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Microbial quality evaluation of awara (Soybean cheese) processed and sold at university of maiduguri campus

Abstract

Microbial quality evaluation of Awara sold within University of Maiduguri, Borno State was carried out. Results indicates that all the samples collected from the four cardinal point were highly contaminated, The South East (Commercial Area) which is the second cardinal point had the highest total aerobic bacteria count of $9.60x10^2$CFU/g and North West (Unimaid Quarters) which is the fourth cardinal point had the lowest count of $4.53x10^2$CFU/g. South East had the highest coliform count of $3.87x10^2$CFU/g while North West had the lowest coliform count of $157x10^2$CFU/g. Total yeast ranged from $3.33x10^2$ in South East to $1.07x10^2$CFU/g in South West (Acada Area). Total mould was high in South East with $2.67x10^2$CFU/g while North West had the lowest mould count of $1.00x10^2$CFU/g. Staphylococcus count was high in North East (Complex Area) which ranged from $3.57x10^2$ to $1.57x10^{2CFU/g}$ in North West (Unimaid Quarters). 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: microbial, quality evaluation, awara, consumption

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Abbreviations: NE, north east; SE, south east; SW, south west; NW, north west; CFU, colony forming unit

Introduction

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.[1]. 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.[1]

Soybean (Glycine max) has been proven suitable for the production of Awara.[2] It is a legume of an exceptionally high protein content ranging between 38% and 42% with lysine constituting a substantial proportion.[2] 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.[3] 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 Osundahunsi et al.,[4] & kolawole et al.[5] Soybeans contain Omega 3 fatty acids, devoid of cholesterol and easily digestible if properly processed. It contains phytoestrogens like isoflavones, genistein that have been reported in the prevention of cancer. Isoflavones are closely related to the antioxidant flavonoids.[6]

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 products.[6] This product due to its nutrient and high moisture content makes it suitable to the growth of microorganism, especially if there is no good manufacturing practices and proper storage that would increase its self-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.

It is important to provide current knowledge and possible future developments in food (Awara) safety. This study tends to investigate the microbiological quality of Awara sold within University of Maiduguri. Awara have been identified to have a limited shelf-life and so a potential health hazard to it consumers. Therefore, microbiological quality evaluation of Awara will be very important in order to ascertain its safety levels. Identifying different microorganism of Awara and its health implication on the consumers is imperative. This research seeks to ascertain the consequence of consuming foods been hawked within the University of Maiduguri campus.

Materials and methods

Study area

This study was conducted inside University of Maiduguri Campus, Borno State of Nigeria. Samples of Awara sold within the University of Maiduguri Campus were collected. The samples were collected from the four-cardinal point of the University of Maiduguri. Each of the three-samples collected from the cardinal point were replicated and analysed as described by Zumbes et.al.[7] All chemicals and reagents were obtained from the Department of Food Science and Technology, University of Maiduguri. Description of food.

The food used is called Awara (Plate 1). It is a ready to eat (RTE) food product which is also called tofu (soybean cheese or cake). It is light brown in colour with rectangular shape and normally in medium chewable sizes. It has a cheese like taste and soft in texture with egg flavour or aromas as describe by Fasoyiro.[8]

Samples and sampling

A total of twelve (12) samples were collected from University of Maiduguri, from four cardinal points (North East, South East, South West and North West). Three (3) samples from each of the four cardinal points were collected from Awara vendors /hawkers within the university. The samples were collected aseptically in a sterile container. They were labelled and immediately transported to the laboratory for the microbial analysis as describe by Jideani and Jideani[9] (Figure 1).

Figure 1 wara.

Sterilization of materials

All glass ware including conical flask, beakers, test tube, and bottles were washed thoroughly with detergents, rinsed with distilled water. They were dried and sterilized in analytical oven at temperature of 160°C for 1hour.[9] The media were also sterilized in autoclave at a temperature of 121°C for 15minutes. All materials were sterilized before being used.[9]

Preparation of culture media

Nutrient agar (NA): This microbial medium was prepared by using 28g of nutrient agar powder in 1000ml of distilled water in a clean flask. The mouth of the flask was plug with non-absorbent cotton wool and then wrapped with aluminium foil, extended up to the neck of the flask. The flask was agitated gently to mix well and was place on a bunsen burner: to boil and dissolve completely. It was sterilized by autoclaving at 121°C for 15minutes and allowed to cool to 45°C. It was aseptically dispensed into pretidish. These media were used for the total bacterial aerobic plate count.[10]

Peptone water (PW): Peptone water was prepared by dissolving 15g of the powder in 1litre of distilled water. It was mixed well and distributed into final bottle and sterilize by autoclaving for 15minutes at 121°C. Peptone water was use as growth medium.[10]

MacConkey agar (MA): MacConkey agar, bile salt, peptone and salt were dissolved in distilled water and agar-agar was added. It was autoclaved at 121°C for 15minutes. The final pH was adjusted to 7.5. Lactose was also added and mixed followed by neutral red in sufficient amount to give a redish-brown colour. It was dispensed in bottles and autoclaved at 121°C for 10minutes, then allowed to cool to 45°C before pouring into plates. This was done to prevent scum from appearing on the surface of the medium. The final mixture was reddish-brown in colour. This was done to determine Coliform count as described by Cheesbrough.[10]

Corn meal agar (GMA): This medium was used to determine yeast count. Corn meal agar was prepared by dissolving 17g of the powder in 1000ml of distilled water. It was heated gently to dissolve completely. One percent polysorbate was added and sterilized in autoclave at 12 I°C for 15minutes. It was allowed to cool at room temperature before pouring into Petri dishes containing the samples as describe by Cheesbrough.[10]

Potato dextrose agar (PDA): The medium was prepared by weighing 39g of potato dextrose agar powder in distilled water. It was heated to boil completely, and then sterilized by autoclaving at 121°C for 15minutes. This was used to determine mould count in the samples.[10]

Mannitol salt agar (MSA): The medium was used for isolation and enumeration of Staphylococcus aureus. For this preparation, 108g of MSA powder (Lab. M. Limited) was weighed and dissolved in 1000ml of distilled water. It was allowed to stay for 10minutes. After that, it was swirled, homogenised and sterilized by autoclaving at 121°C for 15minutes. Then, it was allowed to cool before being poured in to petri dishes.[10]

Serial dilution

Serial dilution was prepared by introducing 1gram of Awara with a sterile blade. A plastic rack was arranged with sterile test tubes containing 9ml of distilled water. A tenfold serial dilution was carried out by homogenizing 1gram of the sample into the test tube and was labelled as 10-l. It was mixed thoroughly and 1ml was taken again from the 10-1 dilution tube and transferred into the next test tube (labelled 10-2). Each test tube was shaken vigorously before each transfer as describe by Jideani.[9]

Plating of culture and incubation

Pour plate method was used for plating the samples. One millilitre from the dilution 10-2 was taken using sterile pipette and then it was introduced into sterile petri dish, these were done in triplicate for each one of the 12 samples of Awara. The prepared media were poured into the Petri dishes containing 1ml of diluted culture. The plates were swirled to mix properly. All plates were allowed to solidify on a bench before incubation.[9]. The plates were incubated at 35°C for 24hours .Similarly Yeast and mould were incubated at room temperature for 7days and were observed daily to allow them grow for easy identification.[11]

Viable cell count

Colony counting machine was used for counting the total bacterial aerobic plate count of the plates.[11]

Gram staining technique

Gram staining reaction has the wide application that is capable of distinguishing virtually all gram positive and gram-negative bacteria. Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was applied for 45second and washed with gentle running water. Lugol's iodine was added for 45seconds and was decolorized with acetone - alcohol and washed with clean water. The slides were counter stained with 30% safranin for 30second and washed. It was then air dried and examined at under oil immersion lens of the microscope used.[10]

Motility test

Motility was performed using agar with concentration of 0.2-0.5% (w/v) was inoculated with the test organism. A stab of each inoculum was made at the centre of each tube. The tube was incubated at 35°C for 24hours. The temperature was reduced for Pseudomonas. A

diffused growth at the place of inoculation is considered as positive and restricted growth is considered as negative.[10]

Catalase test

Catalase test used to determine whether or not a microorganism produces catalase enzyme. A loop full of the culture was placed on a clean grease free slide. The culture was emulsify with a loop full of freshly prepared 3% hydrogen peroxide (H_2O_2) on the slide and the reaction was observe immediately for catalase positive or negative organism.[10]

Coagulase test

Coagulase test is particularly employed to differentiate pathogenic Staphylococcus aureus from the non-pathogenic species. Coagulase test was done by slide method using culture from solid media. A clean grease free slide was divided into two using a grease pencil. A drop of normal saline (0.85%) was placed on each of the portions and 18-hour culture of the tests organism was emulsified in little quantity on each of the drops of normal saline until a uniform suspension was obtained. A drop of rabbit plasma was added to one of the suspensions and stirred for about 5seconds for the presence of Coagulase positive or negative organisms.[10]

Oxidase test

Oxidase test was carried out using petri dish method. A drop of 1% aqueous solution of the reagent was placed on the portion of the culture plate containing the test organism. The reaction was observed between 10seconds.[10]

Urease test

Urease test was carried out to determine if a microorganism produces the enzyme urease. Urease broth was prepared according to direction from the manufacturers and 5ml potion was dispensed into clean test tubes to obtain a slope of I inch built. It was sterilized at 121°C for 15minutes before the test tube was kept in a slanting position to set. The slope surface was inoculated by streaking with a loop full of the peptone water broth culture. It was incubated at 35°C for 24hours before the reaction was observed. Pink colour of the media indicated Urease positive.[10]

Indole test

Peptone water was prepared by adding 10g tryptone to 100ml distilled water. Exactly 0.5g of sodium was added to the solution and the pH was adjusted to final pH of 7.2. Then, 5ml was dispensed into sterile test tubes and were covered loosely and autoclaved at 121°C. It was allowed to cool to 30°C and was inoculated and incubated at 35°C for 48hours. After that, 0.5ml of kovac's reagent was added and was gently shaken. It was allowed to stand for 10minutes. The reaction was observed for red positive colour.[10]

Voges-proskauer test

Glucose-phosphate was prepared and sterilized as the growth medium and allowed to cool to at room temperature for 24hours. Then 1ml of 40% potassium hydroxide and 3 drops of alpha-napthol was added. The tube was shaken well and allowed to stand for 5minutes before observation.[10]

Citrate utilization test

This test was carried out using Simon's citrate agar method. The media was prepared and dispensed into clean test tubes. It was sterilized at 121oC for 15minutes and slopes of about 1 inch were made. The test tubes were kept in a slanted position to set and the surfaces of slopes were inoculated by streaking with a loop full of the peptone water broth culture. They were incubated for 48hours and observed for citrate utilization.[10]

Methyl red test

The glucose phosphate broth was prepared according to manufactures instructions. The medium was, dispense in 9ml amount into clean test tubes and was loosely tightened and sterilized at 121°C for 15minutes. It was allowed to cool to about 30°C then the test organism was inoculated in duplicates. It was incubated for 24hours and a few drops of methyl red reagent wad added into broth culture and observed.[10]

Identification of microbial isolate

Identification of the isolate were performed using classical methods based on their -morphological, physiological, and biochemical characteristics with reference to systematic Bacteriology manuals.[10]

Result and discussion

Microbial load of awara

The microbial count of Awara sampled from four cardinal points of University of Maiduguri is shown in Table 1. The total bacterial aerobic count (TBAC) of Awara from North East (NE), South East (SE), South West (SW) and North West (NW) of University of Maiduguri ranged from $6.27x10^2$ to $9.07x10^2$, $7.40x10^2$ to 9.60, $5.60x10^2$ to $6.67x10^2$ and $4.53x10^2$ to $5.00x10^2$CFU/g, respectively. Whereas Coliform count (CC) of Awara sampled from NE of University of Maiduguri ranged from $2.07x10^2$ to $3.20x10^2$CFU/g, SE ranged from $2.23x10^2$ to $3.83x10^2$CFU/g, SW ranged from $1.77x10^2$ to $2.40x10^2$CFU/g and NW ranged from $1.57x10^2$ to $3.20x10^2$CFU/g. The yeast count (YC) of Awara sampled from NE was between $1.10x10^2$ and $2.67x10^2$CFU/g, while yeast count of Awara from SE ranged from $1.20x10^2$ to $3.33x10^2$CFU/g, SW and NW had total yeast count of $1.07x10^2$ to $2.33x10^2$ and $1.07x10^2$ to $2.33x10^2$CFU/g, respectively. Staphylococcal count of Awara sampled from University of Maiduguri from the four cardinal points ranged from $2.00x10^2$ to $3.57x10^2$, $1.73x10^2$ to $2.33x10^2$, $1.80x10^2$ to $3.13x10^2$ and $1.57x10^2$ to $2.17x10^2$CFU/g for NE, SE, SW and NE, respectively. Awara sampled from NE of University of Maiduguri had mould count ranging from $1.07x10^2$ to $1.20x10^2$, whereas those from SE, SW and NW ranged from 1.23 to $2.67x10^2$, $1.07x10^2$ to $1.33x10^2$ and $1.00x10^2$ to $1.77x10^2$, respectively.

Table 1 Microbiological Count (CFU/g) of Awara obtained from the Four Cardinal Points of University of Maiduguri

Sample	TBAC	CC	YC	SC	MU
North east					
A1	$7.73x10^2$	$3.20x10^2$	$1.20x10^2$	$2.00x10^2$	$1.07x10^2$
A2	$9.07x10^2$	$2.27x10^2$	$1.10x10^2$	$3.33x10^2$	$1.10x10^2$
A3	$6.27x10^2$	$2.07x10^2$	$2.67x10^2$	$3.57x10^2$	$1.20x10^2$

Citation: Bristone C, Mshelia HA, Ogori FA, et al. Microbial quality evaluation of awara (Soybean cheese) processed and sold at university of maiduguri campus. *J Bacteriol Mycol Open Access*. 2018;6(1):30–34. DOI: 10.15406/jbmoa.2018.06.00172

Table Continued....

Sample	TBAC	CC	YC	SC	MU
South east					
B1	$7.40x10^2$	$3.83x10^2$	$2.00x10^2$	$2.33x10^2$	$2.67x10^2$
B2	$7.93x10^2$	$2.23x10^2$	$3.33x10^2$	$2.20x10^2$	$2.33x10^2$
B3	$9.60x10^2$	$2.40x10^2$	$1.^20x10^2$	$1.73x10^2$	$1·23x10^2$
South west					
C1	$5.60x10^2$	$1.77x10^2$	$1.07x10^2$	$1.80x10^2$	$1.07x10^2$
C2	$6.67x10^2$	$3.40x10^2$	$2.33x10^2$	$3.13x10^2$	$1.20x10^2$
C3	$5.87x10^2$	$2.40x10^2$	$2.33x10^2$	$2.40x10^2$	$1.33x10^2$
North west					
D1	$5.00x10^2$	$3.20x10^2$	$1.07x10^2$	$1.57x10^2$	Nil
D2	$4.97x10^2$	$1.67x10^2$	$1.27x10^2$	$2.00x10^2$	$1.00x10^2$
D3	$4.53x10^2$	$1.57x10^2$	$2.33x10^2$	$2.17x10^2$	$1.77x10^2$

Key: TBC, total bacteria aerobic count; CC, coliform count; YC, yeast count; SC, staphylococcal count; MC, mould count

A1 to D3 are replications from the respective cardinal points

From the result obtained in these analyses, South East (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 attributed to presence of heat resistance and post handling contamination.[7] 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 index of poor sanitary conditions during preparation storage and personal hygiene of the food by handlers.[7]

The presence of coliforms from South East (Commercial Area) is high compared to other location and can be as a result of unhygienic practices related to faecal or sewage contaminations through the use of contaminated water, equipment and hand carriage, while North West (Staff Quarters Area) had the lowest coliform count and these could be as a result of different handling practices, water source and environment.[12]

Yeast count was indicated to be high in South east (Commercial Area) and low in South west (Acada area) which is due to direct exposure to air. Sule et al.,[13] reported that, dusty, unhygienic environment coupled with poor handling by vendors are factors contributing to the high,, microbial lead. However, mould count was indicated to be high in South East and low in North West. This may be attributed to humidify air concentration.

Staphylococcus is associated specifically, with the 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 aureus is a pathogenic microorganism which could result in the transmission of diseases.[13]

Morphological and biochemical characteristics of microorganisms isolated from awara

Table 2 shows the morphological and biochemical characteristics of microorganisms isolated from Awara (Soybean Cheese). From the result obtained Staphylococcus aureus is predominantly found. Hence the presence of these organisms indicates contamination from food handlers. S. aureus is a pathogenic and it produces toxin, which causes staphylococcal food poisoning.[14]

Table 2 Morphological and biochemical characteristics of the microorganisms isolated from Awara from four cardinal points of University of Maiduguri campus

Morphological Characteristics	Biochemical Characteristics										Microorganism
	Gram reaction	Motility	Coagulase	Catalase	Oxidase	Urease	Indole	Voges-Proskauer	Citrate Utilization	Methyl red	
Smooth cream, opaque colonies with entire edge	+ Cocci	-	+	+	+	-	-	-	-	+	Staphylococcus aureus
Smooth, pink, circular colonies that ferment Lactose	- Rod	+	-	-	+	+	+	-	-	-	Escherichia coli
Large flat, spread colonies with smell	- Rod	-	+	+	+	-	-	-	+	-	Pseudomonas
Large grey white mucord colonies	- Rod	+	-	-	+	-	-	+	+	-	Salmonella
Pale colour non	- Rod	-	+	+	-	-	-	-	-	+	Shigella

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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. 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.[13–17]

Table 3 shows the percentage occurrence of microorganism isolate from Awara. The occurrence of Staphylococcus aureus in Awara was 37.5%, Salmonella spp 37.5%, *E. coli* 50%, Shigella 3.7%, Pseudomonas spp 25%, Candida albicas 12.5%, Saccharomyce cerevisae 37.5%, Rhizopus oryza12.5%. The occurrence of E. coli, shigella. In Awara was an indication of faecal and environmental contamination and a signal for the presence of other enteric pathogenic.[13] The occurrence of fungi in Awara may be due to contamination from air, dust, packaging material, poor hygiene and sanitation of the processing environment and thus producing mycotoxins which can cause mycotoxicosis in humans Sule et al.[13]

Table 3 The percentage frequency of occurrence of microorganisms isolated from Awara from four cardinal points of University of Maiduguri

Microorganism	University Of maiduguri four cardinal points				% Occurrence
	North East	South East	South West	North West	
Staphylococcus aureus	+	+	+	-	37.50%
Salmonella sp.	+	+	+	-	37.50%
Escherichia coli	+	+	+	+	50%
Shigella	+	-	+	+	37.50%
Pseudomonas sp.	+	+	-	-	25%
Candida albicas	+	-	-	-	12.50%
Saccharomyces cerevisae	+	+	+	-	37.50%
Rhizopus oryza	-	+	-	-	12.50%

Conclusion

From the result obtained in this study, most of the Awara (Soybean cheese) samples obtained from the four cardinal points are not fit for consumption since they have been contaminated by both pathogenic and spoilage microorganisms. It is important to keep cheese fit for consumption by taking adequate measures to prevent contamination during and after production of Awara.

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Conflict of interest

The author declares no conflict of interest.

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