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COMPARATIVE SOFT CHEESE PRODUCTION FROM COW AND CAMEL MILK USING PURIFIED ENZYME PRODUCED BY *Aspergillus niger*

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ABSTRACT
This study evaluates the potential of purified enzyme from Aspergillus niger as an alternative to commercial rennet for production of soft cheese from cow and camel milk. The yield and texture of the cheese were compared. Milk samples from cows and camels were collected from Sidi Mamman Assarakawa farm and Hankom farm Kasarawa and transported to the microbiology laboratory Sokoto State University, in a sealed container with ice pack. A. niger was subjected to solid-state fermentation (SSF) on wheat bran supplemented with skim milk powder and mineral solution, incubated at 30^{o}C for 120 hrs. The crude enzyme was extracted by aqueous shaking and purified via ammonium sulfate precipitation. Milk clotting Activity (MCA) was assessed using a standard skim milk coagulation assay, while proteolytic activity was determined via casein hydrolysis and spectrophotometric analysis at 660 nm. Soft cheese was produced using fungal enzyme (1.0 %) and commercial rennet as a control. The results showed that cow milk cheese produced with A. niger enzyme has a yield of 10.32± 0.03 g/100 ml and 11.96±0.10 g/100 ml by commercial rennet. However, camel milk cheese produced with A. niger enzyme has a yield of 9.4± 0.02 g/100ml and 10.5±0.30 g/100ml by commercial rennet. There is significance differences of the yield between fungal enzyme and commercial rennet (control) at p|>0.05 level of significance This study demonstrates that cow milk showed higher overall yield and firmness, the enzyme from A. niger demonstrated comparable performance to commercial rennet, especially with cow milk, highlighting its potential in sustainable cheese production. These findings suggest a promising alternative for region where rennet availability is limited.
Keyword: Aspergillus niger, enzyme, rennet and cheese

INTRODUCTION

Soft cheese production is a significant aspect of the dairy industry, with various types of milk being used worldwide (Fox *et al*., 2017). Soft cheese is a semi solid and acidic food made from milk, with rennet added to coagulate milk casein. The basic steps of processing milk into cheese for the most varieties are; Milk preparation, Acidification, Coagulation, Dehydration, Molding, Pressing, Salting and Ripening (Fox and Guinee, 2013).

Cheese production relies heavily on rennet, an enzyme complex traditionally sourced from calf stomachs. However, the ever-increasing demand for cheese, shortage of calf rennet, and religious restriction on the consumption of calf rennet-based cheese are major challenges for the dairy industries. This necessitated a search for alternatives to calf rennet. Camel milk, in particular, has gained attention due to its unique nutritional profile and potential health benefits (Konuspayeva *et al*., 2009). *Aspergillus niger* is a well known producer of industrial enzymes, but its application in cheese making remains underexplored. Also *Aspergillus niger*, a fungus, produces enzymes that can be used as a rennet substitute in cheese production (Kumar *et al*., 2010), the use of microbial rennet has gained popularity in recent years due to its potential to reduce costs and improve the sustainability of cheese production (Santos *et al*., 2017).

The majority of these enzymes are mainly produced by genera such as *Aspergillus, Mucor, Entothia, Rhizopus, Penicillium* and *Fusarium*. These fungi are characterized by their adaptation to solid-state fermentation (SSF) using cheap substrates, which improve the recovery of extracellular enzymes from fermentation media with high yields, solve pollution problems and reduce the capital costs of process (Sathya *et al.,* 2009).

This study investigates the potential of a purified enzyme from *Aspergillus niger* in soft cheese production from cow and camel milk, comparing the results with commercial rennet to assess its suitability for cheese production.

MATERIALS AND METHODS

Milk collection

Milk samples from cow and camels were obtained early morning from Sidi Mamman Assarakawa farm and Hankom farm Kasarawa Sokoto. The milk was transported to the microbiology laboratory Sokoto State University in a sealed container in with ice pack. The milk was then stored at 4^{o}C.

Enzyme production

Aspergillus niger was subjected to solid state fermentation (SSF) according to the method of Sathya *et al.* (2009).

The fermentation was carried out in 250 ml conical flask which contained 10g of wheat bran, 2g of skim milk powder, this was moistend with 10ml of mineral solution (g/L) : 2.0, KNO_3; 0.5, $MgSO_4 \cdot 7H_2O$; 1.0, $K2HPO_4$; 0.439, $ZnSO_4 \cdot 7H_2O$; 1.116, $FeSO_4 \cdot 7H_2O$; 0.203, $MnSO_4 \cdot 7H_2O$, and pH was adjusted to pH 5.0. The flask was autoclaved at 121^{0}C for 20 min, and allowed to cool. After sterilization, the flask was inoculated with 1.0 ml of spore suspension and incubated at 30 ^{o}C for 120 h (Sathya *et al.,* 2009).

Extraction of Enzyme

After the fermentation period, 100ml distilled water was added to the solid-state fermentation medium and homogenized by shaking for 40 min. Cell-free supernatant was obtained by centrifuging the extract at 10,000 rpm for 30 min. The centrifuged extract was filtered through Whatman No.1 filter paper to obtain crude enzyme (Ja'afar *et al.*, 2020).

Purification of Extracted Enzyme

The crude enzyme extracted from the samples was purified by subjecting to ammonium sulphate precipitation. The filtrate was taken and 70% fraction of ammonium sulphate was added slowly to the supernatant. While adding the ammonium sulphate, the culture was kept in ice. The mixture was incubated overnight in refrigerator at 4ºC. After 24 hrs, the mixture was centrifuged at 12,000 rpm for 10 mins. The supernatant was collected as a partially purified enzyme (Ramachandran and Arutselvi, 2013).

Determination of Milk-Clotting Activity (MCA)

Milk-clotting activity from the skim milk samples was determined according to Arima *et al,* (1970). Five milliliters (5 mL) of 10% solution of skim milk powder (Itambé) in 0.01 M $CaCl_2$ was pre-incubated at 35°C for 10 min. The enzyme extracts (0.1 mL) was added. Clot formation was observed while manually rotating the test tube. The time at which the first particles were formed was recorded. One milk-clotting unit (MCU) was defined as the amount of enzyme required to clot 1 mL of substrate in 40 min at 35°C and was calculated according to Shata (2005): unit of milk-clotting activity (U) = $2400/T \times S/E$ where T is the time necessary for clot formation, S is the milk volume and E is the enzyme volume (Shata, 2005).

Protease Activity Assay

The proteolytic activity was assayed according to Arima *et al*. (1970). The enzyme extract (0.5 ml) was added to 2.5 mL of 1% (w/v) soluble casein in 20 mM potassium phosphate buffer at pH 6.5, and the mixture was incubated in a water bath at 35°C for 10 min. After the addition of 2.5 mL of 0.44 M trichloroacetic acid to terminate the reaction, the mixture was filtered through the Whatman No.1 (90 mm) filter paper. The filtrate was then mixed with 1 mL volume of three times diluted 2N Folin/phenol reagent and 2.5 mL of 0.55 M sodium carbonate solutions and incubated at 35°C for 20 min to detect color development. Optical density (OD) was measured using a spectrophotometer (UV-vis, Liantrinsat, and Model-CF728YW-UK) at 660 nm. One unit (1 U) of enzyme activity was defined as the amount of enzyme that liberated 1 μg of tyrosine per mL in 1 min:

$$\mathrm{PA\ (U/mL)} = \frac{\mu Tyr * V_t}{V_s * T * V_a},$$

where PA is the protease activity, μTry is the μg of tyrosine equivalent released, V_t is the total volume of the assay in mL (5 ml of the substrate plus 1 ml of the enzyme plus 5 ml of TCA), V_s is the sample volume (i.e., the volume of protease used for the assay in mL), T is the reaction time (i.e., time of incubation in minutes, 10 min), V_a is the volume assayed (i.e., final volume of the product used in calorimetric determination).

Production of soft cheese and yield determination

Soft cheese was made according to the method described by Renner and Abd El-Salam (1991). Pasteurized cow and camel milk was divided into two equal portions. The first part was renneted using calf rennin-coagulate (1g/100 kg) as a control (C). The second part was renneted using

the applicable ratio of fungal enzyme (1.0 %) as experimental treatment (E). All the treatments were kept for clotting until a uniform coagulum was formed. Milk retentate dispensed into plastic bags (500 ml) and held at 38°C until a uniform coagulum was formed. All the cheese samples were stored at 7±2°C and analyzed. The soft cheese produced was weighed immediately and the yield was calculated as follow:

$$\text{Cheese yield (\%)} = \frac{Weight\ of\ cheese}{Weight\ of\ Milk} X\ 100$$

Statistical Analysis

The experimental design adopted for this study was the completely randomized design (CRD). Treatments were replicated three times and data obtained were subjected to analysis of variance (ANOVA) using the SAS (2000) software. P value < 0.05 was considered significant.

RESULTS

Milk clotting activity (MCA) and Protease activity (PA)

Table 1 Shows the Milk Clotting Activity (MCA) of *Aspergillus niger* extract which took 750 ±1.15Sec to initiate milk coagulation which is approximately 12.5 minutes and the value 160 shows the milk clotting activity of the extract, which is a measure of its ability to coagulate milk. The unit of measurement is expressed in soxhlet units (su) and 0.146 $\pm$ 0.00 show the proteolytic activity of the *Aspergillus niger* extract.

Table 2 shows the cheese produced from Cow Milk, i.e. the cheese made using commercial rennet (control) has a higher cheese yield of 11.96±0.10g/100ml compared to the cheese yield produced using *Aspergillus niger* extract 10.32±0.03g/100ml. The fungal enzyme has a slightly higher curd syneresis of 65±1.02ml compared to the commercial rennet (control) with 62±0.01ml. It was observed there is no significant difference (P<0.05) in color and body properties of the two cheese products.

Table 3 shows the cheese produced from Camel Milk, the control cheese made using commercial rennet has a higher cheese yield of 10.5±0.30g compared to the cheese yield produced using *Aspergillus niger* extract 9.4±0.02g. The fungal enzyme has a slightly higher curd syneresis of 75±0.00ml compared to the commercial rennet (control) 70±0.01ml.

Table 1: Milk Clotting Activity and Protease Activity of Purified Enzyme Produced by *Aspergillus niger.*

Sample	MCT (Sec)	MCA (Su)	PA (µ/ml)
A. niger extract	750 ±1.15	160	0.146 ± 0.00
Standard values	2400	500	1.00

Key: MCT - Milk Clotting Time
MCA - Milk Clotting Activity
PA - Proteolytic Activity

Table 2: Production of Cheese from Cow Milk

Treatment	Cheese yield g/100ml	Color	Body properties	Curd Syneresis (ml)
Produced cheese	10.32±0.03	Creamy	Soft	65±1.02
Control Cheese	11.96±0.10	Creamy	Soft	62±0.01

TABLE 3: Production of Cheese from Camel Milk

Treatment	Cheese yield g/100ml	Color	Body properties	Curd Syneresis (ml)
Produced cheese	09.4±0.02	White	Soft	75±0.00
Control Cheese	10.5±0.30	White	Soft	70±0.01

DISCUSSION

The Milk Clotting Activity (MCA) and Milk Clotting Time (MCT) of the partially purified enzyme extracted from *Aspergillus niger* demonstrated a MCA of 160.0 su/ml with a clotting time of 750 ±1.15 Sec. This may be due to enzyme is moderately active. The purification process might have retained sufficient active enzyme, but any residual impurities or incomplete purification could slightly affect its efficiency.

This result is in line with the result reported by Sambo *et al.* (2024) who reported a Milk Clotting Activity (MCA) of 160.8 su/ml at 750 sec Milk Clotting Time (MCT) . Similar result was reported by Carlina *et al.* (2010) who reported highest Milk Clotting Activity (MCA) of 160.3 su/ml. Bensmail *et al.* (2019) also reported highest MCA of 351.2su/ml from *A.tamari strain* and MCA of 398.16 su/ml from *M. circinelloides* which is higher to the result reported in this research. The observed variation in reported MCA values may be attributed to discrepancies in experimental conditions.

The protease activity (PA) generated (0.146 µ/ml) was in line with the result reported by Sambo *et al.* (2023). Emochone *et al.* (2023) also reported a protease activity of 0.32µ/ml. All the result generated are very low as compared with the findings of Rodate *et al.* (2011) who reported protease activity of 13.82µ/ml and 20.9µ/ml from *Aspergillus Ochracious* and *A. Dimorphicus* isolated from fruits. Carolina *et al.* (2010) also reported a low proteolytic activity of 0.6±0.00 (µ/ml). The variation in reported proteolytic activity (PA) of enzymes may be attributed to experimental methods.

The cheese yield reflects the efficiency of Milk conversion into cheese, often influenced by Milk Compositional and Coagulation method.

Jermen *et al.* (2020) reported a cheese yield of 9.0% using commercial rennet and 8.6% with dialyzed fungal coagulant, which are lower than the result reported in this study (10.32) for the cheese produced using *Aspergillus niger* extract and 11.96 for the cheese produced using commercial rennet (control). The result is in line with the result of El Owni (2009) who reported a higher yield of 13.2% and 11.6% for cow milk cheese. Sadia *et al.* (2016) reported a significant higher cheese yield of 19.68% from cow milk. This result is in agreement with the result reported by Ojedapo *et al.* (2014) who reported a cheese yield of 19.26 ± 3.14. Also Olorunnisom (2012) reported a higher yield of 23.41% using Moringa seed extract as a coagulant. However, the camel milk cheese yield reported in this study (9.4% and 10.5%) for both cheese produced using *Aspergillus niger* extract and the cheese produced using commercial rennet (control) were consistent with the result reported by Mihretie *et al.* (2018) who reported a cheese yield from camel milk of 9.86% and 10.94% using commercial rennet. The differences in cheese yield observed may be due to initial milk composition, type of coagulant used and breed of the cow and camels.

Color is a sensory attribute significantly influenced by Milk source and processing methods. Both Cow Milk cheese was described as creamy, while Camel Milk Cheese was described as white in both cheese produced using *Aspergillus niger* extract and cheese produced using commercial rennet (control). The creamy color in Cow Milk Cheese can be attributed to the higher beta-carotene levels in Cow Milk where as Camel Milk lacks this pigments, resulting in a naturally whiter cheese.

The body properties of the cheeses such as texture were consistent across treatments and milk types, with both produced cheese using *Aspergillus niger* extract and produced cheese using commercial rennet from cow and camel were classified as soft. This study is consistent with the study by Hayam *et al.* (2013).

The softness of Camel Milk Cheese aligns with its lower casein Micelle concentration making it harder to achieve firmer textures compared to cow milk cheese as reported by Berhe *et al.* (2017).

Curd Syneresis, the process where whey is expelled from curds, also showed variation. Cow Milk cheese had less syneresis, with 65ml for the produced cheese using *A. niger* extract and 62ml for the produced cheese using commercial rennet. In contrast, Camel Milk cheese exhibited higher syneresis levels, with 75ml for the produced cheese using *A. niger* extract and 70ml for the produced cheese using commercial rennet. This is consistent with the findings that camel milk has weaker curdling properties and forms looser curds leading to greater whey expulsion (Attia *et al.*, 2001).

CONCLUSION

The results demonstrated that cow milk cheese produced with *A. niger* extract had a yield of 10.32±0.03 g/100 ml, which was comparable to the 11.96 ±0.10g/100 ml obtained with commercial rennet. Similarly, camel milk cheese yielded 9.4 ±0.02g/100 ml with *A. niger* extract, compared to 10.5±0.30g/100ml with commercial rennet. However, cow milk cheese exhibited higher yield and firmness than camel milk cheese, and the *A. niger* extract showed a performance close to that of commercial rennet, particularly in cow milk. These findings highlight the potential of *A. niger* –derived enzymes as a viable and sustainable alternative to traditional rennet, especially in regions where rennet availability is limited.

RECOMMENDATION

Further research is needed to optimize the enzyme production process and explore its application in various cheese types.

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