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# Microbiological safety assessment of two types of locally made cheese sold in Malete, Kwara State

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## ABSTRACT

Cheese is a protein rich food with high nutritional benefits. Fulani cheese is a dairy product derived from milk that is produced in a wide range of flavors, textures and forms by coagulation of the milk protein while soy cheese is derived from the coagulation of soya beans milk. The study assesses the microbiological safety of Fulani and soy cheese sold in Malete, Kwara State. The cheese samples were purchased from local sellers in Malete village. Microbial isolation was done through serial dilution and pour plate technique; colonies obtained were identified morphologically and biochemically. The identified bacterial isolates were *Salmonella*, *Enterobacter*, *Serratia*, *Klebsiella*, *Citrobacter*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* and *Proteus* sp while the fungal isolates were *Penicillium*, *Rhodotorula*, *Aspergillus* and *Fusarium* spp. *Staphylococcus aureus* had the highest percentage of occurrence in both cheese samples, followed by *Salmonella* and *Klebsiella* species. Most of the bacterial isolates are human pathogenic organisms. The fungal isolated are known food spoilage agents with potential of releasing mycotoxins in food. It was concluded that both Fulani and Soy bean cheese sold in Malete village are contaminated heavily and could pose health threat to consumer.

**Keywords:** Fulani cheese, Soy-bean cheese, Bacteria, Fungi, Pathogen

## 1. INTRODUCTION

In recent years, small-scale and cottage industries have evolved in cheese production worldwide and Nigeria is no exception (Costanzo et al., 2020). This can be traceable to the increase in demand for ready to eat foods of which local cheese is prominent (Muhammad et al., 2016). The cost of procuring animal protein like meat and fish is almost beyond the reach of the majority in developing countries who often find alternatives in locally produced cheese (Bristone et al., 2018), moreover the love for "quick food" among the students and working class. Food-safety hazard generally refers to the presence of any contaminants in a food which may be biological, chemical or physical agent that could cause adverse health consequences for consumers (McDonald and Scott,

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2018; Costanzo et al., 2020; Owusu-Kwarteng et al., 2020). Such hazards may be introduced into the food at any time during processing.

Consuming microbiologically contaminated food often results in ill health of various status and sometimes death (Bristone et al., 2018; Costanzo et al., 2020). Food contamination has been recognized as a global challenge in several documents and reports (WHO, 2015; Fakuda, 2015; WHO, 2020). An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420, 000 die every year, resulting in the loss of 33 million healthy life years (DALYs) (WHO, 2020). Microbial food poisoning/illnesses occurs as a result of eating food or water contaminated by microorganisms (bacteria, mould, virus, parasites) or their toxic metabolites or both. The symptoms of food poisoning vary and most often depends on the causative agent and most often include but not limited to abdominal cramps, nausea, vomiting, diarrhea, and fever (Owusu-Kwarteng et al., 2020; WHO, 2020).

Dairy cheese is mostly milk curd - substance formed from the coagulation of milk, pressed or molded into a solid mass (LaBarbera, 2012). The major content of cheese is concentrated milk solids, water, rennet, salt and sometimes bacterial cultures and calcium chloride (Farah and Fischer, 2004). Cheese can also be prepared from non-dairy products such as soybean (Schaeffer, 2012; Bristone et al., 2018). Soybean cheese is a healthy, rich and less expensive source of nutrient especially for the developing countries (Tamime et al., 2011; Nazim et al., 2013; Bristone et al., 2018). Soybean cake is made from a legume and legumes are known to be very rich sources of protein. It is the most important and popular food product from soybean in many communities also gaining increasing popularity (Yusuf and Ali, 2013; Bristone et al., 2018).

Soybean cheese was developed some 2000 years ago and has become the world's most popular soy food product due to its high protein content (Egbo, 2012) and to its high nutritional and excellent functional properties. It is very rich in carbohydrates and oil, antioxidants, essential nutrients including many amino acids and well digested if properly processed (Beatrice and Oguntoyinbo, 2012; Kolawole et al., 2015). Soy-bean cheese has been accepted as meat substitute in rural and urban areas of Nigeria. Local processing of soy-cheese is usually done at the home level and usually has no good processing methods (Bristone et al., 2018).

The high protein content and other nutritional content of these products coupled with high moisture content makes it suitable to the growth of microorganisms, especially with no good manufacturing process, poor hygiene of handlers and environment with poor storage and vending conditions. The product though very nutritious is usually of poor shelf life as describe by Fasoyiro, (2011). It is important to provide current knowledge on microbial contaminants and possible health hazards associated with local cheese consumption in Malete, with student community of about 10,000 in population and the location of Kwara State University, this study aims to investigate the microbiological safety of two locally produced, vended and consumed cheese: Fulani cheese and soya beans cheese in Malete.

## 2. MATERIALS AND METHODS

**Collection of Samples**

Fresh samples of both the Fulani and soyabean cheese were obtained from Malete village in clean sterile containers and were conveyed to the laboratory immediately for analysis.

**Isolation of Microorganisms**

Serial dilution was carried out on the samples, plating was done using 0.1 ml of the homogenate inoculated into Petri dishes containing nutrient agar (for general bacteria), Saboraud dextrose (for fungi), Mannitol salt (for *Staphylococcus aureus*), Eosin Methylene blue (for faecal coliform) and MacConkey agar (for total coliform) via pour plate technique. The plates were swirl round for proper mixing before the content was allowed to solidify. The inoculated plates except Sabouraud dextrose agar (SDA) plates were incubated at $37^0$C ($44^0$C for faecal coliform) for 24 hours while the Sabouraud dextrose agar (SDA) plates were incubated on the lab bench for 48 hours. The developed colonies of discrete organisms on plates were observed and counted (Oyeleke and Manga, 2008). The isolates were sub cultured repeatedly to obtain pure culture. The colonies from Fulani cheese were labelled as Fcwhile those from soy cheese were labelled as Sc for the soy bean cheese. The isolates were maintained on slants at $4^0$C for further research.

**Morphological and Biochemical Characteristics of isolates**

Isolates were characterized morphologically and biochemically; the observable characters were used for identifying the organisms by comparing with standard reference organisms (Muhammad et al., 2016). Gram's reaction was done as described by them. Gram

positive cells will stain purple to blue while Gram negative cells stain red to pink. Biochemical tests done were: Catalase test, coagulase test, indole, citrate and oxidase tests and spore detection (Oyeleke and Manga, 2008).

**Identification of Fungi**

The fungal isolates were characterized and identified macroscopically and microscopically using morphological features like hyphal and spore shape, color, texture and other relevant features of the colonies. Staining with a drop of cotton blue in Lactophenol reagent was used to enhance microscopy. The characters obtained were compared with literature for identification using fungal atlas (Hunter and Bamett, 2000).

## 3. RESULTS

**Total Microbial Counts**

The total bacterial counts of Fulani cheese and soy beans are: - Bacteria on nutrient agar 28 and 25 x $10^3$, total coliform are 25 and 29 x $10^3$, *Staphylococcus aureus* 10 and 5 x $10^3$, faecal coliform 22 and 2 x $10^3$ cfu/ml respectively while the fungal count of Fulani and Soy bean cheese were 19 and 21 x $10^3$ cfu/ml. These are shown in Table 1.

**Table 1** Total Bacterial and Fungal Count

| | Total Count x $10^3$ cfu/ml | | | | |
|---|---|---|---|---|---|
| Samples | Bacteria | *Staphylococcus aureus* | Faecal coliform | Total coliform | Fungi |
| Fulani cheese | 28 | 10 | 22 | 25 | 19 |
| Soy bean cheese | 25 | 5 | 2 | 29 | 21 |

**Percentage of Occurrence of Microbial Isolates from Fulani and Soy Bean Cheese**

The percentage of occurrence of bacterial and fungal isolates from Fulani and Soy Bean Cheese are presented in Tables 2 – 4.

**Table 2** Percentage of Occurrence of Bacterial Isolates from Fulani cheese

| Identified organisms | Occurrence of identified organisms | % of occurrence of identified organisms |
|---|---|---|
| *Salmonella* species | 2 | 15.38 |
| *Enterobacter* species | 1 | 7.69 |
| *Serratia* species | 1 | 7.69 |
| *Klebsiella* species | 2 | 15.4 |
| *Citrobacter* species | 1 | 7.69 |
| *Staphylococcus* species | 3 | 23.07 |
| *Escherichia coli* | 1 | 7.69 |
| *Pseudomonas species* | 1 | 7.69 |
| *Proteus species* | 1 | 7.69 |
| Total | 13 | 100 |

**Table 3** Percentage of Occurrence of Bacterial Isolates from Soy Bean Cheese

| Identified organisms | Occurrence of organisms | % of occurrence of identified organisms |
|---|---|---|
| *Salmonella* species | 1 | 20 |
| *Staphylococcus* species | 2 | 40 |
| *Klebsiella* species | 1 | 20 |
| *Salmonella* species | 1 | 20 |
| Total | 5 | 100 |

**Characteristics and Identification of Bacteria Isolates**

Based on morphological and biochemical characteristics; these organisms *Salmonella* sp., *Enterobacter* sp., *Serratia* sp., *Klebsiella* sp., *Citrobacter* sp., *Staphylococcus* sp., *E. coli*, *Pseudomonas* sp. and *Proteus* species were identified. Result is presented in Table 5.

**Table 4** Percentage of Occurrence of Fungal Isolates from Cheese samples

| Identified Organisms | Occurrence of organisms | % of Occurrence of Identified Organisms |
|---|---|---|
| *Penicillium* species | 1 | 25 |
| *Rhodotorula* species | 1 | 25 |
| *Aspergillus* species | 1 | 25 |
| *Fusarium* species | 1 | 25 |
| Total | 4 | 100 |

**Table 5** Biochemical and Morphological Characteristics of Bacteria Isolates from Fulani and Soy Bean Cheese

| Isolates | Oxidase | Citrate | Coagulase | Catalase | Indole | Gram's staining | Spore Staining | Probable organisms |
|---|---|---|---|---|---|---|---|---|
| Fc1N | - | + | - | + | - | - | - | *Salmonella* species |
| Fc2N | - | + | + | + | - | - | - | *Enterobacter* species |
| Fc3N | + | + | - | + | - | - | - | *Serratia* species |
| Fc4N | - | + | + | - | + | - | - | *Klebsiella* species |
| Fc5N | - | + | - | + | + | - | - | *Citrobacter* species |
| Sc1N | - | + | - | + | - | - | - | *Salmonella* species |
| Sc1MS | - | + | - | + | + | + | - | *Staphylococcus* species |
| Sc2MS | - | + | + | + | - | + | - | *Staphylococcus aureus* |
| Fc1MS | - | + | - | + | - | + | - | *Staphylococcus* species |
| Fc2MS | - | + | + | + | + | + | - | *Staphylococcus aureus* |
| Fc3MS | - | + | - | + | - | + | - | *Staphylococcus* species |
| Fc1EMB | - | + | - | + | - | - | - | *Klebsiella* species |
| Fc3EMB | - | - | - | + | + | - | - | *Escherichia coli* |
| Fc4EMB | - | + | - | + | + | - | - | *Pseudomonas* species |
| Sc1EMB | - | + | - | + | - | - | - | *Klebsiella* species |
| Fc1MA | + | + | - | + | + | - | - | *Proteus* species |
| Fc2MA | + | + | + | + | + | + | - | *Salmonella* species |
| Sc1MA | + | + | + | + | + | + | - | *Salmonella* species |

Fc: Fulani cheese, Sc: Soya beans cheese, N: Nutrient Agar, MS: Mannitol Salt Agar, EMB: Eosin methylene blue, MA: MacConkey Agar

**Morphological and Microscopical Characteristics of Fungal Isolates**

Four species of fungal isolates were identified in both the Fulani and Soy bean cheese as members of the genera Penicillium, Rhodotorula, Aspergillus and Fusarium. The result is presented in Table 6.

**Table 6** Morphological and Microscopical Characteristics of Fungal isolates

| Isolates | Morphology characteristics | Microscopic observation | Organisms Isolated |
|---|---|---|---|
| $Fc_1$ | Greenish at the center and whitish at the edge. | It has a brush-like structures with a clusters of flask shapes, the conidia are round, unicellular unbranching chain at the tips of the phialides. | *Penicillium* species |
| $Fc_2$ | Appear pinkish on the plate due to presence of carotenoid pigments. | It is a round cells or oval cells and pseudohyphae are rarely present. | *Rhodotorula* species |
| $Sc_1$ | Conidial are usually whitish growth on the plate | It has radiating conidial heads and the conidiophores appear rough | *Aspergillus* species |
| $Sc_2$ | White mycelium growth on the plate | Some of the microconidia are curved while some were straight | *Fusarium* species |

## 4. DISCUSSION

The cheese samples were found to be heavily contaminated with bacteria and fungi as presented in Table 1. The most prevalent organisms isolated in the present study include *Staphylococcus aureus*, *Salmonella* species and *Klebsiella* species. The isolation of similar pathogens has also been reported by previous workers from various foods raw and ready-to-eat foods including cheese Elfaki and Elhakim, 2011; Ameko et al., 2012; Muhammad et al., 2016). Most of the isolates were members of genera that are pathogenic therefore are of medical importance. Cheese has been reported to be one of the high-risk foods (Ogbolu et al., 2014). The nutritional status and the moisture content of cheese of any type support the growth of many types of microorganisms. Their occurrence in food however could pose various level of danger to the health of their consumers (Muhammad et al., 2016).

The percentage occurrence of *Salmonella* and *Staphylococcus* were high in both cheese samples; beyond allowable limits of organisms in ready to eat foods. Most of the bacterial isolated are enteric in nature though *Staphylococcus aureus* is a commensal and a normal human microbiota (Tables 2 and 3); the source of these microbial isolates could be attributed to lack of the practice of "Good Processing Skill" hygiene standard of the handlers and their environments. This is observation is in line with the work of (Ogbolu et al., 2014; Bristone et al., 2018) who reported heavy microbial contaminants in ready to eat foods. All the bacteria species encountered in these cheese samples are mostly members of organisms regarded as food borne pathogens. Their presence in food and consumption could result in ill health as a result of food infection and intoxication with different kind of symptoms (Owusu-Kwarteng et al., 2020). In severe cases; food poisoning could result in death Willey et al., 2011; WHO, 2020). The presence of proteolytic bacteria like *Pseudomonas, Serratia, Proteus* and *Staphylococcus* species in food can bring about food deterioration and poor shelf life essentially when the food is held at ambient temperature; the cheese tends to lose color, taste, odor and texture. Consumption of food infected with *Staphylococcus aureus* can lead to different types of Staph poisoning.

The presence of organisms of enteric origin is an indication of contamination of the samples with faecal materials. Species of other isolates are widely distributed in air, soil and water (Bristone et al., 2018). Their presence in the cheese samples might have occurred through poor handling by the food handlers/producers and consumers; who will normally touch the food with bare hand before making a purchase. Holding temperature, utensils, container for displaying the cheese and possibly packaging material could also aid the introduction and multiplication of microorganisms in the cheese samples (Adetunji et al., 2008; Ibrahim and Falegan, 2013). Enteric bacteria like *E. coli* and *Salmonella* species are responsible for various types of gastroenteritis and enteric fever. They are majorly responsible for food and water borne diseases (Muhammad et al., 2016; Bristone et al., 2018; WHO, 2020).

The fungal species encountered in both cheese samples are species of genera commonly known to be air borne. Such organisms have been earlier reported as food contaminants and spoilage agents of food and producers of mycotoxin in food essentially *Aspergillus* and *Fusarium.* This finding is in agreement with the work of Tauxe, (2002). *Penicillium* species**,** *Rhodotorula* species, *Aspergillus* species and *Fusarium* species generally produce spores in large number and when these spores get into suitable substates, they easily develop into the vegetative phase. The fungal contaminants could come from the environment and or the food handlers themselves or their utensils. The presence of fungal species in food results more often in food spoilage than food poisoning. When such spoilt foods are however consumed, it could result in food poisoning (Rawat, 2015; Saleh and Al-Thani, 2019).

The production of toxic metabolite by these fungal isolates in food can result in fungal food poisoning though fungal food poisoning is not usually as severe as bacterial food poisoning (Rawat, 2015; Saleh and Al-Thani, 2019). This study has demonstrated that most of the cheese consumed in Malete are contaminated with pathogenic bacteria and food spoilage fungi with potential of toxin production and do not meet the required quality and safety levels. Thus, could pose a health threat to the consumers and efforts to reduce level of contamination of this food are highly recommended because it is not only student from the university that are at risk, but also some individuals from the community.

## 5. CONCLUSION

Most cheese present in the market are not fit for direct consumption, both Fulani and soya bean cheese need to be further processed thoroughly to reduce the microorganisms present in them and make them fit for consumption. This calls for proper education of food handlers on good manufacturing processes, holding temperature, time lapse between production and dispensing of food and food packaging. Food safety measures put in place and strictly follow to enhance compliance will result in production and consumption of microbiologically safe foods and consequently fewer trips to hospital.

**Informed consent**

Not applicable.

**Ethical approval**

Not applicable. Food safety measures are followed.

**Conflicts of interests**

The authors declare that there are no conflicts of interests.

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**Data and materials availability**

All data associated with this study are present in the paper.

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