizopus* sp had the least percentage occurrence of 16.2%.

Discussion

The overall results of this study indicated that, all the samples had mean aerobic mesophilic bacterial counts within the standard limits set by FAO, (2020), that is 10^5 cfu/g, also all the samples had total mean yeast and mold counts within the acceptable limits set by AOAC (2020) which is 10^4cfu/g. The total mean aerobic mesophilic counts in this study for the whole samples is lower than that of the study in Kano, Nigeria by Bukar et al., (2010), who obtained a mean aerobic mesophilic counts of 5.80×10^6 cfu/g in Soybean cake.

The Fungal (yeast and mold) and Staphylococcal counts obtained from this study were lower than the 9.44×10^5 cfu/g as detected by Derar and El-Zubeir, (2013) from camel milk cheese samples. Ready to eat samples were used in this study, whereas Derar and El-Zubeir, (2013) used samples stored in a whey for 21 days prior to examination.

The coliform counts of samples collected from site B, E, F, G and H were not within the acceptable limits of MPN as prescribed in accordance with the provisions of article 17 of the act Governing Food Safety and Sanitation. Nevertheless, the results of MPN obtained from this research were lower than 1275 coliform/g as detected by Yusha'u et al. (2017).

The reasons for these microbial loads might be attributed to the presence of heat resistance organisms and post handling contamination (Zumbes et al., 2014). This agrees with the pact that immense microbial contamination of *Awara* was linked to poor post processing handling practices. Therefore, the microbial load on the *Awara* is an index of poor sanitary conditions during preparation and personal hygiene by handlers (Zumbes et al., 2014). The presence of coliform from all the studied sites can be as a result of unhygienic practices related to the faecal or sewage contamination through the use of contaminated water (Bukar et al., 2010).

Yeast counts was indicated to be high in site G and lower in F which is due to the direct exposure to air of sample G. Sule et al. (2015), reported that, dusty unhygienic environment coupled with poor handling by vendors are factors contributing to high microbial load. However, Mold counts were indicated to be high in site A and low in site D this may be attributed to humidify air concentration.

The percentage occurrence for *E. coli* in this study was 17.1%, Falegan, (2014), reported an occurrence of 25% (50 samples) for *E. coli* in local soybean cake in Ado Ekiti, Ekiti state.

The number of *Samonella* sp positive samples from this study accounted for 16.2% of the total number of bacteria isolated which is lower than that obtained by Falegan, (2014), where recorded the rate of occurrence of 60% for *Salmonella* sp.

S. aureous on the other hand was isolated with a frequency of 26.50% from the samples examined in this study which is lower than the frequency obtained by Idris and Dabo, (2016).

Shigella sp was isolated in this study with percentage of occurrence of 9.4% which is lower than 12.5% as obtained by Bristone et al. (2018).

The presence of *Salmonella* and *Shigella* sp in *Awara* is an indicator of post processing contamination which can cause thypoid fever and other food poisoning. *Staphylococcus aureous* is associated specifically, with hands and nasal cavity. The deposition of this microorganism in *Awara* can occur if good sanitary practices are not followed by the food handlers. *Staphylococcus aureous* is a pathogenic

microorganism which could result in the transmission of disease (Sule et al., 2015).

In this study mold and yeast species was isolated and the percentage obtained are; 21.8% for *A. flavus*, 19.0% for *A. fumigatus*, 23.2% for *A. niger*, 19.7% for *Mucor* sp and 16.2% for *Rhizopus* sp. For the yeast the percentage of occurrences are; 32.8% for *C. albican*, 39.7% for *S. cerevisiae* and 27.6% for *D. hansenii*. Bristone et al., 2018, recorded a low percentage of occurrences of 12.5% for *Rhizopus*, 12.5% for *C. albicans* and 37.5% for *S. cerevisiae*. The occurrence of these organisms in *Awara* may be due to the contamination dust, packaging materials, poor hygiene and sanitation of processing environment and thus, producing mycotoxin which can cause mycotoxicosis in human (Sule et al., 2015).

The results obtained from the current study revealed that there is significant difference in bacterial counts from samples collected in site B to samples collected from the other sites ($p<0.05$), there is significant difference in staphylococcal counts from the samples collected in site I to the samples collected from the other sites ($p<0.05$) and there is no significant difference in mold and yeast counts from sample A to J ($P\geq0.05$).

Conclusion

Conclusively, it was discovered that the results of bacterial counts for all the samples analyzed had counts within the range of satisfactory to marginal level ($<10^6$ cfu/g), Staphylococcal counts shows that most of the samples examined had counts within the marginal (10^2-10^3 cfu/g) level and only few are found to be at unsatisfactory level (10^3-10^4 cfu/g), the results of yeast and mold counts were found to be at satisfactory level ($<10^4$ cfu/g) and results of coliform counts for 50% of the samples analyzed were found to be at satisfactory level (<10 MPN/cfu/g) while the remaining 50% were found to be at unsatisfactory level (≥10 MPN/cfu/g).

It was observed that most of the samples analyzed were contaminated by *S. aureous*, *E. coli*, *Pseudomonas* sp, *Salmonella* sp, *Shigella* sp, *Vibrio* sp, *C. albicans*, *S. cerevisae*, *D. hansenii*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor* sp and *Rhizopus* sp.

Recommendation

The results of this study showed that the *Awara* samples examined were contaminated which render the food unsafe for human consumption. In the light of the results observed in this study, it is recommended that;

The sanitary officers in Wudil town should educate *Awara* handlers on good personal hygiene and good production practice so as to minimize the introduction of coliforms and other pathogens while selling the product.

The government should also regulate the activity of *Awara* sellers so as to check the *Awara* quality and safety within Wudil town.

Conflict of interest: I declared that, there is no conflict of interest.

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