Original Research Article

Proximate Composition and Parasitic Contamination of *Hibiscus sabdariffa* Seed Cake (Roselle Seed Cake): a Soup Condiment Produced by North-Western Community, Nigeria

ABSTRACT

Background: 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. They are abundantly produced in Nigeria especially in North-Western part of the country.
Aims: The aim of this research was to determine the proximate composition and parasitic contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake): a soup condiment produced by North-Western Community, Nigeria.
Study design: Samples were purchased and collected at random from the markets of different places in the study areas, aseptically placed into polythene bags and labelled correctly.
Place and Duration of Study: The study was conducted in Zuru and Sakaba LGAs of Kebbi State, North-Western Nigeria.
Methodology: The proximate analysis carried out include: moisture determination, ash determination, determination of crude protein, lipid determination, fibre determination and carbohydrate estimation by difference. The protozoa present in the samples were identified by direct microscopy.
Results: The proximate composition of *Hibiscus sabdariffa* seed cake in the study areas had a very high level of carbohydrate content (52.44±1.03%), followed by the moisture content (15.43±0.10%), lipid content (12.00±0.50%), fibre content (11.16±0.58%), ash content (7.80±0.05%) and crude protein content (1.16±0.13%) which was the lowest. The highest prevalence rate of the identified protozoa was recorded in *Entamoeba histolytica* 2(50.0%) followed by *Giardia lamblia* 1(25.0%) in Zuru LGA. No protozoa was seen in Zuru sample 0(0.0%). In Dabai sample, *Giardia lamblia* was seen 1(25.0%). While in Bedi sample, *Entamoeba histolytica* 2(50.0%) was seen which was the most prevalent. However, in Sakaba LGA, a total of nine protozoa was seen and all were *Entamoeba histolytica*. Dirin-Daji sample showed 1(11.1%), Doka 2(22.2%), Dankolo 2(22.2%), Janbirni 1(11.1%), Laraba 1(11.1%) and Makuku 2(22.2%) respectively.
Conclusion: It can be concluded that the available carbohydrate and moisture contents were higher than other parameters and the local soup condiments were heavily contaminated with protozoa and *Entamoeba histolytica* had the highest prevalence which can cause serious food-borne diseases in humans in the study areas. Finally, the need to apply good manufacturing practices in processing the condiments in the study areas and North-Western Nigerian Community as a whole is highly recommended.

Keywords: *Proximate Composition; Parasitic Contamination; Hibiscus sabdariffa Seed Cake; Soup Condiment; North-Western Community.*

1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) also called rosella, sorrel or java jute is a fibre crop of the genus *Hibiscus* and family Malvaceae. It is probably native to West Africa although known in the West Indies early in the 16th century and was growing in Asia by the 17th century [1]. Its extensive cultivation in Indonesia began in the 1920s under a government subsidized program established to obtain fibre for sugar-sack manufacture. India, Java, and the Philippines are the world major producers [1, 2]. It is commercially propagated in different parts of the world including USA, United Kingdom and India; while Benin, Sudan, Cote D'Ivoire, Ghana, Niger, Burkina Faso and Nigeria were reported as major areas of Roselle cultivation in Africa [3, 4]. It is grown in East and Central Africa for several thousand years for food and fibre and also been used as a textile fibre source for the production of ropes, twines, bags, rugs, door mats, nets as well as jute substitutes in the manufacturing of sacks for the bagging industry [5]. Roselle is cultivated in various agro-ecological zones of Nigeria but highly concentrated in the North Eastern, North Western and Middle Belt regions [6].

The plant is an erect, annual or perennial herb, bushy herbaceous sub-shrub or woody-based subshrub propagated from seed, widely grown in the tropics and growing to 3 m height (Fern, 2012) or 2.0 – 2.5 m (7 – 8 ft) tall. The leaves are deeply three- to five-lobed, 8 – 15 cm (3 – 6 inches) long, arranged alternately on the stems. The flowers are 8 – 10 cm (3 – 4 inches) in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base; the fruits mature. They take about six months to mature [7].

It is referred to as "Zoborodo" in Northern Nigeria and "Zobo" in Western Nigeria (the Yorubas call the white variety "Isapa"). The two main varieties of *Hibiscus sabdariffa* are *H. sabdariffa* var. *altissima* and *H. sabdariffa* var. *sabdariffa.* The variety *H. sabdariffa* has red or pale yellow inflated edible calyces but a poor quality fibre while variety *altissima* has red or green, spiny calyces which are inedible, and grown for its jute-like fibre [8]. At the base of each flower is a fleshy calyx (sepal of the flowers) which is the part that is harvested and used [9]. In many tropical areas, the red, somewhat acid calyxes of the variety *altissima* are used locally for beverages, sauces, jellies, and preserves while the leaves and stalks are consumed as salads or cooked vegetables and used to season curries [1].

In Nigeria, Roselle cultivation has gained wide acceptability among farmers due to its medicinal [10] and industrial importance [11]. It is used as a digestive agent, purgative and a diuretic [12] and as a folk medicine for cancer, obesity, diabetes and hypertension [13]. According to Mukhtar *et al.*, Roselle calyces are used as digestive and purgative agent and a folk remedy for abscesses, billows, cancer, hypertension etc. [14]. The fresh calyces are known for their unique flavour characteristics that make them appealing to taste. The calyces or petals of the flower are widely used to prepare jam, jelly [15], soup and the popular Zobo drink in Nigeria. Roselle drink had been improved nutritionally by producing fruit-flavoured Roselle drinks which are richer in vitamins and minerals by addition of different fruits with higher consumption acceptability [16]. It is also used in food production such as local non-alcoholic beverages, industrial wine, jam, marmalade and tea [11]. It is also used for making juice, jelly, syrup, gelatin, pudding, cakes, ice-cream, and also dried and brewed into tea as well as flavours and carbonated soft drinks, other acidic foods, spices and used for butter, pies, sauces, fats and other desserts [17]. Production of non-alcoholic beverage (Zoborodo) from dried red Roselle calyces is very popular in Nigeria. A strong fibre obtained from the stem is used for various household purposes including making sackcloth, twine and cord [18]. Roselle is used for the production of bast fibre and as an infusion, in which it may be known as Carcade [19]. The grinded leaves and seed cakes *(Daddawan Batso)* are added to curries as seasoning. Roselle seed has been the main raw material for the production of Roselle seed cake *(Daddawan Batso)* which is a soup condiment produced by North-Western Community, Nigeria.

However, despite the high economic importance of Roselle, especially its potential as a crop with high export value, little attention has been paid to the crop in the areas of important pests and diseases as well as research for improvement.

Diseases have been reported as a limiting factor to the production of Roselle worldwide [20]. Many fungal and few bacterial diseases of Roselle have been reported from various parts of the world including Nigeria and these include damping-off, vascular wilt, leaf spot, stem and foliar blight, leaf, stem, fruit and root rot [21, 22]. Ogunsola *et al.* also reported an incidence of leaf blight, leaf spot, stem wilt, flower decay and leaf discolouration in Roselle plants cultivated in Northern Nigeria [23]. Apart from fungi, a pathogenic bacterium, *Bacillus solanacearum*, has been isolated from Roselle [2].

Daddawan Batso is a soup condiment produced from *Hibiscus sabdariffa* (Roselle) seeds which added aroma and flavour to dishes such as baobab soup *(Miyar Kuka),* fish pepper soup and chicken pepper soup [25].

Soup condiments therefore, 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 [26]. 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 [24, 27]. They are abundantly produced in Nigeria especially in North-Western part of the country.

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 [28, 29, 30]. Roselle seed cake soup condiment was observed to be a delicacy used as food seasoning, flavour enhancer and aroma that was found to be produced locally at commercial scale in North-Western Nigeria.

Yet, there is a popular claim by consumers that the product is always contaminated by micro-organisms at each stage of the production process and also during transportation and marketing [25].

Micro-organisms are major causes of disease which can even lead to human death. *Daddawan Batso* used as local soup condiment can be contaminated by parasites which are capable of causing several ill-healths to man as well as to other animals. The parasites which are causative agents include: *Entamoeba histolytica* and *Giardia lamblia* [31].

The aim of this research was to determine the proximate composition and parasitic contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake): a soup condiment produced by North-Western Community, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area/Location

The study was conducted in Zuru and Sakaba Local Government Areas (LGAs) of Kebbi State, North-Western Nigeria. Both LGAs are located in the Zuru Emirate Council; one of the four (4) Emirates in the State. The Emirate comprises four (4) Local Government Areas (Danko-Wasagu, Fakai, Sakaba and Zuru). Zuru Emirate is located in South-Eastern part of the State with longitude of 5°14′5.78′ E and latitude of 11°26′6.79′ N.

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 [32]. The postal code of the area is 872 [33]. 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.

Sakaba has an area of 1,260 km^2 and a population of 91,728 people based on 2006 national population census [32]. The area council of Sakaba covers communities of Dokan-Kambari, Dirin-Daji, Dirin-Gari, Dokan-Hausawa, Doka, Doka-Bere, Maza-Maza, Gelwasa, Janbirni and Jandutse [32].

The main occupation of the people in the study area especially in the rural areas is agriculture; farming and rearing of animals, which become their source of income.

2.2 Sample Collection

Samples of *Hibiscus sabdariffa* seed cake (Roselle seed cake) were purchased and collected at random from the markets of different places in the study areas. Three (3) places were randomly selected among Zuru Community which include: Dabai, Bedi and Zuru. However, six (6) places were randomly selected among Sakaba Community which include: Laraba, Makuku, Janbirni, Dankolo, Doka and Dirin-Daji.

The condiments were purchased from different sellers at different selling spots in the various markets selected during the market days. Clean hand gloves were used to place the samples of the condiment aseptically into a sterilized polythene bags and were labelled correctly according to the markets. The samples were then transported to Zoology Laboratory, Kebbi State University of Science and Technology, Aliero for proximate analysis and parasitic protozoan identification [34].

2.3 Isolation and Identification of Protozoan Parasites

For direct microscopy, each sample was divided into portion and soaked into sterile distilled water for 30 minutes. The soaked sample was filtered in order to remove large debris. The solid part of the sample was discarded and the liquid part (suspension) was centrifuged at 1500 rpm for 5 minutes. 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 and provided information on the content of the samples [35].

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.

2.4 Proximate Analysis

2.4.1 Moisture Determination (AOAC, 2000)

Principle: This is based on the principle that a known weight of biological material is exposed to heat under controlled conditions. This is achieved by placing the sample in an oven at 105°C for 24 hours. The water from the material evaporates leaving behind the dry matter. The difference in weight after heating gives the moisture content of the material [36].

Procedure: Cleaned petri-dishes were dried in an oven at 80°C for about 30 minutes, cooled in a desiccator and weighed (W_1). 2.0 g of each of the sample was taken into the petri-dish and weighed (W_2). The sample with the container were dried in an oven at 105°C for 24 hours. It was then transferred into a desiccator to cool and weighed (W_3) with minimum exposure to atmosphere.

Calculation:

$$\% \text{ Moisture} = \frac{\text{Loss in weight due to drying}}{\text{Weight of sample}} \times 100$$

The percentage moisture is shown in Table 1.

2.4.2 Ash Determination (AOAC, 2000)

Principle: This is based on the principle that biological materials such as food when heated in a muffle furnace at a high temperature of 600°C have their organic matter burnt off leaving the inorganic substance in the form of ash. The term ash is used for inorganic residue and the weight expressed as percentage [36].

Procedure: 2.0 g of the sample was placed in a clean pre-weight expressed as percentage. The crucible was transferred into a muffle furnace at 600°C for 2 hours. Thereafter, the crucible was placed in a desiccator, cooled and weighed.

Calculation:

The percentage ash content was calculated using the formula:

$$\% \text{ Ash} = \frac{\text{Weight of crucible + ash – weight of empty crucible}}{\text{Weight of sample}} \times 100$$

The percentage ash content is shown in Table 1.

2.4.3 Determination of Crude Protein (Kjeldahl, 1973)

Principle: This process involves the oxidation of organic matter (i.e. protein) with concentrated sulphuric acid (conc. H_2SO_4) and the reduction of nutrient to ammonium sulphate. The subsequent addition of excess amount of NaOH in a closed system neutralizes the acid and releases ammonium which is distillated into boric acid solution and titrated against 0.1N HCl to end point [37].

Procedure: There are three steps involved in this analysis which include digestion, distillation and titration.

- **Digestion:** 2.0 g of the dried (ground) sample was transferred into a micro-kjeldahl flask and digestion tablets were added. The 15 cm^3 of conc. H_2SO_4 was added to the sample mixtures of the micro-kjeldahl flask and heated using a digestion block (heater) in a fumed cupboard continuously until the nitrogen present in the sample reduces to ammonium sulphate.
  Organic matter (sample) + H_2SO_4 → $(NH_4)_2SO_4$ + CO_2

- **Distillation:** The digest was diluted to 50 cm^3 with distilled water. 10 cm^3 of the sample aliquot, 40 cm^3 of the distilled water and 20 cm^3 of 40% NaOH were transferred into a micro-kjeldahl flask. The distillate was collected into a flask containing 10 cm^3 of boric acid and few drops of methyl orange indicator which gives a green colour distillate.

  $(NH_4)_2SO_4$ + $2NaOH \rightarrow$ $2NH_3$ + $2H_2O_{(g)}$ + $Na_2SO_{4\,(aq)}$
  NH_3 + H_3BO_3 → $NH_4^+{}_{(aq)}$ + $H_2BO_3^-{}_{(aq)}$

- **Titration:** The distillate content in the flask was titrated against 0.01N HCl and the colour changed from green to purple at the end point. The titre values were recorded and the average titre value was calculated. This was used to determine the percentage nitrogen.

  Titration: $H_2BO_3^-{}_{(aq)}$ + $H^+{}_{(aq)}$ → $H_3BO_{3\,(aq)}$

Calculation:

If the titre value was found to be (TV), then the concentration of protein can be calculated as follows:

$$\%\ N = \frac{TV \times NA \times 0.014 \times DF}{\text{Vol. of Aliquot} \times \text{sample weight}} \times 100$$

$$\%\ \text{Crude protein (g)} = CF \times \%\ \text{Nitrogen}$$

Where;

Sample weight	=	2.0 g
Vol. of Aliquot	=	10 ml
Conversion Factor (CF)	=	6.25
TV	=	Titre Value
NA	=	Normality of Acid
DF	=	Dilution Factor

The percentage crude protein content is shown in Table 1.

2.4.4 Lipid Determination (Oyeleke, 1984)

Principle: The method of Oyeleke (1984) was employed in this determination. This is based on the principle that non-polar components of the sample are easily extracted into organic solvent, which gives proportion of the true fat present but does not give the particular fatty acid present [38].

Procedure: 250 ml extraction flask was dried in an oven at 105 – 110°C. It was then allowed to cool in a desiccator. The empty extractor flask was weighed as (W_1). 2.0 g of the sample was weighed into a labelled thimble. The porous thimble mouth was covered with cotton wool. 200 ml of n-hexane was then added into the dried 250 ml extractor flask. The covered porous thimble was placed in a condenser and the apparatus assembled. It was extracted for about 5 – 6 hours. The porous thimble was removed with care and the n-hexane collected in the top container for reuse. The thimble containing the sample was oven dried at 105 – 110°C for one hour. It was cooled in a desiccator and the weight is taken.

Calculation:

$$\%\ \text{Lipid} = \frac{\text{Weight loss}}{\text{Sample weight}} \times 100$$

The percentage lipid content is shown in Table 1.

2.4.5 Fibre Determination (AOAC, 2000)

Principle: This is the sequential digestion of the sample with dilute acid and alkaline solution [35].

Procedure: 2.0 g of the sample was introduced into a conical flask. 100 ml of distilled water and 20 ml of 10% H_2SO_4 were added then fixed on a heat for 30 minutes. The sample was filtered in a muslin cloth, rinsed with water and spatula was used to scrap the sample into the flask. It was then heated again for 30 minutes, filtered in a muslin cloth and rinsed with ethanol. It was allowed to drain and the residue was scrapped into a pre-weighed crucible (W_1). It was then put into a muffle furnace to ash for 2 hours at 600°C and allowed in a desiccator and weighed (W_2). The percentage was then calculated.

Calculation:

$$\% \text{ Fibre} = \frac{W_1 - W_2}{2} \times 100$$

Where;
W_1 = Dried weight
W_2 = Ash weight

The percentage fibre content is shown in Table 1.

2.4.6 Carbohydrate Estimation by Difference (Oyeleke, 1984)

Principle: The method of Oyeleke (1984) was employed in this determination. This method is known as estimation by difference [38].

Procedure: The total of carbohydrate in the sample was estimated with calculation by difference that is, by subtracting all the other calculated nutrients like % ash, % crude protein, % lipid and % moisture from 100%. The remainder account for the total percentage of carbohydrate in the sample. This is known as calculation by difference.

Calculation:

Carbohydrate = 100% – (% ash + % protein + % lipid + % moisture).

The percentage carbohydrate content is shown in Table 1.

2.5 Data Analysis

The statistical tools used to analyse the data on proximate composition and parasitic contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake) were mean standard deviation and percentage. The statistical tools were employed in order to calculate the number of parasitic protozoan contamination and the nutritional content from Roselle seed cake samples.

3. RESULTS

Table 1. Proximate Composition of *Hibiscus sabdariffa* Seed Cake (Roselle Seed Cake) in the Study Areas.

Parameter (%)	% Composition of Samples
Moisture	15.43 ± 0.10
Ash	7.80 ± 0.05
Crude Protein	1.16 ± 0.13
Lipid	12.00 ± 0.50
Fibre	11.16 ± 0.58
Available Carbohydrates	52.44 ± 1.03

Key: % **=** Percentage. Values are presented as mean ± standard deviation (n = 9).

Based on the result shown in Table 1, the proximate composition of *Hibiscus sabdariffa* seed cake (Roselle seed cake) in the study areas has a very high level of carbohydrate content (52.44±1.03%), followed by the moisture content (15.43±0.10%), lipid content (12.00±0.50%), fibre content (11.16±0.58%), ash content (7.80±0.05%) and crude protein content (1.16±0.13%) which is the lowest with the respective compositions tabulated in percentage.

Table 2. Protozoan Contamination of *Hibiscus sabdariffa* Seed Cake (Roselle Seed Cake) in Zuru Local Government Area.

Sample	Protozoa	No. of Protozoa	Percentage
Zuru	Not seen	Nil	0.0%
Dabai	*Giardia lamblia*	1	25.0%
Bedi	*Entamoeba histolytica*	2	50.0%

Table 2 above showed the protozoan contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake) in Zuru Local Government Area. No protozoa was seen in Zuru sample 0(0.0%). Therefore Zuru sample showed negative result. In Dabai sample, *Giardia lamblia* was seen 1(25.0%). While in Bedi sample, *Entamoeba histolytica* 2(50.0%) was seen which is the most prevalent.

This showed that Bedi sample has the highest number of contamination.

Table 3. Protozoan Contamination of *Hibiscus sabdariffa* Seed Cake (Roselle Seed Cake) in Sakaba Local Government Area.

Sample	Protozoa	No. of Protozoa	Percentage
Dirin-Daji	*Entamoeba histolytica*	1	11.1%
Doka	*Entamoeba histolytica*	2	22.2%
Dankolo	*Entamoeba histolytica*	2	22.2%
Janbirni	*Entamoeba histolytica*	1	11.1%
Laraba	*Entamoeba histolytica*	1	11.1%
Makuku	*Entamoeba histolytica*	2	22.2%

Table 3 above showed the protozoan contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake) in Sakaba Local Government Area. A total of nine protozoa was seen and all were *Entamoeba histolytica*. Dirin-Daji sample showed 1(11.1%), Doka 2(22.2%), Dankolo 2(22.2%), Janbirni 1(11.1%), Laraba 1(11.1%) and Makuku 2(22.2%) respectively.

4. DISCUSSION

Proximate analysis was carried out on *Hibiscus sabdariffa* seed cake (Roselle seed cake) in the study areas to determine its nutritional compositions (Table 1). The result of proximate composition revealed that the samples had an average moisture content of 15.43±0.10%. This is higher when compared to moisture content of *Hibiscus* seeds reported by Aletan and Kwazo [39]. Moisture content of seeds, fruits and vegetables is indicative of their shelf life. The higher the moisture content, the more susceptible the seeds, fruits and vegetables are to microbial attack and reduce their shelf life [40]. Therefore, the food condiments should be properly dried before storing.

The crude fibre content was found to be 11.16±0.58%. The value obtained is lower than 13.87±0.67% reported for dried *Hibiscus* seed by Tounkara *et al.* [41]. Fibre helps in the maintenance of human health and has been known to reduce cholesterol level, aids digestion and delays emptiness of the stomach [42]. Thus, the fruit is a good dietary fibre and has the potential of providing body requirements of fibre.

The ash content was 7.80±0.05%. The value is higher than 4.40±0.02% reported by Al-Wandawi *et al.* [43]. The result showed that the samples contained a good amount of inorganic matter which is confirmed by the mineral analysis result. This is in accordance to the findings of Fagbohun *et al.* [44] that ash content in vegetables and other samples may be an index of the amount of mineral elements present in the vegetables. The result indicated that the food condiments could supplement the body with some of the macro and microelements required.

The crude protein was found to be 1.16±0.13%. The crude protein content was lower when compared to that of 4.73% in *Hibiscus* seed reported by Oduntan *et al.* [45]. This is an indication that the pulp contains low protein which is known for growth and repair of worn out tissues. Muhammad *et al.* also reported low level of protein in the Roselle seed [46].

Lipid content was found to be 12.00±0.05, which is lower than 23.80±0.50% reported for dried *Hibiscus* seed by Emmy Hainida *et al.* [47]. However, since the condiment contains moderate amount of crude lipid, it could be a good source of edible vegetable oil if well harnessed, and could complement the conventional sources. Lipids provide the body with more energy; approximately twice that of protein and carbohydrate and facilitate intestinal absorption and transportation of fat soluble vitamins [48].

The available carbohydrate content of the soup condiment was found to be 52.44±1.03%, hence is comparable to 52.28% reported for Kenaf seed (African Hibiscus/*Hibiscus cannabinus)* by Khan *et al.* [49]. The condiment has high carbohydrate content. Its consumption could provide the body with fuel and energy that is required for daily activities and exercises [39]. Adequate carbohydrate is also needed for optimum function of the brain, heart, nervous, digestive and immune systems while carbohydrate deficiency causes depletion of these body tissues [39].

The present study carried out on the local soup condiment *(Daddawan Batso)* revealed some of the protozoan species that can possibly contaminate the condiment in the study areas. Table 2 above showed the protozoan contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake) in Zuru Local Government Area. No protozoa was seen in Zuru sample 0(0.0%). Therefore Zuru sample showed negative result. In Dabai sample, *Giardia lamblia* was seen 1(25.0%). While in Bedi sample, *Entamoeba histolytica* 2(50.0%) was seen which is the most prevalent. This showed that Bedi sample has the highest number of contamination. It is quintessential to note that, these soup condiments are produced by different manufacturing personnel, which may be the reason for the result obtained. Dabai and Bedi are extremely rural areas with little or no hygiene. While, Zuru is more urban than both and therefore, no contamination occurred. Again, Dabai and Bedi have little or no enough toilet facilities, defecating randomly and openly. There is possibility that the producers of these condiments also rear animals. As such, flies perch on the human and animal droppings. The flies may carry or bear the ova of these protozoa and perch on the condiments thereby making the condiments contaminated. Table 3 above showed the protozoan contamination of *Hibiscus sabdariffa* seed cake (Roselle seed cake) in Sakaba Local Government Area. A total of nine protozoa was seen and all were *Entamoeba histolytica*. Dirin-Daji sample showed 1(11.1%), Doka 2(22.2%), Dankolo 2(22.2%), Janbirni 1(11.1%), Laraba 1(11.1%) and Makuku 2(22.2%) respectively. However, there is dearth of published information on parasitic contamination of *Hibiscus sabdariffa* seed cake in Nigeria.

Most previous studies were mainly on investigating the nutrient composition of fermented condiments [30, 39, 40, 41, 45, 46, 47, 49]. 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 [50]. 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 [51]. It was generally observed that the water samples used in rinsing the boiled Roselle seeds 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. 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 [52]. In a nutshell, *Entamoeba histolytica* and *Giardia lamblia* were identified as the possible protozoa that can contaminate *Hibiscus sabdariffa* seed cake (Roselle seed cake) in the study areas. 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 to prepare and process these condiments under better hygienic processes.

5. CONCLUSION

It can be concluded based on the results obtained from this study on the proximate analysis of *Hibiscus sabdariffa* seed cake (Roselle seed cake) collected from the nine study areas in Zuru and Sakaba Local Government Areas that the available carbohydrate and moisture contents were higher than other parameters. Again, the local soup condiments were heavily contaminated with protozoa and *Entamoeba histolytica* had the highest prevalence. This revealed that the 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, 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 *Hibiscus sabdariffa* seed cake (Roselle seed cake) is widespread. Therefore, the need for proper hygiene and sanitation is paramount and indispensable.

6. RECOMMENDATIONS

The findings from this research work showed that processed *Hibiscus sabdariffa* seed cake (Roselle seed cake) produced and consumed in the study areas were heavily contaminated with protozoa. 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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