CARD International Journal of Science and Advanced Innovative Research (IJSAIR)
ISSN: 2536-7315 (Print) 2536-7323 (Online)
Volume 1, Number 2, September 2016
http://www.casirmediapublishing.com

Comparative Assessment of the Microbial and Nutritional Qualities of *Wara* (Cheese) Produced from Cow Milk and Soybean Milk

[1]Noah, Abimbola A. & [1]Oduwobi, Oludayo O.
[1]Department of Food Technology, The Federal Polytechnic, Ilaro, Ogun State
[1]Science Laboratory Technology, The Federal Polytechnic, Ilaro, Ogun State
Email: adukechoix@gmail.com

ABSTRACT

An investigation was carried out to determine the microbial and nutritional analyses 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 analyses of the two samples after production showed total bacteria count of $1.0x10^4$cfu/g with no growth of Salmonella, Coliforms, Staphylococcus and fungi. After7days, the results shows that cheese from cow milk has the higher total bacteria count, Staphylococcus count and fungi count which are$23.5x10^4$cfu/g, $9.5x10^4$cfu/g, $12.5x10^4$cfu/g while cheese from soymilk had $18.5x10^4$cfu/g, $5.5x10^4$cfu/g, $2.5x10^4$cfu/g and a high coliform count of $4x10^4$cfu/g than cow milk cheese with $1.5x10^4$cfu/g, there was no growth of Salmonella. The overall assessment shows that the total microbial count did not exceed the standard, hence; the cheese is 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 with 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 good sources of valuable nutrients because of their nutritional qualities.

Keywords: Cheese, soybean milk, cow milk, microbial analysis

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, bacterial 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 this gel is cut into small pieces (Curds), the whey (mostly water and lactose) begins to separate from the curds. Acids production by bacterial cultures 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 nutrients in milk. Milk is an extremely nutritious food. It is an aqueous colloidal 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 western parts of Nigeria is nutritious and is prepared by curdling of the milk extract from soybean (Adejuyitan *et al.,* 2014). 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 several 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).

Nigerian cheese (wara) is a soft white unripe 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 fresh cheese, that is, moist curd that has been cut and drained of the whey but never ripened and unsalted and uncoloured. About one 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 microorganisms and as such, vulnerable to microbial degradation or attack, hence, the need to always watch out for its microbiological safety. Milk, essentially is a complete medium or diet and is capable of supplying all the basic nutrients for growth and survival but milk from animal source is still out of the financial reach of many, hence, the need for a cheaper alternative source that is equally nutritious – plant source.

MATERIALS AND METHOD

Source of Materials

The raw milk was obtained from fresh whole cow milk by hand milking from white 'Fulani' cows in Ilaro, Ogun state, Nigeria. *Calotropis procera* leaves were got 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' market in Ilaro, Ogun state. Good quality and mold-free seeds were selected and stored at ambient temperature.

Preparation of Sample

The raw milk was pasteurised in order to destroy microorganisms and prevent fermentation by pouring the fresh morning milk into a coated-metallic container and heated at 50°C for 30mins. *Calotropis procera* leaves were weighed carefully, washed with distilled water to remove dirt, soaked in 150ml 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 soybean seeds were sorted to remove stones, damage and deformed seeds. The Soybeans were washed 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 and then washed to remove the shafts. The clean beans were milled into paste and water was added. The slurry was drained (filtered) using muslin cloth. The filtrate, the soy milk, was pasteurised by heating at 71°C for 15sec.

Production of Cheese

Production of Cow Milk Cheese

1 Litre of warm raw milk was measured into a metallic pot and 50ml of the *Calotropis procera* juice extract was added to already warmed milk and10ml of starter cultures was 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 68°C for 20mins slowly with intermittent stirring. After heating for clotting to occur, the mixture was cooled to a temperature of 30°C. The curds were separated from the whey using a decontaminated sieve cloth in order not to attract microorganisms.150ml of water at 50°Cwas added to the curd with continuous stirring for 45min in order to remove the whey still left with the curd. The curd was collected in a sieve cloth and left for 15mins to drain out water. When the cheese was firm enough to retain it shape, it was removed from the sieve and placed in a container of cool water as shown in Fig 1. The cheese was then soaked in a salt solution (15% NaCl) for two-three hours. The salted cheese was subjected to organoleptic, microbiological and proximate analyses.

Production of Soy Cheese

1Litre of warm soymilk was measured into a metallic pot,50ml of *Calostropis procera* extract and starter culture were added and placed over a slow burning fire, heated to temperature of 68°C for 20mins. The milk was stirred gently with a wooden spatula during the initial and

subsequent heating and cooking. Salt (NaCl) was added to curdle the milk and heated slowly by intermittently stirring until it coagulated and there was visible separation of curds and whey. The curds and whey were poured into a muslin cloth over a container for whey collection and pressed to separate the whey from the cheese. They were left for 4-5 hours to set and then cut with a knife to the desired shapes and sizes. The soy cheese was subjected to organoleptic, microbiological and proximate analyses.

Microbial Analysis

Using Pour Plate Techniques, 1g of each sample was serially diluted from the tenfold dilution of each sample; 1ml from dilution factor 10^{-4} was 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 sulphite Agar for *Salmonella* count and Potato Dextrose Agar for fungi count. All the plates were incubated at 37°C for 24-48hrs while Fungi plates were incubated at 28°C ± 2°C for 5 days. Colonies were counted on a colony counter. Pure cultures of each isolate 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 tests such as Gram's stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP and Urease and sugar fermentation test methods as described by Lynne(2003) and Cowan(2010).

Proximate Analysis

The proximate composition was analysed as described by AOAC (2000).All the chemicals used were of the analytical grade. Each analysis was carried out in duplicates. Moisture, ash, crude protein, fat, crude fibre and carbohydrate were analysed.

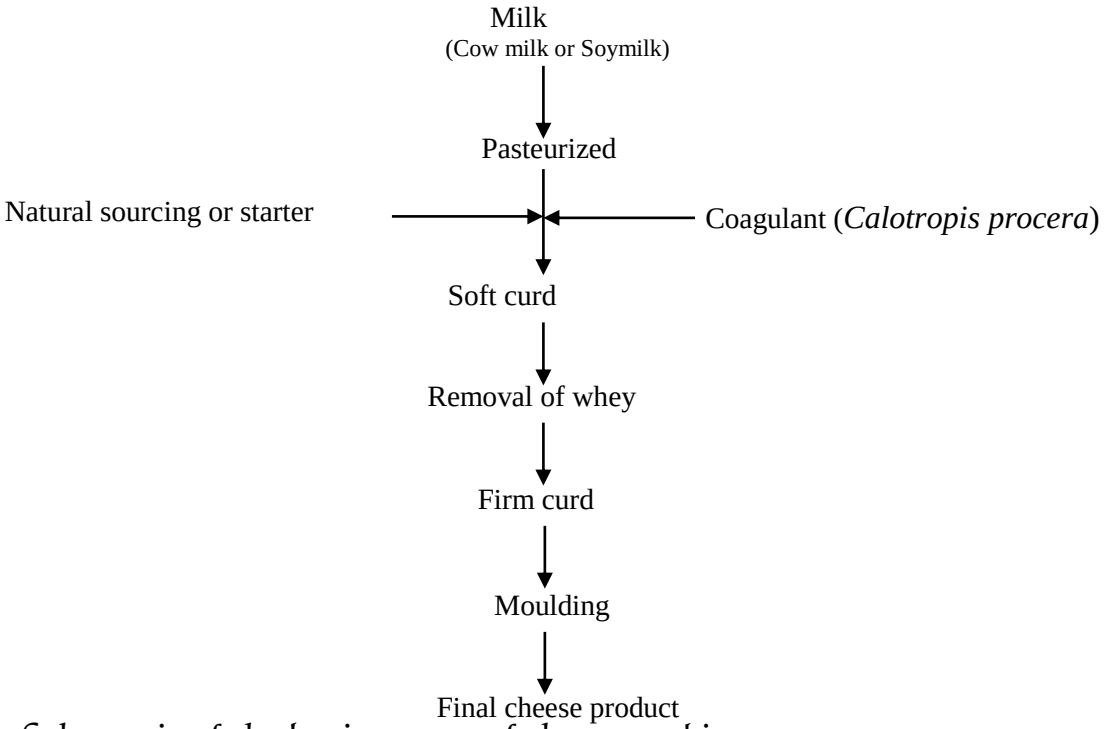


Fig1: Schematic of the basic stages of cheese making

RESULTS AND DISCUSSION

Microbial Analysis

The microbial analysis result is shown in Table 1.At day one, after the production, the total viable count ranged between $1.2x10^4$ and $1.0x10^4$cfu/g and there was no growth of fungi, coliforms and *Salmonella*. Analysis after storage for7days at 10°C showed that cow milk cheese had a high value of total plate count, Staphylococcus count and fungi count than soybeans cheese. The cow milk cheese total viable count was 23.5 $x10^4$, 1.5 x 10^4cfu/g Coliform, 9 x 10^4cfu/g *Staphylococcus*, $12.5x10^4$cfu/g fungi (more of yeast) while soy milk cheese had 18.5 $x10^4$cfu/g Total viable count,$4x10^4$cfu/g Coliform, $5x10^4$*Staphylococcus* and $2.5x10^4$cfu/g Fungi. The two samples recorded no growth of *Salmonella*. The presence of *Staphylococcus* might have been from the equipment used. Since milk is a balanced culture for most micro organisms, contamination easily takes place (Falegan, 2014). In all, the

counts were still at the permissible limit. Microbes isolated were subjected to biochemical tests as shown in table 3 and the bacteria were of the genera *Lactobacillus, Leuconostoc, Streptococcus* and*Enterobacter* while the fungi isolated were *Saccharomyces* and*Rhizopus* which grew on the 7thday.

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 have high moisture content and may be as a result of the moisture of the milk used for processing and subsequent formation of the thick curd. The result is inline with the observation made by previous works on 'wara' (Fasakin and Unokiwedi, 1992). Ash content of the two samples was lower than values recorded by Lawal and Adedeji(2013). Fat content was higher in cow milk. Fats are used by cells of organs and glands to provide energy and in the synthesis of some of their secretions (Lawal and Adedeji, 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 fat by 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.

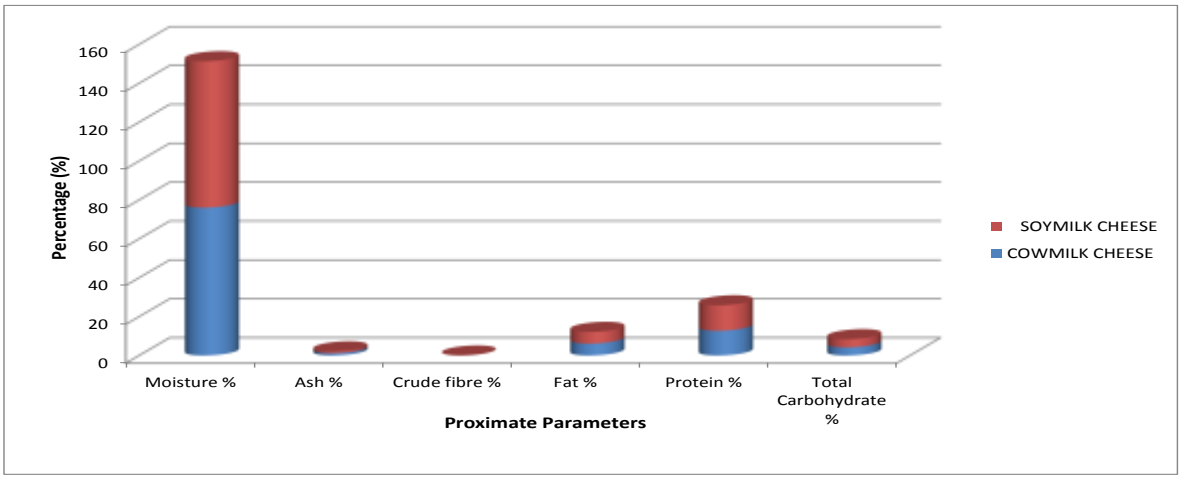
Table 1: Microbial Analysis of Cheese Samples

Sample code	Total viable count cfu/g	Coliform count cfu/g	*Staphylococcus* count cfu/g	*Salmonella* count cfu/g	Fungi count cfu/g	*E.coli* count cfu/g
Day 0						
ATM	1.2×10^4	Nil	Nil	Nil	Nill	Nill
OTS	1.0×10^4	Nil	Nil	Nil	Nill	Nill
Day 7						
ATM	23.5×10^4	1.5×10^4	9×10^4	Nill	12.5×10^4	1.0×10^4
OTS	18.5×10^4	4×10^4	5×10^4	Nill	$2.5X10^4$	Nil

Keys: ATM - Cow milk cheese
OTS - Soymilk cheese

Table 2: Proximate Composition of Cheese Samples

Parameters	Cow milk 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 Testsfor Bacterial Isolates

Suspected Microbes	Shape	Gram's stain	Catalse	Oxidase	Coagulase	Motility	Urease	Indole	Sucrose	Lactose	glucose
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 sp.	Rod	+ve	+ve	+ve	-ve	+ve	+ve	-ve	acid/gas	acid/gas	acid/gas
*Enterobacter*sp.	Rod	-ve	+ve	-ve	-ve	+ve	-ve	-ve	acid/gas	acid/gas	acid/gas

CONCLUSION

From the analyses carried on the samples (cow milk cheese and soymilk cheese), the total microbial count from the samples did not exceed the recommended value of 10^5cfu/g in food (ICMSF) and the overall statistical analysis shows that there is a strong evidence that microbial count, proximate composition and sensory evaluation varied with the samples. However, it can be therefore concluded that the two samples are good sources of valuable nutrients because of their rich nutritional qualities but the cow milk cheese is more accepted than the soymilk cheese.

RECOMMENDATIONS

Research work could be carried out on different packaging materials to determine the best packaging material(s) for the products (cow milk cheese and soy milk cheese). Further research work could also be carried out on the shelf life of the products.

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