Influence of Biological Coagulants and Heat Treatment Duration on the Microbiological Properties of Nigerian Soft Cow-milk Cheese

Abstract

The study examines the microbiological properties of cheese from cow milk as influenced by heat treatment durations and biological coagulant types. The cow milk was pasteurized at 65°C and subjected to further heat treatment for 15, 20, and 25 minutes with the addition of coagulants (lime juice, tamarind pulp and moringa seed paste) to respective samples and allowed to cool for 30 minutes before pressing out the whey. The initial properties of raw cow milk were determined to serve as control and the nine produced cow-milk cheese samples were determined using a 3x3 factorial treatment design. The microbiological properties of the cheese were determined using standard methods. Results obtained were analyzed statistically to determine the influence of heat treatment duration and coagulant sample. The microbial analysis revealed that there was Coliform in samples from cow cheeses, C_{25} (moringa seed paste coagulated cheese at 15 minutes of heat treatment) has the highest Coliform of $1.6x10^3$ cfu/g, B_{15} (tamarind pulp coagulated cheese at 15 minutes of heat treatment) and C_{20} have yeast and mould bacteria of $<1.0 \times 10^2$ cfu/g and mesophylic bacteria was highest at C_{15} of $1.1x10^4$ cfu/g, which are also within the permissible limit of consumption. This study is initiated to determine the effect of heat treatment and local coagulants on the microbial properties of Nigerian soft cow-milk cheese. Hence, investigating the hygienic quality and safety of consuming the diary product.

Keywords: Cow-milk cheese, lime, tamarind, moringa, microbial.

1. Introduction

Cow-milk cheese has been consumed for centuries in different cultures as a regular substitute for dairy food due to several reasons such as health benefits, lactose intolerance and simple taste preference (Grosch, 2008). Hazards associated with the consumption of raw milk includes health hazard due to the unhygienic manner and technique of milking by most local milk processors and the coagulants for cheese production which are locally sourced. This process is not standardized yet and is usually done under unhygienic conditions (Sacks *et al.*, 2006). Thus, there is a need to further study the effects of heat treatment and local coagulants on the hygienic quality and safety of Nigerian soft cheese (Fellows, 2008). In Nigeria, cow-milk cheese consumption per capita is

increasing which has prompted an increase in the production of the product. This seemly large national demand suggests the need to look at the safety of the product been consumed across the country (Akinsoye, 2007). The demand for dairy products is principally based on its perceived health benefits which have influenced the high consumption rate.

Heat treatment is the most widely used processing technology for microbial treatment in the dairy industry (Olorunfemi *et al.*, 2006). Some common methods such as heat treatment of fluid milk products for a predetermined time and temperature main objective is to destroy microorganisms and to ensure the milk is safe for consumption with a reasonable shelf life. Although there have been successes recorded in the use of chemicals such as calcium chloride ($CaCl_2$) in the conversion of milk to cheese, it is necessary to use natural coagulants in the production of cheese. Natural coagulants are biological coagulants that have no consequence on the health of humans but serves as an additive (Savur, 1998). The coagulants are readily available in rural areas and are very cheap and highly effective in the coagulation of milk (Iwalokun *et al.*, 2004; Olorunfemi *et al.*, 2006). These biological coagulants include moringa kernel cake, lime juice and tamarind pulp. The health benefits of lime include weight loss, skin care, improved digestion, relief from constipation, peptic ulcer, respiratory disorders, urinary disorders (Beresford *et al.*, 2001). (Kanerva, 2000) states that whole tamarind seed and kernels are also rich in protein (13-20%) and the seed coat is rich in fibre (20%) and tannins 20% (Cogan, 2000).

2. Materials and Methods

The materials used for the study include cow-milk, moringa seed, lime, tamarind fruit, water, cooking pots, spoons, stirrer, stove, microwave, cheese knife, bowls, cheesecloth, mortar, pestle and blender. The local coagulants used in this study are as shown in Fig 1.

(i)  **(ii)**  **(iii)** 

Figure 1: (i) Lime fruit (ii) Tamarind fruit, and (iii) Moringa seed.

2.1 Sample collection and preparation

The cow-milk samples used for the study were obtained at Monday market, Kaduna. The ingredients, moringa seed, lime and tamarind fruit were obtained from the same market. The cow-milk cheeses were produced at Crop Processing Laboratory, Food Technology Department, Kaduna Polytechnic, Nigeria. The samples and raw milk (control) were kept in sterile plastic containers and taken to the laboratory for microbial determination.

2.2 Instruments and reagents

The equipment used for the experiment are: laboratory oven dryer, microscope, autoclave, electric blender, flame photometer, calorimeter, incubator, fume cupboard, digital weighing balance, soxhlet apparatus, muffle furnace, burette, retort stand, funnel, conical flask, pipette, measuring cylinder, beaker spatula, the crucible, desiccators, soxhlet flask, filter paper, Petri dishes, and thermometer. The reagents used include phenolphthalein, formaldehyde, potassium sulphate, petroleum ether, potatoes dextrose, agar, mackonkey agar, nutrient agar, ringer table, tartaric acid, and copper sulphate.

2.3 Preparation of coagulants

Moringa oleifera seeds were dehulled after which it was weighed and introduced into a pot to be toasted for 3 minutes. The seed was milled to powdered form using an electrical blender and then the oil was extracted by the manual method: by the use of hot water (little quantity) and the oil expressed manually. The seed paste obtained was used as a coagulant. The fresh lime was sorted, graded, washed, sliced, and squeezed to extract the juice, which was used as a coagulant for the production of cheese from cow milk. About 75 ml of the extracted juice was used in the production. The tamarind seed pod was sorted, washed and soaked in warm water for about six (6) hours and sifted to obtain the pulp. The pulp was then used as a coagulant for the production of cheese from cow milk. Also, 75 ml of the pulp was used in the production.

2.4 Microbial analysis

The standard method of AOAC (2010) was employed. About 1 g of the different cheese samples was aseptically weighed using a weighing balance and carefully introduced into 9 ml of the sterile distilled water. This was shaken manually to have a homogenous suspension. 1ml of this was taken and introduced into the second tube, followed by series of dilutions up to 10^{-10} dilutions. 1ml was taken 10^{-4} dilution and introduced into sterile plates and modern agar (50 ^{o}C) added by pour plate method using the following agar and incubation periods. Potato Dextrose Agar was used for the

enumeration of mould and yeast in the sample. The plates were incubated at 30°C for 24 hours for yeast and 2 - 5 days for mould. Nutrient Agar was for the determination of total viable bacterial in the sample. The plates were incubated at 37 ºC for 24 - 48 hours. McConkey Agar was used for the enumeration of total Coliform organisms in the sample. The plates were incubated at 37°C for 24 to 48 hours.

2.5 Experimental procedure for cow-milk cheese production

The raw cow-milk was pasteurized in a microwave at 65°C for 10 minutes before the coagulants were added and subjected to further heat treatment for 15, 20, and 25 minutes to form the curds. It was then left to cool for 30 minutes and were later separated using a cheese mould. The cheese was removed from the mould, packed and stored. A 3^2 factorial treatment design was applied to analyze the collated data and examine the experimental results. The test yielded about nine (9) experimental runs for the cow-milk cheese sample. The results were analyzed with a completely randomized design with 3 replicates. All data were subjected to variance analyses using SPSS 20.0 statistic program. Table 1 shows the codes for cheese production from cow milk and the experimental layout for the study is shown in Table 2. The volume of milk used for the milk sample is 1000 ml per run.

Table 1: Layout for production of cheese produced from cow-milk.

No.of runs	**Codes**	**Meaning**
1	A_{15}	Lime coagulated cow-milk cheese for 15 minutes Heat Treatment duration
2	A_{20}	Lime coagulated cow-milk cheese for 20 minutes Heat Treatment duration
3	A_{25}	Lime coagulated cow-milk cheese for 25 minutes Heat Treatment duration
4	B_{15}	Tamarind pulp coagulated cow-milk cheese for 15 minutes Heat Treatment duration
5	B_{20}	Tamarind pulp coagulated cow-milk cheese for 20 minutes Heat Treatment duration
6	B_{25}	Tamarind pulp coagulated cow-milk cheese for 25 minutes Heat Treatment duration
7	C_{15}	Moringa seed paste coagulated cow-milk cheese for 15 minutes Heat Treatment duration
8	C_{20}	Moringa seed paste coagulated cow-milk cheese for 20 minutes Heat Treatment duration
9	C_{25}	Moringa seed paste coagulated cow-milk cheese for 25 minutes Heat Treatment duration

Table 2: Layout of experimental design

Coded Values			
	High	Middle	Low
Parameters	1	0	-1
Heat Treatment Duration	25	20	15
Coagulants	Lime (A)	Tamarind pulp (B)	Moringa seed Paste (C)

Note: -1, 0, and 1 signify: low, mid and high ranges, respectively.

3. Discussion of Results

The results of the microbiological properties of the cheese produced from cow milk when subjected to various heat treatment durations and different biological coagulants show the mesophilic aerobic bacteria (total plate count) as presented in Tables 3, 4, and 5. The result in Table 3 indicates the presence of colonies of variation between $2.1x10^2$ and 5.2×10^4 cfu/g which is in line with the findings of James *et al* (2016). The raw milk shows high colonies which may be due to the low heat treatment applied but the main products have colonies that are lower than that of the raw milk. However, this is within the acceptable range for ready to eat food of $<10^5$ cfu/g as reported by the Centre for Food Safety (CFS, 2014). The results showed the influence of heat treatment duration and coagulant types on the reduction in the total colonies counted. A_{25} has the highest colonies of 1.1×10^4 cfu/g and C_{25} has 9.0×10^3 cfu/g, this is similar to results obtained by Olorunfemi *et al.* (2006). The overall results showed that the coagulants and heat treatment reduced the mesophilic aerobic bacteria of cow-milk sample.

Table 3: Mean value for enumeration of mesophilic aerobic bacteria (Total Plate Count) of cheese produced from cow milk at different heat treatment durations and different coagulants

Sample Code	**Cfu/g/ml**	**Coded**
A_{15}	$8.8x10^2$	-1
B_{15}	$2.1x10^2$	0
C_{15}	$9.0x10^2$	1
A_{20}	$1.0x10^3$	-1
B_{20}	$7.4x10^2$	0
C_{20}	$4.0x10^2$	1

A_{25}	$1.1x10^4$	-1
B_{25}	$8.0x10^3$	0
C_{25}	$9.0x10^3$	1
Control	$5.2x10^4$	-1

A=Lime coagulant cow-milk cheese, B=Tamarind coagulant cow-milk cheese, C=Moringa coagulant cow-milk cheese, 15 = 15 minutes heat treatment duration, 20 = 20 minutes Heat treatment duration, 25 = 25 minutes heat treatment duration.

The result in Table 4 shows that there was a reduction in the coliform bacteria which may be as a result of the heat treatment applied, Fox *et al* (2000) also reported a similar reduction in coliform bacteria. At 15 minutes, only B_{15} has Coliform bacteria $<1.0x10^2$ cfu/g, C_{20} has Coliform of 2.4×10^3 cfu/g whereas at 15 minutes A_{15} has no Coliform bacteria. The cheese when subjected to high temperature during frying will destroy the Coliform bacteria present. The control has coliform bacteria when subjected to heat treatment with the inclusion of coagulants. However, the result indicated that the samples are within the acceptable standard required for cheese consumption according to (FAO, 2000) and (German and Euir, 2009).

Table 4: Mean values for coliform bacteria of cheese produced from cow milk at different heat treatment duration and different coagulants.

Sample Code	**cfu/g/ml**	**Coded**
A_{15}	Nil	-1
B_{15}	$<1.0x10^2$	0
C_{15}	$2.0x10^2$	1
A_{20}	$4.3x10^2$	-1
B_{20}	$1.7x10^2$	0
C_{20}	$2.4\ x10^3$	1
A_{25}	$6.4x10^2$	-1
B_{25}	$6.0x10^2$	0
C_{25}	$1.6\ x10^3$	1
Control	$1.1\ x10^3$	-1

A=Lime coagulant cow-milk cheese, B=Tamarind coagulant cow-milk cheese, C=Moringa coagulant cow-milk cheese, 15 = 15 minutes heat treatment duration, 20 = 20 minutes Heat treatment duration, 25 = 25 minutes heat treatment duration.

The results in Table 5 showed that there was no yeast and mould found at 15 minutes heat treatment duration for A and C, at 20 minutes there was no yeast and mould found in the samples of A and B, at 25 minutes there was no yeast and mould found in the samples of A, B and C. But sample B and C showed the presence of yeast and mould of $<1.0 \times 10^2$ cfu/g at 15 and 20 minutes respectively, this result agrees with the finding of James *et al* (2016) which is still within the permissible level for cheese consumption (3.0×10^5 cfu/g) according to (FAO, 2000) and (McCane and Windowson, 2002).

Table 5: Mean values for enumeration of yeasts and moulds of cheese produced from cow milk at different heat treatment duration and different coagulants.

Sample Code	**cfu/g**	**Coded**
A_{15}	Nil	-1
B_{15}	$<1.0x10^2$	0
C_{15}	Nil	1
A_{20}	Nil	-1
B_{20}	Nil	0
C_{20}	$<1.0x10^2$	1
A_{25}	Nil	-1
B_{25}	Nil	0
C_{25}	Nil	1
Control	$<1.0x10^2$	-1

D = Lime coagulant cow-milk cheese, E = Tamarind coagulant cow-milk cheese, F= Moringa coagulant cow-milk cheese, 15 = 15 minutes heat treatment duration, 20 = 20 minutes Heat treatment duration, 25 = 25 minutes heat treatment duration.

4. Conclusion

The following conclusions were drawn from the study:

i. The microbiological analysis revealed that the cow-milk cheese samples were all fit for consumption at all the HTD.

ii. The microbial analysis revealed that there was Coliform in cow-milk cheese samples. C_{25} (moringa seed paste coagulated cheese at 15 minutes of heat treatment) has the highest Coliform of $1.6x10^3$ cfu/g which is within the permissible limit of consumption.

iii. B_{15} (tamarind pulp coagulated cheese at 15 minutes of heat treatment) and C_{20} have yeast and mould bacteria of $<1.0 \times 10^2$ cfu/g which are also within the permissible limit of consumption.

iv. Mesophylic bacteria was highest at C_{15} of $1.1x10^4$ cfu/g which is within the permissible limit of consumption.

Recommendation

Nigerian soft cow-milk cheese made with local coagulants should therefore be recommended for consumption. Further experimental tests could be carried out on the effect of heat treatment and local coagulants on the sensory, mineral and proximate properties of the sample.

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