



Production of Soft Cheese from *Aspergillus tamarii* Enzyme and Sensory Perception

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ABSTRACT

Microorganisms from naturally-occurring acid habitats are expected to provide new strains producing enzymes that are active and stable in acid conditions. The increasing demand for rennin for cheese making and ethical constrain on the use of porcine rennet justified the need to search for renin substitutes from sources other than animals. Making cheese is a great way to preserve milk supply as some types of cheese can be aged for two years or more without refrigeration. Therefore, screening for organisms that can produce milk clotting enzymes can add to the country's economy because it is affordable. Partially purified protease of *Aspergillus tamarii* was used for comparative cheese production with commercial rennet, using eighteen (18) experimental buckets according to the types of coagulants and Milk samples. Production of cheese with the enzyme using Camel and Cow Milk was better with the combination of the two milks at 1:1 ratio when compared to commercial rennet. The enzyme produced cheese yield of $17.32 \pm 0.16\%$. The sensory perception by panelist for cheese produced placed Cow Milk +Extracted Enzymes Cheese as the best for their choice and the 1st by Kruskal Wallis mean ranking.

INTRODUCTION

Cheese can be defined as a consolidated curd of milk solids in which milk fat is entrapped by coagulated casein (Adam and Moss, 2000). This description comprises a heterogeneous group of fermented milk-based products, which are elaborated worldwide and present a broad range of flavours and forms. The main ingredient in cheese is milk. Cheese is made using cow, goat, sheep, buffalo or a blend of these milks. The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid sources such as acetic acid (the acid vinegar) or

gluconodelta-lactone (a mild fool acid), is used. For rennet cheese, calf rennet or, more commonly, rennet produced through microbial source, calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk (cheese production/milk Facts.info). Aspartic proteases, also known as acidic proteases have been isolated from diverse sources, including virus, bacteria, fungi plants and animals (Chen *et al.*, 2009). They are use as milk-coagulating enzyme for the manufacture of cheese. Hence some of the aspartic proteases are termed as microbial rennet like milk clotting enzymes that catalyse milk coagulation substituting calf rennet,

which is the main enzyme employed in cheese production (Patilpallavi *et al.*, 2012). Milk coagulation is a basic step in cheese manufacture using *Mucor* and *Penicillium* strains mainly as adjuncts in different types of cheese, and in these recent years other strains like *Rhizopus* prove interesting as producers of rennet – like enzymes (Abebe *et al.*, 2014). The worldwide increase of cheese production coupled with a reduced supply of calf rennet has prompted a search for calf rennet substitutes including microbial and plant rennet (Cavalcanti *et al.*, 2004). The finding of this research would create awareness and job opportunities in dairy industries for teaming population. It would also developed a protocol of preserving milk through cheese production aver along period of time.

Therefore, this study aimed at production of cheese from camel and cow milk and combination of both using the extracted enzymes (EE) produced and compare with commercial rennet. And further evaluate sensory perception of cheeses produced using Hedonic scale for individual responses.

MATERIALS AND METHODS

Collection and Analysis of milk Sample for cheese production

Ten (10) liters each of camel and cow milk were collected early morning from free range camel and cow herds at a Fulani settlement in Sokoto. The milks were sieved in a double layered cheese cloth and poured in plastic containers surrounded with ice block and transported to laboratory, for pH determination, mineral analysis and cheese production.

Production and Purification of Enzymes for Cheese Making

Aspergillus specie was subjected to Solid State Fermentation (SSF) according to the method of sirividya (2012) with a little modification using Banana Peel Powder (BPP) as substrates to produce protease that was used for comparative cheese making. After fermentation, enzyme protease was extracted using the method of Sirividya (2012) and

purified according to the method of Ramachandra and Arustselve (2013).

Assay of enzyme and Protease activity (Milk clotting activity)

The Milk clotting activity (MCA) of the enzyme extracted was measured by method described by Otani *et al.*, (1991) and the milk-clotting activity was calculated by using the formula of Kawai and Mukai (1970). Protease activity of each protease was measured using the method of Kunitz (1947) which was adopted by kademi *et al.* (2013).

Soft Cheese Making and yield determination

Partially purified enzymes of *Aspergillus tamaris* (EE) were used for the preparation of comparative milk curds. A 500ml of fresh milk each of cow (CWM) and camel (CM) were poured separately into eighteen (18) experimental buckets which were labeled according to the type of milk and coagulant. The cheese produced from each experiment was weighed on an electrical weighing balance; percentage cheese yield was thus calculated using the formula:

$$\frac{\text{Grams of cheese produced}}{\text{Grams of milk used}} \times 100$$

Akinloye and Adewomi, 2016

Sensory Evaluation of the Soft Cheeses Produced

Each produced cheese was examined after 72 hours of ripening and sixty member trained panelists were employed for the sensory tests. Evaluation was based on five attributes: colour, taste, flavor, texture and overall acceptability, scores were based on 9 - point hedonic scale (Badmos and Joseph, 2014)

RESULTS

Figure 1 revealed that, there is no significant difference between pH values 6.7 and 6.6 of cow and camel milk.

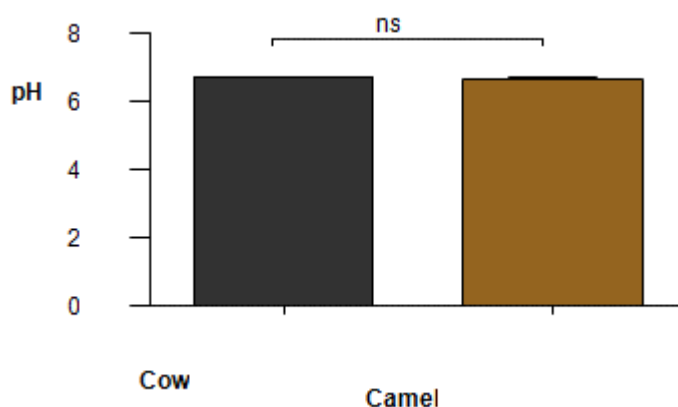


Figure 1: pH of Cow and Camel Milk

pH of cow and camel milk values are mean $\pm$ SD of three replicates, means values of the two milk are not significantly different (ns) to one another at $P > 0.05$ using one way ANOVA.

Table 1 shows that there was no significant difference in terms of calcium contents of cow and camel milk samples, but there was significant difference in all other mineral compositions ($P > 0.05$).

Table 1: Mineral Contents of Cow and Camel Milk Samples

Sample	Mineral composition (mg/kg)				
	Sodium	Potassium	Calcium	Magnesium	Phosphorus
Cow	25.33 $\pm$ 0.58 ^a	346.70 $\pm$ 23.09 ^a	0.38 $\pm$ 0.03 ^a	0.12 $\pm$ 0.03 ^a	1.43 $\pm$ 0.01 ^a
Camel	33.67 $\pm$ 1.50 ^d	520.00 $\pm$ 40.00 ^c	0.33 $\pm$ 0.03 ^a	0.23 $\pm$ 0.03 ^c	1.89 $\pm$ 0.02 ^d

Values are mean $\pm$ SD of three replicates. Mean value with different superscript letters in columns are significantly ($p < 0.05$) different to one another (Unpaired t test). b: significant ($p < 0.05$); c: significant ($p < 0.001$); d: significant ($p < 0.0001$).

Table 2 present results of milk-clotting properties of partially-purified enzymes of *Aspergillus tamaraii*. The average clotting time was 382 sec. The milk-clotting

activity (on the average using 5ml portion of milk and 0.5ml of enzymes) was 351.2 with a protease activity of 0.35U/mL.

Table 2: Milk Clotting Properties of Partially Purified Protease Produced from *Aspergillus tamaraii* for Cheese Production

Sample	Milk Clotting Time (MCT) (sec)	Milk Clotting Activity (MCA) (SU)	Protease Activity (PA) (U/mL)
BPP + <i>Aspergillus</i>	382	351.2	0.35

Values are mean $\pm$ SD of three replicates. Mean value with different superscript letters in columns are significantly ($p < 0.05$) different to one another (Unpaired t test). b: significant ($p < 0.05$); c: significant ($p < 0.001$); d: significant ($p < 0.0001$).

BPP =Banana Peel Powder

Aspergillus = *Aspergillus tamaraii*.

SU = Soxhlet Unit

Table 3, presents the percentage cheeses yield for the six samples produced from camel and cow milk. The highest percentage yield (17.32 $\pm$ 0.16%) was obtained from a combination of the two milks treated with the extracted enzyme (EE) of *Aspergillus tamaraii*, followed by cheese produced from combination of the two milks treated with commercial rennet (CR) (16.89 $\pm$ 0.13%). The least percentage yield was from camel milk treated with CR (15.26 $\pm$ 0.35). However, it

was also observed from the table that EE (15.67 $\pm$ 0.16) was better with camel milk than CR (15.26 $\pm$ 0.35), while CR was better with cow milk than EE (although the difference was not significant). All the enzymes were good with the combination of CM and CWM as the yields was very close (17.22 $\pm$ 0.16% for EE and 16.89 $\pm$ 0.13% for CR). There was a significant difference among the various cheese yields except in CWM treatments.

Table 3: Soft Cheese yield from *Aspergillus tamaraii* Enzymes and Commercial Rennet

Source	Yield (%)				
	Cow	Camel	Cow + Camel	Cow	Camel
CR	16.50 $\pm$ 0.25 ^a	15.26 $\pm$ 0.35 ^a	16.89 $\pm$ 0.13 ^a		
EE	16.21 $\pm$ 0.15 ^a	15.77 $\pm$ 0.16 ^b	17.22 $\pm$ 0.16 ^c	16.21 $\pm$ 0.15 ^a	15.87 $\pm$ 0.16 ^b

Values are mean $\pm$ SD of three replicates. Mean value with different superscript letters in columns are significantly ($p < 0.05$) different to one another (Unpaired t test). b: significant ($p < 0.05$); c: significant ($p < 0.001$).

The sensory evaluation data were subjected to Kruskal Wallis test to rank the products as selected by different panelists. Both the Kruskal Wallis rank points and chi-square values were used to rank the products in ascending order based on the panelist acceptability and its relationship with the sensory attributed.

The Kruskal Wallis mean ranking (Table 4) showed that cheese produced from CW + EE was the best of panelists' choice. So it was ranked 1st (as it has the highest mean scores for taste, flavor and general acceptability (GA) which were 216.90, 204.64 and 222.58 respectively). This was followed by cheese

produced from a combination of CWM+CM+EE, which had the highest mean score for texture (205.48) and was next to CWM+EE in terms of taste and flavor. Hence it was positioned as 2nd. Cheese from CWM+CR was ranked 3rd and the best in terms of colour with the highest score of 203.76. The 4th position on the Kruskal Wallis mean ranking was the cheese product from CWM+CM+CR. Then finally, the test in relation to the panelists' choice left behind cheese products from CM+EE and CM+CR as the 5th and 6th positions respectively.

Table 4: Sensory Evaluation of Soft Cheeses Produced from Camel and Cow Milk

Products	Colour	Texture	Taste	Flavour	GA	Rank
Cow Milk + EE	177.95	199.44	215.90	202.65	222.58	1 st
Camel Milk + EE	185.50	170.24	163.73	177.81	178.39	5 th
Cow Milk + Camel Milk + EE	155.17	204.48	194.49	184.77	166.44	2 nd
Cow Milk + CR	203.76	161.38	193.13	166.54	171.27	3 rd
Camel Milk + CR	180.45	156.35	135.83	166.65	157.89	6 th
Cow Milk + Camel Milk + CR	181.18	186.77	178.91	182.58	181.20	4 th
Chi-square value	6.921	11.785*	22.241*	5.617	14.850*	
Chi-square Critical Value						11.070
P-value	0.227	0.038*	0.000*	0.345	0.011*	

*values with asterisk are significantly different

DISCUSSION

pH result for CWM 6.7 is within the East African community 2006 reference range of 6.6 to 6.8, it is also very close to the mean pH of cow milk samples reported by Gwandu *et al.*, (2018). While pH 6.6 for CM is within the range of pH (6.4-6.7) reported by Wangoh (1997) for fresh camel milk, which further stated that it was similar to that of sheep milk, but slightly lower than that of Bovine milk.

The MCA and MCT of the supernatant enzymes from BPP treated with *A. tamari* supernatant proved to have a highest MCA of 351.2SU/mL with a short clotting time of 382 sec. The shortest clotting time of *A. tamari* is related to the findings of Benlounissi *et al.*, (2012) who reported that *A. tamari* and *A. niger* fermented industrial whey of cheese to clot milk within 5 minutes. Mirian *et al.*, (2011), also reported high clotting activity from *A. ochraceous*. Sethi *et al.*, (2016) identified banana peel (*Musa paradisiaca* L. Family: Musaceae) as most suitable for proteases (pectinase) biosynthesis with activity of 6500±1116.2 µg/L. The milk clotting activity 351.2 SU/ML from *Aspergillus tamarii* strain is also related MCA of 398.16 SU/ML from *M. circinelloides* was reported by Bensmail *et al.* (2019) using wheat bran as substrate.

The PA generated 0.35 U/ML for the enzymes of *A. tamari* on treatment with BPP are very low when compared with the findings of Rodate *et al.*, (2011). Protease activity of 13.82U and 20.90U for *Aspergillus ochraceous* and *Aspergillus dimorphicus* isolated from fruits and use casein as substrate to assay for their PA at 30°C, pH 7.0 for 2 hours. The time coarse and pH could be a reason for the

variation, that is 30 minutes, pH 6.0 (used in this research)

The soft cheese produced from CM + CR was extremely white and CM + EE cheese was also white. This is contrary to the report of Shahein *et al.*, (2014) who noted no difference in appearance of CM+BM (buffalo milk) and their individual respective milks soft cheese. The difference in the cheese texture could be attributed to the initial composition of the milk, e.g., high acidity, proteins and total solids contents generally make cheese harder and less easily deformed (Kehagis *et al.*, 1995). The reason why cheese produced from CWM and combinations was more formed and a bit harder than the cheese produced from CM was because the protein and total solid contents were more in the CWM than in the CM (as indicated in physiochemical properties of the two milks). The tastes of the cheeses were typically slighter to moderately sour for all except the product produced from CM + CR, which was having a kind of bitterness prior to swallowed and remained some times after swallowing. However, Ramet (1994) highlighted that temporary bitterness has been noted in some soft and semi-hard cheeses. The defect has been detected mainly after the cheese has been swallowed, adding that bitterness in dairy products might be caused by factors such as alkalis of ingested plants, salts of external origin (mainly calcium and magnesium chlorides) and carbonates or bitter peptides (generated by casein hydrolysis) and presence of proteolytic residues, which accumulate when the pH of the cheese is low and a high residual proteolytic activity from clotting enzymes remains in the curd.

The soft cheese produced from CM + CR is extremely white; CM + EE cheese is also white, this is contrary to the report of Shahein *et al.*, 2014 who noted no difference in appearance of CM+BM (Buffalo milk) and their individual respective milks soft cheese. The sensory evaluation by 60 panelists has revealed that individual CM cheese was the least choice where by CM + EE occupied 5th position and CM + CR 6th position by kruskal Wallis mean ranking. While Siddiq *et al.*, (2016) reported that mixing CWM with CM in the ratios 1:1 to produced Jibnabeida cheese did not decrease the acceptability of the final product.

CONCLUSION

The strain *Aspergillus tamarii* isolated in this study is versatile producer of extra-cellular protease that produced EAs at pH 5.0 (0.309±0.21 U/mL), 6.0 (0.351±0.096U/mL) and 8.0 (0.146±0.020). Hence, it could be exploited as bioactive agent and could further be checkmate for further industrial usage. The study revealed that protease of *Aspergillus tamarii* had produced cheese which is the best of panelist choice from cow milk and ranked 1st by Kruskal Wallis test. Findings in this study indicated that in terms of cheese production CM yield a better product when combined with CWM at 1:1 ratio. There is need for soft cheese producers to use coagulant that promote taste and texture as they are the attribute that indicated significant difference in the sensorial evaluation of the cheeses produced.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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