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Proximate Composition and Microbial Evaluation of *Kindirm*o Sold in Okada, Benin City

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Abstract: *Kindirmo is* a traditional fermented dairy product among the Fulanis and Hausas of Northern Nigeria. This study was carried out to evaluate the proximate composition and microbial load of *kindirmo* sold in Okada in Ovia North-East Local Government Area. Eighty (80) *kindirmo* samples were collected from Okada, bulked, homogenized and analyzed. Samples from four treatments (T1 - January, T2 - February, T3 – March, and T4 - April) were evaluated chemically according to AOAC and microbiologically with five replicates per treatment, using the standard plate count method. In addition, 0.1 mL of samples were collected from dilutions 5 and 6, inoculated in plates containing NA and PDA and observed for 24 and 48 hours, respectively. Colony forming units were calculated, microorganisms were identified biochemically, culturally, and morphologically. Data obtained were subjected to one way ANOVA using the Genstat statistical package. Results of pH ranged between 3.68- 4.08, and the range of moisture content, ASH, NFE, total solids values are 79.87-81.10%, 1.65-1.72%, 5.58-6.84% and 10.55-10.58%, respectively, with non-significant variations from January to April. Significant variations were recorded for fat and CP values. Fungal load ranged from 6.20-43.00 x 10^6 CFU/mL, while bacteria load ranged from 19.80 -74.80 x 10^6 CFU/mL, both significantly above acceptable limits (100,000 CFU/mL of bacteria in raw milk after pasteurization) approved by the Food and Drug Administration (FDA). Microbial examination results revealed the presence of both beneficial (*Bacillus subtilis*) and pathogenic (*Acinetobacter* and *Aspergillus flavi, Samonella spp and Mucor*) microbial species in the samples analyzed during the four months under study. In conclusion, the microbiological load of the *kindirmo* samples was above the acceptable limits, and therefore, stringent hygienic standards and improved processing practices/methods are recommended to reduce microbial contamination to safe levels before *kindirmo* can be considered safe for consumption.

Keywords: *Kindirmo*; Microbial evaluation; Biochemical test; Microbial load; Microorganisms.

INTRODUCTION

According to Adewumi et al. (2015), Nigeria's dairy industry generated an estimated 450, 000 tons of milk annually, and Federal Ministry of Agriculture and Rural Development in 2021 put the estimated milk yield at 700,000 metric tons, which is insufficient to meet Nigerians' needs for dairy products. The Nigerian dairy sector is largely disjointed, unproductive and inefficient despite its size (Posthumus et al., 2018). Though smallholder dairy households (i.e. pastoralists) produce most of the raw milk in Nigeria, the end market is controlled by large multinationals that use imported milk in over 97% of products consumed (2019). In Nigeria, most of the raw milk produced is sold or processed informally by Fulani women into local products such as *Kindirmo, Mai-Shanu, Nono,* *Fura da nono* by applying heat treatment to extend the product's bacterial shelf life and homogenization to extend the physical shelf life by delaying fat separation (Juffs *et al.,* 2007). Only a small percentage estimated at 5% is purchased by a few formal processors.

Kindirmo, a fermented dairy product also known as local yogurt, is a dairy and popular treat among northerners in Nigeria. Fermented dairy products are now widely recognized to provide nutrients to both young and old individuals worldwide (Adesokan *et al*., 2011). This is because some of the microorganisms involved in the processing of these products are capable of producing several metabolites that not only add value in the form of taste, aroma, and firm consistency that are unique and appealing to consumers during fermentation but also have positive health implications (Igwegbe et al., 2015; Agunwa et al., 2019; Anyanwu et al., 2019).

MATERIALS AND METHODS

Study Location

This study was conducted in Okada town in Ovia North East Local Government Area of Edo state, with an area of 2,301 km^2 which lies on Longitude 5ºº23'40.1" E, and Latitude 6º44'05.6" N. It is located in the rain forest region of Southern Nigeria; average annual rainfall of about 2000 mm; temperature range 25-35ºC; and relative humidity 75-95%. The State is bordered in the north by Kogi State, in the south and east by Delta State, and in the west by Ondo State. The experiment was carried out in the Animal Science laboratory, University of Benin, Benin City, Edo State, Nigeria.

Figure 1: Map of Study Area

Sample Collection

Eighty (80) samples of *kindirmo* were bought fresh and used for this experiment. Twenty (20) samples each were collected monthly for four (4) months (January, February, March and April, respectively) from the Fulani cattle community in Okada. The samples were collected in sterile bottles and stored at 4ºC for about one week before the commencement of proximate analysis.

Sterilization of work area and materials

All laboratory work was carried out under aseptic condition while following laboratory rules and regulations. The work bench was disinfected with 70% ethanol, and glass wares were properly washed and sterilized in an autoclave at 121°C at 15 mmHg for 15 psi before use. Chemical analysis was performed using the method of AOAC (2016)

Microbial Analysis

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

Preparation and sterilization of culture media

The media used were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C at 15 psi for 15 minutes. The media used were nutrient agar and potato dextrose agar.

Preparation of Nutrient Agar

Twenty-eight grams (28 g) of nutrient agar (NA) powder was dissolved in 1000 mL of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 mL of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 minutes at 121°C. After sterilization, the flask containing the medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes.

Preparation of Potato Dextrose Agar (PDA)

PDA medium (39 g) was dissolved in 1000 mL of distilled water in a conical flask and then closed with a cork stopper. The suspension was first dissolve completely by shaking and then sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool, then dispensed aseptically into sterile petri dishes. The petri dishes were covered and allowed to solidify (Needam *et al*., 2019).

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Enumeration and isolation of total heterotrophic bacterial

One-thousand-fold serial dilution of the fish and meat samples were prepared aseptically in sterile physiological saline. An aliquot of 1ml was inoculated using the pour plating technique. Appropriate media were used for fungal and bacterial enumeration. Nutrient agar (supplemented with fluconazole) and Potato dextrose agar was used for bacteria and fungi load assessment, respectively. Plates were cultured at 37±2^{o}C for 24 hours.

The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula shown below (Bardosa *et al.,*2008):

$$\text{cfu/mL} = \frac{\text{Number of colonies x Dilution fold/series}}{\text{Volume of inoculum}}$$

Colony forming units were calculated using a colony counter to determine the microbial load from distinct colonies in pure cultures obtained from plates incubated with samples for one day at 28°C. Microorganisms were identified culturally (shape, elevation, margin, colour and size); morphologically (gram staining, cell type and cell arrangement) and biochemically (catalase, oxidase, urease, citrate utilization and indole tests)

Statistical Analysis

Data collected were subjected to one-way ANOVA using the GENSTAT (12th edition) statistical package and means were separated using Duncan Multiple Range Test, where significant differences existed and were tested at 5% significance level.

RESULTS

Results of the study are as presented. Plates 1, 2, 3, 4, 5 and 6 shows the different microorganisms from the pure cultures of the samples as well as the respective months during which they were predominant.

Plate 1: *Phycomycetes /Mucor sp* sub-cultured from plate (JANUARY AND FEBRUARY)

Plate 2: *Saccharomyces/ Yeast sp* subcultured plate (JANUARY/FEBRUARY/APRIL)

Plate 3: *Acinetobacter sp* sub-cultured from plate (FEBRUARY)

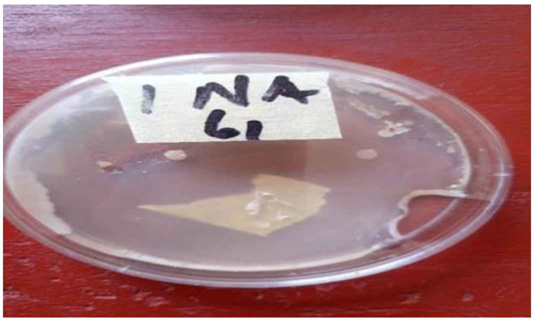

Plate 4: *Bacillus subtilis sp* sub-cultured from plate (JANUARY/FEBRUARY)

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Plate 5: *Alternaria sp* sub-cultured from plate (APRIL)

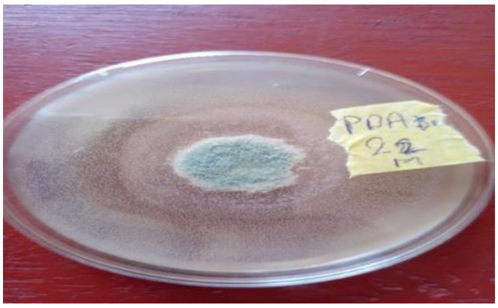

Plate 6: *Aspergillus flavus* sub-cultured from dish (FEBRUARY /MARCH)

Table 1 presents the proximate composition of *Kindirmo* samples collected in Okada from January-April. The moisture contents values, which ranged from 79.87-81.10% and the ash values ranging from 1.650-1.722%, were not significantly affected by the months during which the samples were collected. *Kindirmo* samples collected in April recorded the highest crude protein (CP) value (12.25%), which was significantly higher than the CP values obtained in January, February and March. The fat content was highest in the month of March, though not significantly different from values recorded in January and February. Similarly, NFE values obtained in March was not significantly different from that obtained in February but differed from NFE values recorded in January and April.

Table 1: Proximate composition of *Kindirmo samples* collected from January-April in Okada Community in Edo State

MONTHS	MC	CP	ASH	FAT	NFE
JANUARY	81.10	10.51^{b}	1.710	1.100^{ab}	5.580^{bc}
FEBRUARY	80.32	10.53^{b}	1.720	1.100^{ab}	6.360^{ab}
MARCH	80.11	10.50^{b}	1.722	1.200^{a}	6.480^{a}
APRIL	79.87	12.25^{a}	1.650	0.970^{b}	5.260^{c}
SEM	2.32	0.337	0.086	0.041	0.245

*ab for each parameter, means with different superscripts along columns vary significantly. SEM= standard error of mean; ASH= Ash content; CP= Crude Protein; NFE= Nitrogen-free extracts; MC= Moisture content

Table 2: Microbial Load of *Kindirmo* Samples Collected from January- April in Okada Community in Edo State

	TREATMENTS (MONTHS)				
Microbial Load	**January**	**February**	**March**	**April**	**SEM**
Fungi	$15.20 \times 10^{6\,b}$	$6.20 \times 10^{6\,b}$	$27.40 \times 1^{6\,ab}$	$43 \times 10^{6\,a}$	8.05
Bacteria	$19.80 \times 10^{6\,b}$	$20.00 \times 10^{6\,b}$	$74.20 \times 10^{6\,a}$	$74.80 \times 10^{6\,a}$	10.20

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Table 3: Cultural and Morphological Characteristics of Fungal Isolates from January - April in Okada Community

	Months			
Parameters	**January**	**February**	**March**	**April**
Colour of mycelium on agar plate	Cream front color	Greenish-blue, woolly with profuse growth	Black mass of mycelium	*Pyenidia* black
Colour of plate culture reverse	Dark cream	Dark	Dark brown	Darkish
Nature of hyphae	Septate	Septate	Septate	Non-septate
Type of Spore	Haploid spore	Conidiospore	Conidiospore	Sporangiospor e
Conidia	Absent	Present	Present	Absent
Rhizoids	Absent	Absent	Absent	Absent
Spore colour	Absent	Absent	Absent	Absent
Appearance of special structure	Fruiting heads	Dark	Brown	White
Class of fungi	Saccharomycetes	Ascomycetes	Dothideomycetes	Phycomycetes
Possible Identity	*Yeast*	*A.flavus*	*Alternaria* spp.	*Mucor*

Table 4: Cultural, morphological and biochemical characteristics of bacteria isolates

	MONTHS		
	JANUARY/FEBRUARY	**JANUARY/MARCH**	**FEBRUARY/APRIL**
Cultural tests			
Shape	Circular	Filamentous	Circular
Elevation	Flat	Flat	Umbonate
Margin	Entire	Lobate	Entire
Colour	Cream	White	Green
Size	Small	Big	Small
Morphological tests			
Gram staining	+	-	-
Cell type	Rod	Rod	Rod
Cell arrangement	Pairs	Pairs	Cluster
Biochemical:			Biochemical:
Catalase	+	+	+
Oxidase	-	-	-
Urease	-	+	-
Indole	-	-	-
Citrate	+	+	+
Fermentation:			Fermentation:
Glucose	+	+	+
Lactose	+	-	-
Sucrose	+	-	-
Gas	-	-	-
H_2S	-	+	-
Possible identity	*Bacillus subtilis*	*Salmonella sp.*	*Acinetobacter sp*

+ = positive/present; - = negative/absent

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DISCUSSION

Results obtained for the moisture content of *kindirmo* samples collected at Okada from January to April are in consonance with the work of Abubakar *et al*. (2023) whose values ranged from 74.90-78.10% as well as the values (78.62-82.41 %) reported by Igbabul et al*.* (2014). However, the values were lower than the moisture content values (87.5-90.4%) reported by Maikano and Umar (2019) and the 88.0-92.5% reported by Diko et al (2011) for *kindirmo*. The variation of the moisture content may be attributed to the volume of water used during dilution in the course of *kindirmo* preparation, the duration of storage and the weather condition of the months during which the *kindirmo* was produced, because January and February were drier months compared to March and April (advent of rains). The ash values obtained during this study from January to April were higher and at variance with those of Abubakar *et al*. (2023); Dandare et al. (2014) and Maikano and Umar (2019), which were 0.68%-0.74%, 0.73% and 0.47-0.79%, respectively. This higher mineral content may be due to milk production systems; percentage of fermentable bacteria in the sample; variations in water quality and feed; mineral availability in different areas and environmental factors like dust, dirt and contaminated equipment. The fat content values of *kindirmo* sampled between January and April showed no significant variations and were at variance and lower than the 4.40% recorded by Okeke et al. (2016). The Fat content recorded for *kindirmo* samples used during this study were also lower than the 3.20-3.70 % fat content recorded by Abubakar et al. (2023) and 3.4-3.6% recorded by Maikano and Umar (2019). The lower fat content obtained during this study can be attributed to the age and stage of lactation of the cow that was milked.

Results of protein content obtained during this study showed significant variations among the months. The highest protein value was recorded in April and varied significantly from the values obtained in January, February and March. However, protein values recorded for *kindirmo* in this work are consistent with the 11.60% reported by Okeke et al. (2016). This can be attributed to the non-dilution of the sample with water after fermentation by the vendors (Aliyu et al., 2019). Furthermore, this variation can be attributed to difference in feeding regime, milk protein concentration and milk urea concentration (Nichols et al., 2018). The nitrogen free extract (NFE) values of *kindirmo* samples obtained from this study were at variance with and lower than the value of (12.98%) reported by Maikano and Umar (2019). The carbohydrate values obtained from this study is in variation with a study by Shibdawa et al. (2018) where the carbohydrate of higher values was between 11.31-12.49 %. The lower carbohydrate values could be attributed to the process of fermentation which converts carbohydrates, basically lactose to lactic acid. This makes the *kindirmo* an ideal food for lactose intolerance individuals (Ehirim and Ndimantang, 2004). The pH of *kindirmo* obtained from this study in the first month was the most acidic with a pH value of 3.68, followed by values of 3.89 and 3.92 for the third and fourth months, respectively with the second month being the least acidic with a pH value of 4.08. Generally, the variation in moisture, fat, protein, ash, and carbohydrate in all the *kindirmo* samples can be associated with differences in feeding regime, breed, age, stage of lactation, and health status of the milked cows.

Microbial load of *Kindirmo*

Microbial results obtained during this study were higher than and at variance with the Food and Drug Administration (FDA) recommended safety levels of 100,000 cfu/mL of bacteria in milk, 300,000cfu/mL for Commingled, and 30,000 CFU/mL for total bacteria. Total bacterial counts obtained were higher than the 3.82×10^6 CFU/mL (John et al., 2024) in *kindirmo* samples sold in Jos Metropolis, Nigeria, as well as the and bacterial counts ranging from 3×10^3 to 24×10^6 CFU/mL reported by Egwaikhide et al. (2009), although their values were also above the maximum recommended level of log10 6.30 CFU/mL (2.0×10^6 CFU/mL) as the standard set by East Africa Community Standards (EAS 67: 2007), and log10 5.70CFU/mL (5.01×10^6 CFU/mL) given by Thai Agricultural Standard (TAS 6003—2010). The variations in the microbial counts of the samples over the sampling period (January-April) may be due to the presence of contaminants possibly from lactating cows, milking equipment, storage containers, poor handling practices at farm level as well as production stage, unsuitable storage condition, use of unclean water, lactating cows with unclean udder/ or teats and poor personal hygiene of the milkers (Bukuku, 2013; Kenyaka, 2014). The higher load of micro-organism reported during this study could also be as a result of the spontaneous fermentation, which takes place during the traditional method of production of *kindirmo* (Igwe et al., 2016)

Cultural, morphological and biochemical analyses of fungi and bacteria isolates in *Kindirmo*

The results of this study's cultural, morphological, and biochemical analyses of fungi isolates in *kindirmo* showed that *yeast* was present in the January samples and, it is a beneficial organism in *kindirmo* production because it aids in the fermentation of lactose and sucrose and the hydrolysis of milk casein (Fleet and Mian, 1987). *Aspergillus flavus*, was seen in samples collected in February, and has been proven to be harmful because it produces aflatoxins, which are hazardous to human health (Bennett and Klich, 2003). *Alternaria sp*. was also found in the *kindirmo* samples collected in March. It has been proven to be detrimental because it produces host-specific toxins (HSTs) and non-host specific toxins (nHSTs) that target various areas of cells such as mitochondria and the nucleus (Meena *et al*., 2017). *Mucor*, another detected fungal species, is harmful rather than useful since it spoils dairy products such as yogurt, resulting in significant food waste and economic losses (Shi et al 2022).

The results of the analysis of cultural, morphological, and biochemical characteristics of bacteria isolates of *kindirmo* obtained from this study showed that *Acinetobacter* was found in samples collected in February and April. The bacteria isolates were found to be harmful in dairy as it represents a serious health problem, especially because of

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their resistance to colistin, an anti-biotic treatment for pneumonia (Malta *et al.,* 2020). *Bacillus subtilis* was also identified in January/February samples. *Bacillus subtilis* are a beneficial bacteria species because they are frequently added to fermented foods such as yogurt, which aids the body's digestion and nutrient absorption (Adamski *et al*., 2023). *Salmonella* sp*,* was identified in *kindirmo* samples collected in January and March. This bacterial species is harmful and remains a major concern for the dairy industry because contamination in dairy products causes salmonellosis, a bacterial disease that affects the intestinal tract and causes diarrhea (Center for Disease Control and Prevention, 2024). Knowledge of these microbial loads of *kindirmo* helps to reveal the safety of the food as well as protect consumers from spoilage organisms and food borne pathogens samples.

CONCLUSION

This study demonstrated that while *Kindirmo*—a fermented milk product offers significant nutritional benefits to consumers, its fermentation, a highly traditional production process remains highly susceptible to contamination. Its proximate composition showed comparable results to those found in previous research. However, the microbial loads were found to be significantly higher than recommended safety limits, making its consumption a potential health risk.

Over a four-month collection period, various fungi and bacteria were identified, some of which are dangerous to health when consumed. To mitigate these risks, fermentation practices should be improved and standardized. Stricter hygiene protocols, from initial milk collection through to the final product, are essential to reducing the microbial load and thus recommended to ensure the safety of *Kindirmo* for human consumption."

Conflict of Interest

There are no conflicts of interest declared by the authors.

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