Comparative assesment on microbial and nutritional quality of wara cheese from cow milk and soybean milk

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**ABSTRACT**

An investigation was carried out to determine the microbial and nutritional analysis of wara cheese from cow milk and soybean milk .The aim of this study was to compare the microbial quality and proximate composition of cow milk cheese with soy milk cheese. Microbial analysis of the two samples after production shows Total bacteria count of 1.0x104 cfu/g with no growth of *Salmonella,Coliform,Staphylococcus* andfungi. After7days, the results shows that cheese from cow milk has the highest Total bacteria count,*Staphylococcus* count and fungi count which are23.5x104cfu/g, 9.5x104cfu/g, 12.5x104cfu/g while cheese from soymilk has 18.5x104cfu/g, 5.5x104cfu/g, 2.5x104cfu/g and high coliform count of 4x104cfu/g than cow milk cheese 1.5x104cfu/g and with no growth of *salmonella*. The overall assessment shows that the total microbial count does not exceed the standard hence the cheese was good and safe for consumption. Proximate analysis shows that, fat content is lower and protein content is higher in soymilk cheese 6.01+0.04,13.09+0.01than cow milk cheese 6.13+0.03, 12.75+0.01. Moisture, ash, fat and total carbohydrate are significantly higher in cow milk cheese than soymilk cheese. Crude fiber was not found in both samples. This research work shows that the samples are concentrated source of valuable nutrients because it has good nutritional qualities.

*Keywords:* Cheese, soybeanmilk, cow milk, sensory evaluation

**INTRODUCTION**

Cheese has been defined as a product made from the curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes in the presence of lactic acid produce by added microorganism, from which part of the moisture has been removed by cutting, cooking and/or pressing which has been shaped in a mould, and then ripened by holding it for some time at suitable temperature and humidity. The essential ingredients of cheese are milk, coagulants, bacteria cultures and salt (Augustine et al.,2014).

The coagulant causes the milk protein to aggregate and ultimately transform fluid milk to a semi firm gel. When these gel is cut into small pieces (Curds), the whey (mostly water and lactose) begins to separate from the curds. Acids production by bacteria culture is essential to aid expulsion of whey from the curd and largely determines the final cheese moisture, flavour and texture (Raheem et al.,2009).The physicochemical parameters like pH, water activity, and salt concentration in cheese are responsible for texture and flavour inconsistencies (Steijns,2001). Cheese is a concentrate source of many nutrient in milk. Milk is an extremely nutritious food.It is an aqueuoscolloidial suspension of proteins, fat and carbohydrate that contain numerous vitamin and minerals such as calcium, phosphorous, sodium, potassium, magnesium (Sangoyomi et al., 2010).Milk proteins are ideal in that, they are complete and have high essential amino acids composition. Although milk and its various derivatives such as butter, yoghurt and cheese are vital human foods. It provides an excellent medium for growth of many kinds of microorganism (Adesokan et al., 2009). The continuous increase in population and inadequate supply of protein has inadvertently increased the occurrence of malnutrition in developing countries (Siddhuraju*et al*., 1996). However, in order to meet the protein demands in developing countries, where animal protein is also grossly inadequate and relatively expensive, research effort is geared towards finding alternative sources of protein from legume seeds. It must be stressed that for the selective few that are able to afford animal milk, there is always an increasing concern about its fat and cholesterol contents. This factor has made vegetable milk to become an alternative source of milk. In this regard, soymilk has been recognized as being nutritionally helpful. For instance, soy cheese (a product from soymilk) accords advantages in terms of nutrition and health, since it contains no cholesterol or lactose and only small quantities of saturated fatty acids ( Nazim,et al.,2013).

Soybean cheese also known as ‘beske’ in the westerns parts of Nigeria is nutritious and is prepared by curdling of the milk extract from soybean(Adejuyitanet al.,201.**)**. Soybean, the basic raw material for the product, has great nutritional (Source of proteins, minerals etc.), and therapeutic values e.g. prevention of chronic disease such as cancer, and it is also beneficial in products like soy milk and soy cheese ( Liu et al., 2006, Anderson et al., 1991, Setchell and Cassidy, 1999). Sodium chloride is the basic component of sufu which provide the traditional flavour and the product safety against pathogenic microorganism (Han, 2001).Cheese is made in almost every country of the world and these are more than 2000 varieties, made from milk of serveral mammals, processed industrial or by traditional methods. However, despise the large number of varieties, the basic steps required in any cheese processing are essentially the same, and slight variations in any of these steps may result in products of different general quality (Guinee and Wilkinson, 1992).

Nigeria cheese (wara) is a soft white unripen cheese that originated from Fulani cattle rearers in the northern part of the country.It is commonly produced by Fulani women from unpasteurised cow milk and sold along the major street of Nigeria. ‘Wara’ is a fresh cheese , that is, moist curd that as been cut and drained of the whey but never ripened and unsalted and uncoloured.Aboutone kilogramme of cheese will be obtained from about five litres of milk (Sangoyomi et al.,2009,Adetunji and Alonge,2009). Cheese is an excellent source of protein fat and mineral such as calcium, iron and phosphorous ,vitamins and essential amino acids and therefore is an important food in the diet of both young and old people.

Microbiological spoilage of cheese is one of the important reasons that render the nutritious and tasty cheese not only inedible but also a potential source of infection, The spoilage may be due to bacteria or fungi .The defect is due to contamination may arise from the surface of the cheese showing visual and organoleptic changes or it may be hidden internally (Falegan,2014).

**Statement of the Problem**

Milk is an enriched medium for microorganism and as such of organism may still harbourin it there is need to look for another source milk, soy bean milk in the production of cheese andanalyse, to minimize the microbial load.

**MATERIALS AND METHOD**

## Source of Materials

The raw milk was sourced from fresh whole cowmilk through hand milking from white Fulani cows in ilaro, ogun state, Nigeria. The*Calotropisprooceera* leaves were source from the Fulani local cheese producers from a plantation in ilaro. The sodium chloride used was of analytical grade.Soybeans seeds were obtained from Sayedero markets in ilaro, ogun state. Good quality and mold free seeds were selected and ambient temperature to usage.

## Preparation of Sample

The raw milk pasteurised in order to destroy microorganism and prevent fermentation by pouring the fresh morning milk into a metallic and heating 50oC for 30mins.*Calotropisprocera* leaves was weight carefully wash with distilled water to remove dirty, it was then drop in 150mm of warm water, in order for the extract to be collected, and left for 5min. After 5min,the mixture was further sieved to collect the juice extract.

**Production of soymilk:** The raw soy beans seed was sorted to remove stones, damage and deformed seeds. The Soy beans was wash and soaked in distilled water (500g in a litre) for12hr. The water was then drained and the beans were dehulled by rubbing them firmly between the palms of the hand, it was then washed to remove the sharft. The clean beans were milled into a paste and water was added. The slurry was strained (filtered) using muslin cloth .The filterate, the soy milk was pasteurised by heating at 71oC for 15sec.

## Production of Cheese

## Production of Cowmilk Cheese

1 Litre of warmed raw milk was measured into a metal pot and 50mm of the *Calotropisproceera* juice extract was added to already warmed milk and10ml of starter culturewas added to the mixture (the culture determine the characteristic of the PH and inhibit the growth of undesirable bacteria in the medium). The heating was carried at a 68OC for 20mins slowly with intermittent stirring.After heating for clotting to occur, the mixture was cooled to rennecting temperature of 30OC.The curds was separated from the whey using a decontaminated sieve cloth in order not to attract microorganism.150ml of water at 50OCwas added to the curd with continuous stirring for 45min in order to remove the whey still left with the curd.Curd was collected in a sieve cloth and left for 15mins to drain out water. When the cheese is firm enough to retain it shape it was remove from the sieve and placed in a container of cool water as shown in Fig 1. The cheese was then soak in a salt solution(15% Nacl) for two- three hours. The sailted cheese was subjected to organoleptic microbiological and proximate analysis.

**Production of Soy Cheese**

1Litre of warmed soymilk was measured into a metallic pot,50ml of Calostropisprocesera extract and starter culture was added and placed over a slow burning fire,heated to temperature of 680C for 20mins. The milk is stirred gently with a wood spatula, during the initial and subsequent heating and cooking. Salt (Nacl) was added to curdle the milk and heated slowly with intermittent stirring until it coagulates and there is visible separation of curds and whey. The curds and whey was poured into a muslin cloth over a container for whey collection and pressed to separate the whey from the cheese. It was left for 4-5 hours to set and then cut with knife to desire shape and size. The soy cheese was subjecteds to organoleptic, microbiological and proximate analysis.

## Microbial Analysis

Using Pour Plate Techniques, 1g of each sample was serially diluted.from the tenfold dilution of each sample,1ml of each dilution four (104) were pour plated aseptically in triplicates using Nutrient Agar for Total viable count, MacConkey agar for Total Coliform counts, Baird Parkers Agar for Staphylococcus count, Bismuth sulfite Agar for Salmonella count and Potato Dextrose Agar for fungi count. All the plates were incubated at 37oC for 24-48hrs while Fungi plates were incubated at 28oC ± 2oC for 5 days. Colonies were counted on colony counter. Pure cultures of each isolates were obtained by streaking the specific colonies on suitable media and incubated appropriately (Lynne, 2003).

**Identification of Microbial Isolates:**Colonies were selected randomly and were characterized using morphological and biochemical test such as gram stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP and Urease and sugar fermentation test methods described by (Lynne, 2003 and Cowan, 2010).

###### Proximate Analysis

The proximate composition were analysed as described by AOAC(2000).All chemicals were of the analytical grade. Each analysis was carried out in duplicates.Moisture, Ash, Crude protein.Fat, Crude fibre and Carbohydrate were analysed.

Final cheese product

Moulding

 Firm curd

 Removal of whey

 Soft curd

 Natural sourcing or starter culture

 Coagulant (*Calotropisprooceera*

(Cow milk or Soymilk)

 Pasteurized

Milk

MM Milk

**Fig. 1** Schematic of the basic stages of cheese making

## RESULT AND DISCUSSION

## Microbial Analysis

 The microbial analysis result is shown in Table 1.At day one after the production the total viabl count range 1.2x104 and 1.0x104cfu/g and there was no growth of fungi, coliform and salmonella.Analysis after storage for7days at 10oC showed that cow milk cheese has high value of total plate count, Staphylococcus count and fungi count than soybeans.The cow cheese Total viable count was 23.5 x104, 1.5 x 104cfu/g Coliform, 9 x 104cfu/g Staphylococcus, 12.5x104cfu/g fungi (more of yeast) while soymilk cheese has 18.5 x104cfulg Total viable count,4x104cfu/g Coliform, 5x104 Staphylococcus and 2.5x104cfu\g Fungi. The two samples recorded no growth of Salmonella. The presence of Staphylococcus might have been from the equipment. Contamination from water under of the cow during milking, handling during processing,since milk is a balanced culture for most micro organisms hence contamination easily takes place (Falegan, 2014). In all, the count were still at the permissible limit. Microbes isolated were subjected to biochemical test as shown in table 3 and the bacteria were of the general *Lactobacillus, Leuconostoc, Streptococcus,Enterobacter*and fungi isolated were *Saccharomyces* and*Rhizopus* which grow at the 7day.

## Proximate Composition

The result of the proximate analysis carried out on the samples is shown in Table 2. This showed that moisture, ash,fat and carbohydrates are significantly higher in cow milk cheese than soymilk cheese,on the other hand protein is slightly higher in soymilk cheese than cow milk cheese.The high level of protein as observed from soycheese thus gives an indication that they could meet up with the protein requirement required by the body. The samples as high moisture contentand may be as a result of the moisture of the milk used for processing andsubsequent formation of the thick curd. The result is inline with the observation made by previous workers on wara(Fasakin and Unokiwedi, 1992). Ash content of the two samples were lower than values recorded by( Lawal and Adedeji 2013)Fat content were highest in cowmilk,Fat are used by cells of organs and glands to provide energy and in the synthesis of some of their secretions( LawalandAdedeji 2013).Recent studies have shown that a high fat meal may impair vaso activity and transiently impair endothelial function (Nazim et al.,2013). So people may reduce the risk of animal’s fatby consuming this Soya product.In recent studies, Soy protein contributed to control of hyperglycemia and reduced body weight. Hyperlipidemiaand hyperinsulinemia (Bhathena and Velasquez, 2002). These characteristics may be useful to both non diabetic and diabetic person in control of obesity and blood sugar.Crude fibre was not found in both samples.

#### Table 1: Microbial Analysis of Cheese Samples

**Sample Total viable Coliform Staphylococcus Salmonella Fungi E.coli**

**code count cfu/g count cfu/g count cfu/g count cfu/g count cfu/g count cfu/g**

**Day 0**

ATM 1.2 x 104 Nil NilNilNillNill

OTS 1.0 x 104 Nil NilNilNillNill

**Day 7**

ATM 23.5 x 104 1.5 x 1049 x 104 Nill 12.5 x 104 1.0 x 104

OTS 18.5 x 104 4 x 1045 x 104 Nill 2.5X104 Nil

ATM - Cow milk cheese

OTS - Soymilk cheese

**Table 2: Proximate Composition of Cheese Samples**

# Parameters Cowmilk cheese Soymilk cheese

Moisture % 76.08+0.02 75.28+0.8

Ash % 1.20+0.10 0.93+0.02

Crude fibre % ND ND

Fat % 6.13+0.03 6.01+0.04

Protein % 12.75+0.01 13.09+0.01

Total Carbohydrate % 4.23+0.1 4.20+0.01

Values were means of duplicate determination (mean+SD)



**Fig. 2**Comparing the proximate composition of cheese samples

#### Table 3: Biochemical Test For Bacteria Isolate

**Suspected shape Gram’s Catalse Oxidase Coagulase Motility Urease Indole Sucrose Lactose glucose**

##### Microbes staining

Streptococcus Cocci +ve -ve -ve -ve -ve -ve +ve acid/gas acid/gas acid/gas

Lactobacillus Rod +ve -ve -ve -ve -ve -ve +ve acid/gas acid/gas acid/gas

Staphylococcus Cocci +ve +ve -ve +ve -ve +ve +ve acid/gas acid/gas acid/gas

Bacillus spp Rod +ve +ve +ve -ve +ve +ve -ve acid/gas acid/gas acid/gas

Enterobactersp Rod -ve +ve -ve -ve +ve -ve -ve acid/gas acid/gas acid/gas

**CONCLUSION**

From the analysis carried on the samples (cow milk cheese and soymilk cheese), the total microbial count from the samples does not exceed the recommended value 105cfu/g in food (ICMSF) and the overall subjected to statistical analysis shows that there is strong evidence that microbial count, proximate composition and sensory evaluation varies with the samples. However, it can be therefore concluded that, the two samples is a concentrated source of valuable nutrients because, it has a good nutritional qualities but, cow milk cheese was more accepted than soymilk cheese and there is significant difference in the means of the samples as p <0.05 in all attribute rated.

## RECOMMENDATION

Research work should be carried out on different packaging materials to know the best packaging materials for the product (cow milk cheese and soymilk cheese). More research work should also be carried out on the shelf life of the product.

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