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Evaluation of Bacterial and Protozoan Contamination of Local Soup Condiment (Daddawa) Produced by North-Western Community, Nigeria

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***Abstract*—**Soup condiments are edible food items added to dishes, used as thickeners for soup and as food supplements such as sauce added to food to impact specific flavours. They are abundantly produced in Nigeria especially in its North-Western part. The aim of this research was to determine the bacterial and protozoan contamination of local soup condiment (Daddawa) produced by North-Western Community, Nigeria. Samples were randomly collected from three sellers each in Dabai, Bedi, Senchi and Zuru, aseptically placed into polythene bags and labelled correctly. 8g of nutrient agar powder was weighed and dissolved in 350mls of distilled water, hot plated and sterilised by autoclaving at 121°C for 15 minutes and allowed to cool. 20mls of the prepared nutrient media was poured into a sterile petri dish and allowed to solidify. The protozoa present in the samples were identified by direct microscopy. A total of 12 samples from the four study areas were examined. The mean bacterial count of the samples in Dabai, Bedi, Senchi and Zuru were 125.3, 85.0, 75.3 and 39.7 respectively. A total of four bacterial species were isolated with 43 isolates. 19(44.1%) was recorded in *Staphylococcus aureus* which was the most prevalent in the study areas followed by *Shigella dysenteriae* 13(30.2%), *Escherichia coli* 8(18.6%) and *Salmonella typhi* 3(6.9%). The highest prevalence rate of the identified protozoa was recorded in *Entamoeba histolytica* 2(50.0%) followed by *Giardia lamblia* 1(25.0%). In conclusion, this study had confirmed that local soup condiments are contaminated by bacteria and protozoa. *Staphylococcus aureus* and *Entamoeba histolytica* respectively were the most prevalent in the study areas. It was recommended that urgent review of the entire process in the study areas and North-Western Nigerian Community as a whole be carried out to ensure awareness and that all the condiments are produced by following the standard operation procedure.

Keywords—Evaluation, Bacteria, Protozoa, Contamination, Local Soup Condiment & North-Western Community

I. INTRODUCTION

Soup condiments are edible food items which are added to dishes, used as thickeners for soup and also as food supplements such as sauce that is added to food to impact specific flavours [1]. In some cultures, they are used to compliment some dishes. The terms originally describe the condiments as preserved foods, but their meaning has changed over time [2], [3]. They are abundantly produced in Nigeria especially in North-Western part of the country.
Food condiments or spices are strong smelling, sharp tasting substances usually used to improve or adjust the flavour of food. They are usually of vegetable origin. Common examples include mustard, nutmeg, ginger, garlic, coriander, locust bean etc. [4].

Food condiments made from vegetable protein may be a good source of certain vitamin B, but are deficient in ascorbate and some fat-soluble vitamins, which are lost during fermentation. It is evident that fermented condiments are good source of nutrients and could be used to produce complementary food supplements and contribute to enhance food quality [5].

Condiments add flavour, make the food tasty and palatable [6]. They also serve as appetizers in food [7]. Condiments possess essential stimulating qualities, rendering them peculiarly fitted for inducing reflex action, the secretion of alimentary juice. As the function of condiment, such as pepper, mustard, spices, potherbs, that besides their stimulating properties, they give flavour to food and then indefferent food is made palatable, and digestion accelerated. Letheby enumerated it as the aid of digestion – proper selection of food, according to the taste of the individual [3].

Condiments were known in ancient Rome, ancient India and ancient China, where there was myth that, before food preservation techniques became widespread, pungent spices and condiments were sold as wet paste, used to give the very strong traditional taste, making the food palatable [8].

There are different types of condiments which include pickle foods, which date back to the ancient world in Europe as well as Asia. Common pickle foods used as condiments today include ginger used by Japanese, chutney in Asia and cucumbers. Almostn all the vegetables have been pickled and used as condiments in some forms. They are served whole, in slices or diced in relish [8].

There are spicy and hot condiments which are black pepper, chilli pepper, mustard garlic and onions [9]. Black pepper is a product from Asia which is commonly used from shakers throughout the western world in a dried state and is usually ground and coarse or fine state before consumption. Like salt, pepper is used in dishes and also as based ingredient in some pepper sauce. Chilli pepper is a product of the Americas employed in dried form on diverse foods [10].

Fresh chopped chilli pepper is also the based ingredient in a number of other based condiments, including salsa. In Pre-Columbian times, a number of sauces based on chilli pepper were employed along with tomatoes and grounded pumpkin seeds. After the Spanish conquest in 1521, fusion foods developed including salsas, which combined salt with ground tomatoes and chilli peppers from the new world and vinegar from the old world.

African locust beans (*Parkia biglobosa*) and other oil-seeds such as melon seed, castor seed, soybean seed etc. are fermented to produce condiments [11]. Fermented locust bean is a well-known condiment with characteristic ammoniac odour and flavour which enhances the taste of traditional soups and sauces especially those used as accompaniments to starchy foods. It is known to contribute to the calorie and protein intake [12], [13], [14], [15], [16], [17]. It is generally added to soups as low cost meat substitute by low income families in parts of Nigeria.

The nutritional content of locust bean showed that protein and fat increases whereas the quantity of carbohydrates decreases. Increase in levels of amino acids was also reported except for arginine, leucine and phenylalanine. Soluble products increase during fermentation resulting in high digestability of the fermented product. Improved nutritive values were attributed to the increase in amino acid profiles due to fermentation [5].

II. RELATED WORK

Generally, it is recognised that sicknesses due to the consumption of contaminated foods are assumed to be the most widespread health problems in the contemporary world today, and a significant cause of reduced economic productivity. Recent studies examining the morbidity and mortality of food-borne diseases have confirmed the significant public health burden posed by these diseases. Microbial food safety is an increasing public health concern worldwide [18]. In developed countries, it is estimated that one-quarter to one-third of the population are made ill each year because of food-borne diseases. Studies on food-related illness and death in the United States were conducted [19]. In the developing countries, including Nigeria, the burden is much more severe. Therefore, there is need to regularly survey the food preparation, handling and storage techniques to ensure the safety of the food on one hand, and the health of the consumers on the other hand.

At this juncture, several studies had been conducted by various researchers in Nigeria and abroad on the contamination and proximate composition of local soup condiments [1], [2], [6], [11], [13], [14], [15], [16], [20], [21], [22], [23].

III. METHODOLOGY

Study Area/Location

The study was conducted in Zuru Local Government Area of Kebbi State, North-Western Nigeria. Zuru is located on latitude 11°35 and 11°55 N and longitude 40°45 E. It has an area of 653 km^2 (252 square miles) and a population of 165,547 people based on 2006 national population census [24]. The postal code of the area is 872 [25]. It is bordered by Anka Local Government Area of Zamfara State to the North, to the South-West by Rijau Local Government Area of Niger State and West by Koko-Besse Local Government Area. It is surrounded by mountains which serve as walls for Zuru people. The climatic condition lies within the tropical Sudan Savannah. The minimum temperature of the area ranges from 15° - 24°C, while the maximum temperature ranges from 32° - 39°C. The annual rainfall ranges from 560 – 1300 mm. The first rainfall usually begins from April and lasts for five (5) to six (6) months.

Sample Collection

Samples were randomly collected from the four study areas in Zuru Local Government Area of Kebbi State, North-Western Nigeria. The four study areas include: Dabai, Bedi, Senchi and Zuru. Random collections were made from three sellers to make one sample each on their various market days making a total of 12 samples. The samples were then aseptically placed into polythene bags, labelled correctly and transported to Microbiology Laboratory, Kebbi State University of Science and Technology, Aliero [26].

Materials and Reagents

The glasswares, materials and reagents used in carrying out this practical work include: petri dishes, conical flasks (250mls, 500mls and 1000mls), test tubes, glass slides, distilled water, inoculating needle, cotton wool, aluminium foil, hand gloves, face mask, masking tape, labelling pencil, cover slips, spirit lamp, marker, gram staining

reagents, ethanol, microscope, Lugol's iodine, acetone, safranin, Bunsen burner, immersion oil, hydrogen peroxide solution, sterile urea broth, oxidase reagent and filter paper.

Media Preparation

Preparation of Nutrient Agar

The media used for the isolation of bacteria was nutrient agar prepared according to the manufacturer's procedure. 8g of nutrient agar powder was weighed and dissolved in 350mls of distilled water. The mixture was hot plated for the solution to dissolve properly. The mixture was sterilised by autoclaving at 121°C for 15 minutes and was allowed to cool. 20mls of the prepared nutrient media was poured into a sterile petri dish and allowed to solidify. The glasswares used in carrying out this practical work include: petri dishes, conical flasks (250mls, 500mls and 1000mls), glass slides, distilled water, test tubes, measuring cylinders, inoculating needle, cotton wool, aluminium foil, face mask, masking tape, cover slip, microscope, spirit lamp, labelling pencil and ethanol.

Inoculation of Bacteria

Part of the samples were cut and serially diluted. One mile from the test tubes was inoculated into the designated media plates in duplicates and incubated at 37 degrees for 24 hours. Colonies of bacteria appeared on the surface of the media and the total bacterial count was determined by standard plate count method after incubation at 37 degrees centigrade for 24 to 48 hours.

Smear Making

A smear was prepared by transferring a colony with a sterilised wire loop from agar plate to a clean glass slide. The procedure for smear making is as follows: a wire loop was sterilised with a Bunsen burner flame until heated to redness and allowed to cool; a drop of distilled water was placed on the centre of a glass slide; a little of the colony was picked and transferred at the same point where a drop of distilled water was added; a sterilised wire loop was used to emulsify the growth over an area for about 1cm radius in order to obtain a very thin smear. The slide was allowed to air dry and the glass slide was heated by passing it twice over the Bunsen burner flame.

Gram Staining Method

This method is used to distinguish nearly all bacteria in gram negative or gram positive bacteria using crystal violet and subsequently with iodine (gram positive cell appears purple while a gram negative cell appears red). A thin smear of bacteria was made on a glass slide; air dried and heat fixed on a hot air oven for seconds. The smear was covered with crystal violet for 60 seconds, washed rapidly with distilled water. The glass slide was allowed to tip off all water and the smear was covered with Lugol's iodine for 60 seconds. The iodine was washed off with clean water and was decolourised with acetone and washed off immediately. The smear was covered with safranin for 60 seconds and washed off with clean water; the back of the slide was cleaned and placed on a rack to air dry. The dried slide was viewed with immersion oil microscopically using the ×100 objective lens microscope [27].

Biochemical Tests

Catalase Test

3mls of hydrogen peroxide solution was dropped into a test tube; using a sterile wire loop, similar colonies of the test organisms were immersed in the hydrogen peroxide solution. Evolution of white bubbles as gas indicates catalase positive reaction. Absence of these bubbles indicates a negative catalase reaction [28].

Coagulase Test

A drop of distilled water was placed on each end of the slide or on two separate slides. A colony of the test organism was emulsified in each of the drop to make two thick suspensions. If a loop full of plasma is attached to one end of the slide, clumping of the organisms will be seen [28].

Urease Test

The test organism was heavily inoculated in a bottle containing 3mls of sterile urea broth. It was incubated at 35 – 37 degrees centigrade for 3 hours. A pink colour indicates positive urease test while the absence of pink colour indicates negative [28].

Oxidase Test

A piece of filter paper was placed on a clean petri dish and 3 drops of freshly prepared oxidase reagent were added. A wire loop was used to remove the colony of the test organism and was smeared on the filter paper. There was a blue-purple colour within 10 seconds which indicates positive oxidase test while the absence of the blue-purple colour indicates negative oxidase test [28].

Isolation and Identifcation of Protozoa

For direct microscopy, each sample was divided into portions and soaked in sterile distilled water for 30 minutes; the soaked sample was filtered. After filtration, the solid part of the sample was discarded and the liquid part was centrifuged. After centrifuging, a drop of the supernatant was placed on a clean grease-free glass slide and examined microscopically. ×10 and ×40 objective lenses were used for viewing the parasites. These allowed the detection of motile trophozoite forms of parasitic protozoa [29].

The protozoan species were identified using the identification keys provided by Zoologist Georg August Goldfuss in 1818. In some cases, staining was used to increase contrast and get a clearer view. Some of the stains used here include: methylene blue, carmine powder, Bismarck brown and bromothymol blue.

Data Analysis

The statistical tools used to analyse the data on bacterial and protozoan contamination of local soup condiment (Daddawa) were mean standard deviation standard plate count and percentage. The statistical tools were employed

in order to calculate the number of bacterial and protozoan contamination from local soup condiment samples and their respective percentages.

IV. RESULTS AND DISCUSSION

Table 1: Isolation of Bacteria Associated with Contamination of Local Soup Condiment (Daddawa) in the Study Areas

Sample	Count	Total Count (cfu/g)	Mean
A	150	1.50×10^2	
	130	1.30×10^3	**125.3**
	96	9.60×10^4	
B	130	1.30×10^2	
	85	8.50×10^3	**85.0**
	40	4.00×10^4	
C	90	9.00×10^2	
	76	7.60×10^3	**75.3**
	60	6.00×10^4	
D	64	6.40×10^2	
	30	3.00×10^3	**39.7**
	25	2.50×10^4	

Key: cfu = colony forming unit; g = gram

The table above gives a summary of the total bacterial count of local soup condiment (Daddawa) in the the four study areas (Dabai, Bedi, Senchi and Zuru) labelled as A, B, C and D respectively.

Sample A (Dabai) has the highest count followed by sample B (Bedi), sample C (Senchi) and sample D (Zuru).

Table 2: Identification of Bacteria Associated with Contamination of Local Soup Condiment (Daddawa) in the Study Areas

Sample	Isolates	No. of Isolates	Percentage
A	*Staphylococcus aureus*	19	**44.1%**
B	*Shigella dysenteriae*	13	**30.2%**
C	*Escherichia coli*	8	**18.6%**
D	*Salmonella typhi*	3	**6.9%**

The table above shows the types of bacteria associated with contamination of local soup condiment (Daddawa) in the study areas, their number of isolates and their respective percentages calculated.

Sample A (Dabai) has the highest percentage of bacterial isolates followed by Samples B, C and D (Bedi, Senchi and Zuru respectively).

Again, *Staphylococcus aureus* recorded highest percentage followed by *Shigella dysenteriae*, *Escherichia coli* and *Salmonella typhi* in Dabai, Bedi, Senchi and Zuru respectively.

Table 3: Biochemical Identification of Isolated Bacteria from Local Soup Condiment (Daddawa) in the Study Areas

Gram Stain	Cat	Coagu	Urea	Oxi	Organisms Identified
+Cocci	+	+	+	-	*Staphylococcus aureus*
-Rod	+	-	-	-	*Escherichia coli*
-Rod	+	-	-	-	*Shigella dysenteriae*
-Rod	+	-	-	-	*Salmonella typhi*

Key: Cat = Catalase, Coagu = Coagulase, Urea = Urease, Oxi = Oxidase

The table above shows the biochemical identification of isolated bacteria from local soup condiment (Daddawa) in the study areas. The biochemical result gives the correct insight of the bacteria isolated.

Table 4: Identification of Protozoa Associated with Contamination of Local Soup Condiment (Daddawa) in the Study Areas

Sample	Protozoa	No. of Protozoa	Percentage
A	Not seen	Nil	0.0%
B	Not seen	Nil	0.0%
C	*Entamoeba histolytica*	2	50.0%
D	*Giardia lamblia*	1	25.0%

The table above identifies the protozoa associated with contamination of local soup condiment (Daddawa) in the study areas. No protozoa were seen in sample A and B (Dabai and Bedi respectively), but *Entamoeba histolytica* was identified in sample C (Senchi) and *Giardia lamblia* was identified in sample D (Zuru).

Entamoeba histolytica recorded highest prevalence (50.0%) than *Giardia lamblia* (25.0%).

Discussion

It is generally recognised that diseases due to the consumption of contaminated foods are the most widespread health problems in the modern world today, and an indispensable cause of reduced economic productivity. Recent studies examining the morbidity and mortality of food-borne diseases have confirmed the significant public health burden posed by these diseases. Microbial food safety is an increasing public health concern worldwide [18]. In developed countries, it is estimated that one-quarter to one-third of the population are made sick each year because of food-borne infections. In the developing countries, including Nigeria, the burden is much more severe; therefore, there is need to regularly survey the food preparation, handling and storage techniques to ensure the safety of the food on one hand, and the consumers' health on the other hand.

In the present study, the results of the bacterial and protozoan contamination of local soup condiment (Daddawa) revealed some of the bacterial species that can

possibly contaminate the condiment in the study areas. These bacterial species isolated from the four study areas (Dabai as sample A, Bedi as sample B, Senchi as sample C and Zuru as sample D respectively) were *Staphylococcus aureus, Shigella dysenteriae, Escherichia coli* and *Salmonella typhi.* Sample A has the highest mean bacterial count (125.3) followed by sample B (85.0), sample C (75.3) and sample D (39.7) (Table 1). This is consistent with the study of Ogbadu and Okagbue which showed that *Staphylococcus aureus, Shigella dysenteriae, Escherichia coli* and *Salmonella typhi* were the possible bacteria responsible for contamination of local soup condiment (Daddawa) [20].

The identification of bacteria associated with contamination of local soup condiment (Daddawa) in the study areas was calculated in percentage where sample A representing Dabai has the highest percentage of *Staphylococcus aureus* (44.1%), sample B representating Bedi shows *Shigella dysenteriae* (30.2%), sample C representing Senchi shows *Escherichia coli* (18.6%) and sample D representing Zuru shows *Salmonella typhi* (6.9%). Again, based on the number of bacterial isolates, *Staphylococcus aureus, Shigella dysenteriae, Escherichia coli* and *Salmonella typhi* have 19, 13, 8 and 3 respectively (Table 2). This is also consistent with the study conducted by Ogbadu and Okagbue on "Bacterial Fermentation of Soya Bean for 'Daddawa' Production" [30].

The biochemical identification of isolated bacteria from local soup condiment (Daddawa) in the study areas revealed that sample A (Dabai) has gram stain of +Cocci, positive catalase, positive coagulase, positive urease and negative oxidase and the bacterium identified as *Staphylococcus aureus.* Sample B (Bedi) has gram stain of –Rod, positive catalase, negative coagulase, negative urease and negative oxidase and the bacterium identified as *Escherichia coli.* Sample C (Senchi) has gram stain of –Rod, positive catalase, negative coagulase, negative urease and negative oxidase and the bacterium identified as *Shigella dysenteriae.* Sample D (Zuru) has gram stain of –Rod, positive catalase, negative coagulase, negative urease and negative oxidase and the bacterium identified as *Salmonella typhi* (Table 3). Most previous studies were mainly on investigating the nutrient composition of fermented condiments. However, the relatively high recovery of the gram negative bacteria indicators and manitol-fermenting, coagulase-positive *S. aureus* are of clinical importance. Many gram-negative bacteria such as *Escherichia coli, Salmonella serovars; Campylobacter* spp., *Enterobacter* spp. *Klebsiella* spp. etc. have been implicated in food borne diseases [19]. Similar report of Ogunshe *et al.* also isolated such gram-negative bacteria of clinical importance from a fermented food condiment [21]. *Staphylococci* have been known worldwide to cause food borne intoxication and poisoning [31]. There is virtually no documented information available on the involvement of *S. aureus* in food borne disease out-breaks of fermented food condiment origin due to virtually non-existing system in Nigeria. *E. coli* isolated in this study has become a significant public health concern with a worldwide distribution [19]. Other gram-negative bacteria isolated in this study have also been implicated in acute bacterial diarrhoea and food poisonings. *S. dysenteriae* and *S. typhi* which were also isolated from the fermented soup condiment (Daddawa) in this study have been previously implicated as opportunistic pathogens and have become of increasing importance [31]. The fermented condiment preparation is still a traditional family art done in households and the fermentation that do not require conscious introduction of microbial flora in fermenting environment, thereby leading to the relative significant recovery of these indicator bacteria from the fermented condiment as indicated by the study [20]. The high recovery rates of *S. aureus* and the total coliform in the fermented condiments; the wholesomeness in the fermented condiments, which may result in an increased risk of transmission of the disease to the humans who consume them.

The identification of protozoa associated with contamination of local soup condiment (Daddawa) in the study areas revealed that protozoa were not seen in sample A and B (Dabai and Bedi respectively) but *Entamoeba histolytica* was identified in Sample C (Senchi) with 50.0% prevalence rate and *Giardia lamblia* was identified in sample D (Zuru) with 25.0% prevalence rate (Table 4). Therefore, *Entamoeba histolytica* and *Giardia lamblia* were identified as the possible protozoa that can contaminate local soup condiment (Daddawa) in the study areas. However, there is dearth of published information on protozoan contamination of local soup condiment in Nigeria. Estimates of food-borne disease deaths are subject to uncertainty because the number of deaths caused by unidentified pathogenic agents in the food supply is unknown. However, in the influential study of food-borne diseases in the United States by Mead *et al.*, it was estimated that unknown food-borne agents caused 3400 deaths per year or 65% of the estimated 5200 annual deaths from food-borne illnesses [19]. No matter how alarming these estimates from a developed country like the United States may be, more alarming will be the estimate from developing countries like Nigeria.

Common sources of food-borne diseases are bacterial and protozoan contamination of food by food handlers. However, the safety aspects of fermented condiments are not adequately documented and appreciated in developing countries like Nigeria [22]. It was generally observed that the water samples used in rinsing the boiled locust beans prior to fermentation were highly polluted. It is a common practice among the Nigerian elites to wash the fermented seeds in clean water before adding to culinary, due to sandy mouth feel usually encountered while chewing such prepared foods; meanwhile, the portions washed off are nutritious portions of the condiments. Again, some pathogens (bacteria or protozoa) may not be removed from the condiments by just mere washing. Previous study indicated that, salt can be used in preserving the condiments to prevent the high level presence of food

pathogens. Therefore, there is need for producer and consumer education about the safety of indigenous fermented food condiments.

The results obtained in this study agree with the study conducted by Liman *et al.*, who demonstrated the impact of environmental conditions on the quality of some processed locust beans, where contaminants like bacteria and protozoa can be gotten either from the water used in washing the seeds, the handlers or from utensils, flies and a lot [23]. Therefore, there is need to prepare and process these condiments under better hygienic processes.

V. CONCLUSION AND FUTURE SCOPE

It can be concluded based on the results obtained from this study that the local soup condiments (Daddawa) collected from the four study areas were heavily contaminated with bacteria and protozoa with bacteria having the highest occurence. This revealed that the bacteria and protozoa present can cause serious food-borne diseases in humans in the study areas. Contamination of these local soup condiments can draw the attention of both the producers and the consumers when they significantly establish proofs concerning the contamination. On the basis of the overall results from this investigation, bacteria and protozoa are justified to contaminate local soup condiments. When produced correctly, i.e. by maintaining proper hygiene, the local soup condiments are considered to be a bit safe from contamination. People are greatly concerned about how the condiments are produced, how neat the production process is, and how safe are they when consumed because most people prefer using them than the modern soup condiments. The use of local soup condiment (Daddawa) is widespread. Therefore, the need for proper hygiene and sanitation is paramount and indispensable. It is therefore recommended that urgent review of the entire process in the study areas and North-Western Nigerian Community as a whole be carried out to ensure awareness and that all the local soup condiments are produced by following the standard operation procedure. There is also an urgent need to educate producers of food condiments, vendors and consumers on the dangers of poor food handling and storage and the need to apply good manufacturing practices in processing the condiments in the study areas and North-Western Nigerian Community as a whole.

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