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BACTERIOLOGICAL ASSESSMENT OF SOYA-BEAN CAKE (*AWARA*) SOLD IN FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE (BOSSO CAMPUS)

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ABSTRACT

Soy bean cake (*Awara*) is a ready to eat snack consumed by students on campus usually due to convenience or acclaimed nutritive value. The bacteriological assessment of a*wara* was carried out using pour plate technique. The highest mean bacterial count was $7.50x10^5$cfu/g and the lowest was $4.5x10^5$cfu/g. Bacteria isolated from samples were *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp, the predominant bacteria was *S. aureus*. The presence of these pathogens indicates contamination which can have a negative impact on public health. Therefore, there is need to sensitize students on the health hazard pose by the consumption of contaminated street vended snacks, educate the handlers on personal hygiene and environmental cleanliness.

Keywords: Bacteriological, Soya beans, Pathogen, Hygiene

INTRODUCTION

Food is complex mixture of substances required for the survival of an organism. It is an excellent source of nutrients and hence a good environment for the growth of microorganisms as they also require nutrients for their growth (Yusha'u *et al*., 2017). The main contributing factor to the wellbeing of an individual is the maintenance of a balanced diet of which protein, a body building nutrient is essential (Ahmad *et al*., 2020). Soy bean (*Glycine max*) is a legume of an exceptionally high protein content ranging between 38% and 42% (Oranusi *et al*., 2013). They are considered as a good source of protein having all the essential amino acids, relatively low in crude fibre, rich source of vitamins and minerals, oil, Omega 3 fatty acids, devoid of cholesterol and easily digestible if properly processed

(Bintu *et al*., 2021). Soy bean 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 (Bristone *et al.,* 2018). It is a very good source of dietary protein that ranks high among the great world protein sources, which are meat, fish, eggs and milk (Yusuf and Ali, 2013).

Soya bean cake is a soft- cheese -like food obtained by coagulating fresh hot soymilk with one or more coagulant (Eze *et al*., 2018). The snack is said to originate from Asia where it is called *Tofu*, which is popularly known as *Awara* in Northern Nigeria and *Beske* in the Western parts of the country (Bagirei *et al*., 2021). It is produced by first preparing soymilk and further precipitating the milk with a suitable coagulant. Traditionally, steep water from *Ogi* is used as a coagulant, the coagulant used help to mobilize the protein and oil in the soymilk to form curds. These curds are pressed to remove excess water, cut into blocks and deep fried in hot oil if necessary (Bagirei *et al*., 2021; Bintu *et al*., 2021).

In Nigeria, *awara* has been regarded as a cheap source of protein that is readily available and affordable for a common man as compared to animal food products. This product due to its nutrient and high moisture content makes it suitable for the growth of microorganisms, especially if there is no good manufacturing practices and proper storage that would increase its shelf-life (Oranusi *et al*., 2013).

In developing countries, it is a challenge to ensure food security but more to that is the safety of such foods (Acácio *et al*., 2021). Food borne illnesses of microbial origin are a major health problem associated with street foods causing heavy burden of diseases and mortality (Mamdouh *et al*., 2021). Furthermore, an estimate of over 200 types of diseases are estimated to be caused or spread by food, occasionally causing long-term health problems in vulnerable groups such as the elderly, pregnant women, children and immunocompromised individuals (Acácio *et al*., 2021). Food pathogens contaminate food

at different stages in a food production chain, the contamination can be from the raw material, water, soil, or air as well as unsafe food storage and processing (Hammuel *et al*., 2022). Local processing of soy bean cake is usually done at the home level and usually has no good processing methods. The traditional processing methods that are used in preparation, inappropriate holding temperature, lack of formal education, no or very little knowledge about the cause of food-borne diseases and poor personal hygiene of food handlers are also contributing factors. Consumers who depend on such foods are more interested in its convenience and usually pay little attention to its safety, quality and hygiene (Kharel *et al*., 2016; Temesgen *et al*., 2016). Thus, it is important to ensure food safety as a public health measure. This study tends to investigate the bacteriological quality of soya bean cake sold within Federal University of Technology, Mnna, Niger State (Bosso campus).

MATERIALS AND METHODS

Study area

This study was conducted within Federal University of Technology, Minna, Niger State (Bosso campus) located between Latitude 9˚39`3.82``N to 9˚39`25.90``N, Longitude 6˚31`27.65``E in Bosso Local Government Area of Minna, Niger State.

Collection of Samples

A total of 12 samples of *awara* were collected from four different hawkers around the classrooms and hostel blocks. The hawkers were designated A, B, C and D. The samples were transported to Microbiology laboratory in the Department of Microbiology, Federal University of Technology Minna for analysis.

Sterilization of Materials

Glass wares such as conical flask, beakers, test tubes and bottles were washed with detergents, rinsed with water, air dried and sterilized in hot air oven at 160°C for 1 hour. Wire loops were sterilized by heating them to redness in Bunsen burner flame.

Preparation of Culture Media

The culture media used were prepared according to manufacturer's instruction and sterilized by autoclaving at 121° C for 15minutes.

Serial dilution

One gram of each sample was homogenized with 9 ml of Nutrient broth and serial dilutions of each sample homogenate were made to 10^{-5}dilutions. Each test tube was shaken vigorously before each transfer.

Isolation and Identification of Bacteria

This was carried out according to the method described by Idris and Dabo (2016) with slight modifications.

Using a sterile syringe 1ml of the dilutions (10^{-5}) was transferred to appropriately labeled petri dishes, followed by pouring aseptically molten nutrient agar. The dishes were swirled to ensure proper mixing and then the media was allowed to solidify. The plates were incubated at 37˚C for 24 hours. After the incubation time, the culture plates were examined for microbial growth. Colonies were counted using the colony counter and different morphological attributes of the colonies were observed and recorded. Discrete colonies were isolated and purified by repeated sub-culturing. Pure cultures were stored on slants at 4ºC for further characterization. The identities of the bacteria were confirmed by Gram staining and biochemical tests. References were made to standard identification keys and atlas.

Gram staining techniques

On clean, grease-free slides, a thin smear of each pure 24-hour-old culture of the bacterial isolates were made and heat-fixed by passing over flame. Two drops of crystal violet solution were added to each heat-fixed smear, then allowed to stain for 60 seconds before being washed with water. The smears were then flooded with Lugol's iodine for 60 seconds, decolourized by flooding with 70% alcohol for 15 seconds before being rinsed with distilled water. The slides were counter stained with Safranin for 60 seconds, then rinsed with water

and left to air dry. The smears were mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while gram positive organisms appeared purple (Fawole and Oso, 2004).

Catalase test

With the use of a sterile inoculating loop, a small amount of 24 hours-old culture was transferred into a drop of 3% hydrogen peroxide solution on a clean slide. The presence of the catalase enzyme was indicated by gas seen as white foam (Cheesbrough, 2006).

Coagulase Test (slide test)

A colony of the bacterial isolate was used to make a thick smear on two separate slides to which a drop of distilled water initially placed. A loopful of plasma was placed on one slide and gently mixed with a sterile wire and the other slide was used as a control. After 10 seconds, clumping of the organisms in the first slide indicates it is coagulase positive and the other control slide was used to differentiate any granular appearance of the organism from true coagulase (Cheesbrough, 2006).

Citrate Utilization Test

Bijou bottles were used to make Simmon's citrate agar medium slants. The slopes of the slants were first streaked with a saline suspension of the bacterial isolates and then the butts were stabbed using sterile needle. A bright blue colour in the medium indicates positive citrate test and no colour change indicates negative result after incubation at 35°C for 48 hours (Cheesbrough, 2006).

Indole Test

Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37°C for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added and shaken gently; it was allowed to stand for 20

minutes to permit the reagent to rise. A red-violet colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result (Cheesbrough, 2006).

Oxidase Test

A piece of filter paper was soaked with few drops of oxidase reagent. With the aid of a sterile inoculating loop, a colony of the test organism was smeared on the filter paper. Oxidase producing organisms appeared deep purple colour (Cheesbrough, 2006).

Urease Test

A small quantity of 24 hours old culture was inoculated in a urease agar and incubated at 37°C for 48 hours, the development of a red-pink color indicates a positive result (Oyeleke and Manga, 2008).

RESULTS AND DISCUSSION

Mean Bacterial Count of soya bean cake (*awara*)

The mean bacterial count of *awara* is shown on Table 1. The samples collected from hawker B had the highest mean bacterial count followed by soya bean cake collected from hawker D and then soya bean cake samples from hawker A while the soya bean cake samples collected from hawker C had the lowest mean bacterial count.

Table 1: *Mean Bacterial Count of Awara*

Hawker	Dilution factor (10^{-5}) (cfu/g)
A	5.0×10^5
B	7.5×10^5
C	4.5×10^5
D	6.00×10^5

The mean bacterial count in this study ranged from 7.50 x 10^5cfu/g and 4.5x 10^5cfu/g which is similar to the finding of Bristone *et al*. (2018) but relatively higher than the results reported by Yushau *et al*. (2017). Such variations are largely attributed to methods of preparation, seasonings and condiments used, handling and serving protocols of vendors. In addition, diversity in the environments/climatic conditions could be contributing factors Temesgen *et al*. (2016)

Table 2: *Colony Morphology, Gram reaction and Biochemical Characteristics and Bacterial Isolates*

Table 2 shows the colony morphology, results of Gram reaction and biochemical tests.

IS	CM	GMR	CA	CO	CU	IN	OX	UR	Suspected organism
1	Whitish with glistening edges	+ Cocci	+	+	+	-	-	+	*Staphylococus aureus*
2	Greyish white with moist surface	- Rod	+	-	-	+	-	-	*Escherichia coli*
3	Greyish white with smooth surface	- Rod	+	-	-	-	-	-	*Salmonella* spp
4	Whitish with Glistening edges	+ Cocci	+	+	+	-	-	+	*Staphylococus aureus*
5	Greyish white with moist surface	- Rod	+	-	-	+	-	-	*Escherichia coli*
6	Whitish with glistening edges	+ Cocci	+	+	+	-	-	+	*Staphylococus aureus*

Key: IS: Isolates, GMR: Gram staining reaction, CA: Catalase Test, CO: Coagulase Test, CU: Citrate Utilization Test, IN: Indole Test, OX: Oxidase Test, UR: Urease Test, +: Positive, -: Negative.

In this study, the samples were found to be contaminated with food borne pathogens and indicator organisms including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. A similar study by Temesgen *et al* (2016) on street ready to eat food revealed the presence

of these organisms. The work of Bristone *et al* (2018) corroborates the result of this study with *S. aureus* being the dominant pathogen found. Idris and Dabo (2016) also reported the same pathogens in their work.

Staphylococcus aureus is found in the nasal cavity, hair and skin of more than 50% of healthy individuals (Daniyan *et al.,* 2011). The deposition of this microorganism in the samples may have resulted from excessive handling or lack of good sanitary practices. Staphylococcal food poisoning is one of the few causes of bacterial food poisoning that is usually attributed to a food handler. The individual who inadvertently inoculates the food with *S. aureus* may have a clinical infection, but usually is colonized asymptomatically (Isaac and Buddy 2023). If the microbes are allowed to incubate in food, a situation called Temperature abuse, they grow and release enterotoxins which leads to food intoxication and ultimately food poisoning (Tortora *et al*., 2011). This toxin is heat stable, being tolerant to boiling for 1 hour and incubation time after ingestion is 1-6 hours, this triggers the brain vomiting reflex centre causing vomiting, abdominal cramps and diarrhea ensues (Tortora *et al*., 2011, Doyle, 2013)

The presence of *Salmonella* sp and *Escherichia coli* which is a feacal coliform is an indication of faecal contamination of the foods as it was suggested by Temesgen *et al*. (2016). This is attributed to a number of factors such as unclean surroundings and utensils, contaminated water, improper handling and processing of food items and lack of proper storage as well as unhygienic display of food (Sharma and Mazumdar 2014). This can result in the spread of diseases such as gastroenteritis and typhoid fever. Furthermore, *awara* is prepared locally at home commonly among the lower class that lack basic social amenities, so this predisposes the product to these pathogens because such environments are suitable for their growth and transmission.

Though part of the normal human intestinal microbiota, some strains of *E. coli* are commonly associated with food poisoning outbreak. Human acquire the virulent strains of *E.coli* from contaminated water and foods (Hariri, 2022). Once the bacteria are in the body, they multiply and invade the intestinal mucosa where they produce enterotoxin and or cytotoxin that destroys epithelial cells. Most prominent symptoms include abdominal cramps, diarrhea, nausea, vomiting and fever which persist for 2-5 days but can last for several weeks. Excessive loss of fluid can lead to severe consequences including death (Willey *et al*., 2011).

Salmonellae are ubiquitous human and animal pathogens causing salmonellosis, in humans the disease usually takes the form of a self-limiting food poisoning (gastroenteritis), but occasionally manifests as a serious systemic infection (enteric fever) which requires prompt antibiotic treatment (Giannella, 1996). The microbes first invade the intestinal mucosa and multiply there, occasionally passing the intestinal mucosa to the cardiovascular and lymphatic systems. The incubation time is about 12-36 hours, there is usually a moderate fever accompanied by nausea, abdominal pain and diarrhea (Giannella, 1996; Tortora *et al*., 2007). Normally, recovery will be complete in few days but patients will shed the organisms in their faeces for months (Tortora *et al*., 2007).

Prevention of food poisoning must include good personal hygiene, proper handling, cleaning and disinfection of equipment, use of clean water and proper storage of food product after processing.

CONCLUSION

The results of this study showed that the samples examined were contaminated with pathogens capable of causing food borne illness. In view of the above, the students should be sensitized on the health risk of consuming of the contaminated street vended food, the producers and vendors should be educated on good personal hygiene and good

manufacturing practice and the government should provide the basic amenities for lower class to reduce the contamination of not only *awara* but other foods they sell or consume.

REFERENCES

Acácio, S., Ana, C. S., Luísa, B. and Manuel, M. (2021). Microbiological assessment of street foods at the point of sale in Maputo (Mozambique), *Food Quality and Safety*, 5:30. https://doi.org/10.1093/fqsafe/fyaa030

Ahmad, A., Abdulrahman, I. T., Abdulaziz, U. K. and Dinesh, C. S. (2020). Effects of processing methods on proximate compositions and organoleptic properties of soybean curd (*awara*) refined in Zamfara, Northern Nigeria. *International Journal of Food Science and Nutrition*, 5(2): 116-119

Bintu, P. B., Falmata, A. S., Maryam, B. K., Nnenna, R. O., Hasina, A. L., And Modu, S. (2021). Nutritional quality of *awara* made from fermented banjara beans and soybean. *Open Journal of Bioscience Research*, 2(2): 66 - 78. https://doi.org/10.52417/ojbr.v2i2.298

Bagirei, S. Y., Mohammad, Y. S., Maude, M. M., Abdulkadir, F. and Umar, S. A. (2021). Evaluation of sensory qualities of *awara* and performance on yield and functional properties of fresh and dried soy-curds. *International Journal of Science for Global Sustainability*, 7(1), 6

Bristone, C., Mshelia, H. A., Ogori, F. A., Badau, M. H. and Ogori, J. (2018). Microbial quality evaluation of *awara* (soybean cheese) processed and sold at University of Maiduguri campus *Jornal of Bacteriological* Mycology, 6(1):30–34. doi: 10.15406/jbmoa.2018.06.00172

Cheesbrough M. (2006). District laboratory practice in tropical countries (2nd ed.) Cambridge, UK: Cambridge University Press

Daniya, S. Y., Abalaka, M. E., Momoh, J. A. and Adabara, N. U. (2011). Microbiological and physiological assessment of street vended soyabeans cheese sold in Minna Niger state. *International Journal of Biomedical and Advance Research,* 2(1): 26-30

Doyle, M. P. (2013). Food Safety: Bacterial Contamination. *The Encyclopedia of Human Nutrition*: (3rd Ed.). Amsterdam, Netherlands: Elsevier Publishing Company

Eze, N. M., Okwume, U. G., Eseadi, C., Udenta, E. A., Onyeke, N. G., Ugwu, E. N., Akubue, B. N., Njoku, H. A. and Ezeanwu, A. B. (2018). Acceptability and consumption of

Tofu as a meat alternative among secondary school boarders in Enugu State, Nigeria. *Medicine (Baltimore)*, 97(45): e13155. doi: 10.1097/MD.0000000000013155

Fawole, M. O. and Oso, B. A. (2004). Characterization of Bacteria: Laboratory Manual of Microbiology (4th ed.). Ibadan, Nigeria: Spectrum Book Ltd

Giannella, R. A. (1996). *Medical Microbiology* (4th Ed.) University of Texas Medical Branch: Galveston (TX)

Hammuel, C., Aso, R. E., Briska, J., Daji, M. and Anguwa, E. L. (2022). Occurrence of *Staphylococcus* species from '*awara'* sold in Wukari old market, Taraba State. *Journal of Food Microbiology*, 6(4):117. doi:10.35841/aafmy-6.4.117

Hariri, S. (2022). Detection of *Escherichia coli* in food samples using culture and polymerase chain reaction methods. *Cureus*, 14(12): e32808. DOI: 10.7759/cureus.32808

Idris, A. and Dabo, N. T. (2016). Microbiological and nutritional analyses of soybean cake (*awara*) and camel milk cheese (*chukwui*) local snacks, vended in Kano Metropolis, Nigeria. *UMYU Journal of Microbiology Research*, 1(1):7-14

Kharel, N., Uma, P. and Prakash, J. T. (2016). Microbiological assessment of ethnic street foods of the Himalayas. *Journal of Ethnic Foods* 3(3): 235-24. https://doi.org/10.1016/j.jef.2016.01.001

Mamdouh, A. B., Talib, M. B., Mohammed, E., Rasha, A. B., Razaz, A. Q., Amal, T., Maher, K., Mohammed, A. K., Ahmed, A. and Fayez, B. (2021). Assessment of microbiological quality of food preparation process in some restaurants of Makkah City. *Saudi Journal of Biological Sciences*, 28 (10): 5993-5997. Retrieved from https://www.sciencedirect.com/journal/saudi-journal-of-biological-sciences

Noah, N. (2005). *Encyclopedia of Human* Nutrition: Food Safety/Bacterial Contamination (2nd Ed.). Amsterdam, Netherlands: Elsevier Publishing Company

Oranusi, S. U., Oguoma, O. I. and Agusi, E. (2013). Microbiological quality assessment of foods sold in student's cafeterias. *Global Research Journal of Microbiology*, 3 (1): 1–7

Oyeleke, S. B. and Manga, B. S. (2008). Essential of Laboratory Practical's in Microbiology (1st ed.). Minna, Niger State: Tobest Publishers S.W

Sharma, I. and Mazumdar, J. A. (2014). Assessment of bacteriological quality of ready to eat food vended in streets of Silchar City, Assam, India. *Indian Journal of Medical Microbiology*, 32(2): 169-171. doi:10.4103/0255-0857.129809

Temesgen, E., Haimanot, T., Derese, D. and Gebre, K. (2016). Bacteriological quality of street foods and antimicrobial resistance of isolates in Hawassa, Ethopia. *Ethiopian Journal of Health Sciences* 26(6): 533–542. doi: 10.4314/ejhsv26i6.5

Yusha'u, M. B. Atabor, D. W., Taura, L. G. and Abdulrahman, I. (2018). Bacteriological quality of soya bean cake (*awara*) sold at Bayero University, Kano. doi:10.17758/EIRAI.F0217702

Tortora, G. J., Funke, B. R. and Case, C. L. (2007). Microbiology: An Introduction (9th Ed). San Francisco: Pearson Benjamin Cummings

Willey, J. M., Sherwood, L. M. and Woolverton, C. J. (2011). Prescott's Microbiology (8th Ed). New York City: McGraw Hill

Yusuf, H. L. and Ali, A. I. (2013). Production, Proximate and Sensory Assessment of *Awara* Produced from Fresh and Sun Dried Soybean Curd Mix. *Bayero Journal of Pure and Applied Sciences*, 6(2): 146 – 148. http://dx.doi.org/10.4314/bajopas.v6i2.30