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Effect of lactic starters on microbiological, physicochemical and sensory characteristics of cheese from goat milk

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ABSTRACT

Lactic acid bacteria are highly beneficial to human health and enhance some vital qualities of foods. In the study a total of ten isolates were obtained from sample are identified as *Lactobacillus plantarum, Lactobacillus rhamnosus, Lactococcus cremoris, Lactococcus lactis, Lactobacillus delbrueckii and Streptococcus thermophillus.* The amount of Lactic acid and diacetyl obtained by the isolates ranged between 0.36g/litre to 1.51g/litre and 0.2g/litre to 0.67g/litre respectively. The highest quantity of lactic acid was produced by *Lactobacillus plantarum* while *Lactococcs cremoris* produced the highest quantity of diacetyl. The entire LAB grew best at the 40 ^{0} C and pH 6. *Streptococcus thermophilus* showed the highest enzyme activities. The nutritional, physicochemical, mineral and sensory properties of cheese samples were evaluated. The cheese sample D had the highest protein (16.25± 0.11%) content and had the least carbohydrate content of 1.8 ± 0.08%. In the mineral composition, cheese D had the highest Calcium content of 49.00 ± 0.31%, Magnesium content of 13.50 ± 0.28 %, Iron content of 0.04 ± 00%, total titratable acidity of 0.16% ± 0.01 and pH of 4.39 ± 0.01. Cheese sample E had the highest reducing sugar of 4.00 ± 0.05% while sample D had the least (3.60 ± 0.09%). Sensory analysis of cheese revealed that sample D had the best rating based on the taste and aroma (2.6 ± 0.31) while sample E had the lowest rating. Conclusively, the use of combined lactic starters of *Streptococcus thermophillus, Lactococcus cremoris, Lactococcus lactis,* and *Lactobacillus delbrueckii* enhanced the nutritional, mineral and sensory cheese produced.

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1. Introduction

Food fermentation had existed for centuries as a way to conserve perishable foods products; it is a technique of strengthening the keeping quality of food for more than six centuries which serve to increase the nutritional value, improve shelf-life, safety, sensory quality and general acceptability of a food product. These were made possible with aid of fermentation conditions and starter culture (Hansen, 2002).

Lactic starters are mixtures of large numerous live Lactic acid bacteria, which are added to accelerate a fermentation process. Being adapted to the substrate, a typical starter facilitates improved control of a fermentation process and predictability of its products. The starter cultures are chosen particularly from a natural source or raw material like animal or plant sources or from their products for example of milk, roots, grains, beans, meat, and tubers (Holzapfel, 1997). Milk a liquid substance, except colostrum's, produced by mammals for the growth and development of their young. Colostrum is a much more concentrated liquid containing up to 25% total solids which is obtained after the birth of the offspring. Milks are highly perishable foods because; its composition offers a complete food for growth of most microorganisms including fastidious pathogens (Martin and Maurice, 2008). Milk is usually found in mammals which include Human, Monkey, Donkey, Camel, Horse, Dog, Goat and Sheep etc. Milk obtain from goats have a greater quantity of medium-chain fatty acids it is known to anti-microbial and disintegrate fat accumulation in the body and highly digestible (Silanikove *et al*., 2008). The nutrient in milk is conserved when cheese is produced (Martin and Maurice, 2008).

Cheese is an aggregated curd of milk solids which contains agglutinated casein enclosed by milk fat. Unlike fermented milks, the observable feature of cheese differs from the milk. This is as a result of enzymatic activity from proteins, fats protein coagulation and the use of lactic starters. The enzymes that are often used in cheese production are *Streptococcus thermophillus, Lactococcus lactis, Lactobacillus delbrueckii, Lactococcus cremoris, Lactobacillus rhamnsus, Lactobacillus plantarum, Lactobacillus helveticus, and Lactobacillus casei* these microorganisms are selected from their subtrates or raw material (milk) based on their potentials (Hoier *et al*., 1999).

Many factor that influences milk composition also influences coagulation properties, the coagulation that is often used in cheese production include calf-rennet powder, some plant extracts (Sodom apple) *Calotropis procra* and specific amount of Calcium Chloride ($CaCI_2$) is also added to improve the rennet activity and cheese texture development (Aworh, 2010).

2. Materials and Methods

Sample Collection

Goat milk (*Sokoto gudali* breed*)* was sourced from goat ranch Bodija animal market in Ibadan, South West part Nigeria, Oyo state and brought into laboratory in sterile conical flasks for immediate use.

Pasteurization of raw goat milk

One point seventy-five (1.75) litre of raw goat milk were dispense into five conical flasks and plugged with cotton wool in a water bath. The goat milk sample was boiled at 65^0C for 30 minutes (Mallantou *et al.*, 2003).

The preparation of cheese from raw goat milk

The raw milk was filtered with a sterile muslin cloth at ambient temperature to remove foreign materials. The milk sample was dispensed into a heat vat for pasteurization at 65^0C for 30 minutes. The pasteurized milk was now allowed to cool to 38^0C and 2.5ml of 40%v/v of $CaCI_2$, was added followed by addition partially purified vegetable rennet and stirred for about 2 minutes. The lactic starter was then inoculated and curd was formed. The curd was then poured into sieve cloth for draining; it was drained at 20^0C for about 6 hours to remove the whey. The cheese was removed from sieve cloth, it was then treated with salt 15%w/w for 48 hours then allow for ripening at temperature 18-20^0C for the period of 6 to 10 days. The cheese was now packed in to plastic containers which were transported into cold room for storage.

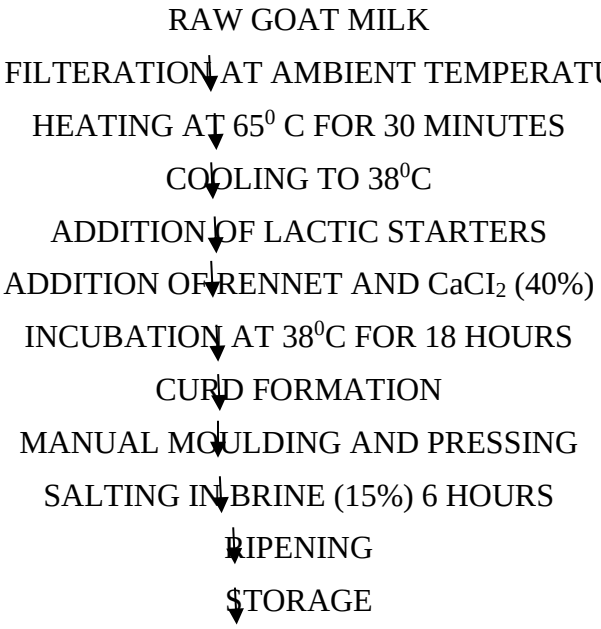

Figure1. The flow chart of Goat milk Cheese using different lactic starters.

Selection for Starter Cultures

The selection of lactic starters used in the production of goat milk cheese was based on the following parameters: Enzymes profile of LAB isolates, quality of lactic acid and diacetyl produced, growth of the Lactic Acid Bacteria isolates at different temperatures and growth at different pH.

Enzymatic screening of the LAB isolates

The Lactic Acid Bacteria enzymatic profiling was carried out using the technique of Arora *et al*. (1990) using API ZYM manufactured by BioMerieux galleries by experimenting for the activity of the following 19 enzymes: Alkaline Phosphatase, Esterase (C 4), Esterase Lipase (6), Lipase (14), Leucinearylamidase, Valinearylamidase, Cytsinearylamidase, Trypsin, α-chymotrypsin, Acid phosphatase, Naphthnol - AS-BI-phossphohydrolase, α-galatosidase, β-galatosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-ficosidase.

Laboratory Production of Goat Milk Cheese

Five treatments of the sample were treated using the above described procedure. The cooled pasteurized raw goat milk was cultured with 1% washed cells of the lactic starters containing approximately 10^6-10^7cfu/ml. The samples were fermented anaerobically in a screw capped conical flask at 38^{0}C for 18h. Lactic starter was introduced into the fifth bottle and this serve as the control sample for the research work.

Cheese Sample **A** was cultured with combine lactic starter, *Streptococcus thermophillus* and Lactobacillus *lactis*,

Cheese Sample **B** was cultured with combine lactic starter, *Streptococcus thermophillus* and *Lactobacillus delbruckii.*

Cheese Sample **C** was cultured with combine lactic starter, *Streptococcus thermophillus* and *Lactobacillus cremoris*

Cheese Sample **D** was cultured with combine lactic starter *Streptococcus thermophillus* and *Lactobacillus cremoris, Lactococcus lactis, Lactobacillus delbrueckii* and

Cheese Sample **E** was the Control (Un-inoculated and unpasteurized milk sample).

The treatments tested, and thus the resultant cheeses, were designated as A, B, C, D and E depending on the starter culture used. 350ml of raw goat milk was poured into five sterile conical flasks in a water bath and heated at 65^0C for 30 minutes. After heating the milk was cooled to 38^0C, and inoculated with lactic starter cultures. At the same time, 2.5ml of 40%v/v $CaCl_2$ and partially purified vegetable rennet was added and mixed well for about 3 minutes. At 18 hours of incubation and at temperature 38^0C coagulation occurred and curd was formed. The curd was transformed a sieve cloth bag, using a ladle, which was then drained at temperature 20^0C for about 6 hours. After draining, the curd was taken out from the bag on a clean board, it was then treated with salt (15% w/w) for 48 hours, wrapping and ripening at 18-20^0C for 6-10 days.

Determination of Physicochemical characteristics of goat milk cheese using lactic starters

A digital pH meter made up of glass electrode was used to determine the pH of the cheese, the digital pH meter was calibrated using standard buffer solutions (Meerck) at pH 4.0 and 7.0.

Potentiometric determination of titratable acidity was carried out using Nout *et al* 1989 technique by titrating 10ml of the fermented sample against NaOH of concentration 0.1M using 3ml of phenolphthalein as the indicator.

Determination of mineral constituent of goat milk cheese using lactic starters.

Spectrophotometric determination of minerals was carried out using A.O.A.C (1980) procedure. Potassium, Sodium, Calcium, Iron, Copper, Zinc, Magnesium and Phosphorus were the minerals analyzed.

Sensory evaluation of goat milk cheese

The sensory evaluation of goat milk cheese was carried out to ascertain the public perception of the product.

The goat milk cheese was subjected to sensory assessment by eight-member panel. The assessor of the goat milk cheese asked for the sample one after the other and taste. It was graded based on the questionnaire provided. The criteria used for evaluating the sample include texture, taste, aroma and the overall acceptance. Eight points hedonic scale was used to grade the sample base on the criteria 8 depicting like extremely to 1depicting dislike extremely.

To evaluate the significant difference between the means, the data obtained from the research work was analyze using ANOVA (Analysis of Variance) SPSS 17.0 version and it was expressed mean ± standard error. The level of significance was set at $P \leq 0.05$.

3. Results

Table 1: The proximate and physicochemical analyses of Cheese Produced from goat milk using different lactic starters.

PARAMETERS	Cheese Samples				
	A	B	C	D	E
PROTEIN (%)	15.58 ± 0.04^{C}	15.80 ± 0.05^{b}	15.00 ± 0.02^{d}	16.25 ± 0.11^{a}	15.50 ± 0.03^{C}
FAT (%)	13.65 ± 0.11^{b}	13.58 ± 0.20^{b}	13.16 ± 0.21^{b}	14.25 ± 0.02^{a}	12.80 ± 0.21^{C}
LACTIC ACID (Titratable Acid) (%)	0.13 ± 0.01^{a}	0.14 ± 0.01^{b}	0.13 ± 0.01^{a}	0.16 ± 0.01^{a}	0.11 ± 0.01^{a}
MOISTURE (%)	52.00 ± 0.63^{b}	51.22 ± 0.04^{bc}	50.44 ± 0.26^{c}	48.61 ± 0.098^{d}	56.64 ± 0.05^{a}
Ph	4.41 ± 0.06^{bc}	4.52 ± 0.04^{b}	4.47 ± 0.02^{bc}	4.39 ± 0.01^{c}	5.84 ± 0.01^{a}
REDUCING SUGAR	3.80 ± 0.17^{bc}	4.00 ± 0.05^{ab}	3.88 ± 0.04^{abc}	3.60 ± 0.09^{c}	4.2 ± 0.05^{a}
ASH (%)	3.76 ± 0.35^{b}	3.60 ± 0.07^{b}	3.91 ± 0.01^{a}	3.8 ± 0.01^{ab}	3.72 ± 0.04^{b}
CARBOHYDRATE (%) (lactose)	2.60 ± 0.09^{b}	2.50 ± 0.16^{b}	2.28 ± 0.09^{b}	1.80 ± 0.08^{c}	3.60 ± 0.09^{a}

Mean Value ± Standard Error, Similar superscript across rows are not significantly different.

A Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcuslactis,*

B Cheese made with lactic starters *Streptococcus thermophillus* and *Lactobacillus delbrueckii*

C Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus cremoris*

D Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus lactis*, *Lactobacillus delbrueckii* and *Lactococcus cremoris* **E** Cheese made without any lactic starter (Control)

Table 2 The mineral composition produced from goat milk using different lactic starters

PARAMETERS	Cheese Samples				
ELEMENTS (mg/100g)	A	B	C	D	E
SODIUM (Na)	35.77 ± 0.12^{b}	35.85 ± 0.02^{b}	35.65 ± 0.14^{b}	36.59 ± 0.05^{a}	35.00 ± 0.00^{C}
COPPER (Cu)	0.02 ± 00^{ab}	0.02 ± 00^{ab}	00.02 ± 00^{b}	0.03 ± 00^{a}	0.02 ± 00^{C}
CALCIUM (Ca)	47.69 ± 0.05^{b}	47.80 ± 0.11^{b}	46.70 ± 0.35^{c}	49.00 ± 0.31^{a}	46.00 ± 0.31^{c}
IRON (Fe)	0.30 ± 00^{b}	0.03 ± 0.0^{b}	0.02 ± 00^{b}	0.04 ± 00^{a}	0.02 ± 00^{c}
MAGNESSIUM (Mg)	12.70 ± 0.23^{a}	12.80 ± 0.17^{a}	12.55 ± 0.20^{a}	13.50 ± 0.28^{a}	11.00 ± 0.57^{b}
POTASSIUM (K)	60.50 ± 0.40^{ab}	61.00 ± 1.15^{ab}	60.00 ± 0.57^{ab}	62.00 ± 0.57^{c}	59.00 ± 1.15^{b}
ZINC (Zn)	0.32 ± 0.01^{a}	0.34 ± 0.01^{a}	0.33 ± 0.01^{a}	0.36 ± 0.02^{a}	0.30 ± 0.02^{a}

Mean Value ± Standard Error, The same superscript across rows are not significantly different.

A Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcuslactis,*

B Cheese made with lactic starters *Streptococcus thermophillus* and *Lactobacillus delbrueckii*

C Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus cremoris*

D Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus lactis, Lactobacillus delbrueckii* and *Lactococcus cremoris*

E Cheese made without any lactic starter (Control)

Table 3 The Sensory characteristics of cheese produced from goat milk using different lactic starters

Sensory Characteristics	Cheese Samples				
	A	B	C	D	E
TASTE	3.5 ± 0.22^{b}	3.9 ± 0.23^{b}	3.5 ± 0.27^{b}	2.6 ± 0.31^{C}	5.5 ± 0.27^{a}
AROMA	$3.8 \pm 0.13^{b, c}$	4.0 ± 0.25^{b}	$3.2 \pm 0.25^{c, d}$	2.7 ± 0.21^{d}	5.7 ± 0.21^{a}
TEXTURE	4.3 ± 0.26^{bc}	$4.5 \pm 0.26^{a, b}$	$4.5 \pm 0.17^{a, b}$	3.7 ± 0.21^{c}	5.1 ± 0.18^{a}
APPEARANCE	4.2 ± 0.25^{b}	4.2 ± 0.29^{b}	4.1 ± 0.23^{b}	3.3 ± 0.36^{c}	5.1 ± 0.23^{a}
GENERAL ACCEPTANCE	3.8 ± 0.13^{c}	4.4 ± 0.26^{b}	3.7 ± 0.21^{c}	2.8 ± 0.20^{d}	5.5 ± 0.16^{a}

Mean value with the superscript are not significantly different along rows. Means value ± Standard error. **A** Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus lactis,*

B Cheese made with lactic starters *Streptococcus thermophillus* and *Lactobacillus delbrueckii*

C Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus cremoris*

D Cheese made with lactic starters *Streptococcus thermophillus* and *Lactococcus lactis, Lactobacillus delbrueckii* and *Lactococcus cremoris*

E Cheese made without any Lactic starter (Control) Using 8 points hedionic scale, 1 - Like very extremely, 2 - Extremely, 3 - Very good, 4 - Good, 5 - fair, 6 - Neither good nor fair, 7 - Poor, 8 - Dislike Extremely

4. Discussion

The LAB reported in this study produced a moderate range of enzymes which are vital in sensory development, protein breakdown and texture development, aid rapid ripening and predictable product (which is in line with the work of Martin and Maurice, 2008). None of the isolates showed activity B-glucosidase but most LAB isolates show lipolytic and proteolytic activity which agreed with the work of Gonzalez *et al*, 2000 and Anderson *et al.*, 1995.

Table 1 revealed the total titrable acidity (lactic acid percentage) increase and pH decreased as a function of fermentation time, which is in agreement with Martin and Maurice, 2008. Lactic acid produced decrease in the proliferation of undesirable microbes. There are no significant differences in proximate analysis for Carbohydrate, reducing sugar, pH and fat for the treatments A, B and C. However, treatment D and E were significantly different for the same parameters (Carbohydrate, reducing sugar, pH and fat). Fermentation by lactic acid bacteria was able to improve the nutritional qualities of the cheese as observed in the noticeable increase in protein and fat. There was a decrease in carbohydrate content which is based on the lactose. The protein and fat of the cheese Sample D is higher when compared to that of cheese Sample E (control). This is similar to the works of Cabeza *et al* (2005) and Fedaku *et al* (2005), they produced Manchego and Colby goat milk cheese with different starter and an increase in fat and protein content was observed. It was observed there was a decrease in carbohydrate and reducing sugar as a result of combination of lactic starters. This is similar to the work of Olasupo *et al.*, 1997. The decrease or low pH

of the cheese Sample A, B, C and D in this work agrees with the reports of Ten Brink *et al* 1994, Ene-Obong and Carnovale, 1992.

Table 2 also revealed increase in mineral elements confirms the fact that fermented foods constitutes product of microbial decomposition resulting in the mineralization of higher organic component to release these elements. There are no significance difference in mineral composition (Potassium and Zinc) among the five treatments also there are no significant difference among treatment A, B and C for Magnesium, Iron, Copper and Sodium but there were significant differences for treatment D and E for the same element. In this study, it was observed that the mineral content of cheese Sample D increases when compared with other cheese Samples. The slight increase in vital mineral nutrients of goat milk shows that lactic acid fermentation enhanced nutritive value of goat milk cheese which is similar work to the works of Sanna *et al* (2005) and Izco *et al* (2002).

Table 3 The sensory analysis revealed that cheese Sample D had higher general acceptability based on aroma, texture, taste and appearance when compared with other cheese Samples produced, which is the interplay of three biochemical events (proteolytic, lipolysis and glycolysis) along with numerous concomitant secondary transformation which is in line with the works of Park, 2001, Park *et al.*, 2007; Akalin *et al.,* 2002 and Uduak *et al.,* 2007. There is no significant difference in sensory evaluation (Taste, Texture and Appearance) in treatment A, B and C. However, the cheese made with the treatment D and E where significantly different for the same characteristics, similar observation was also reported by Aworh, 2010.

Conclusion

In conclusion, fermentation activities aided by lactic starter enhance beneficial value, the safety and general acceptability of goat milk cheese. The research work buttresses the stand on goat milk cheese as an excellent source of proteins, fat and mineral, an excellent quality cheese can be produced using starters comprises of *Streptococcus thermophillus, Lactococcus lactic, Lactobacillus delbrueckii and Lactococcus cremoris* and storage at the refrigerated temperature will prolong the shelf life of cheese so produced.

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