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GROWTH KINETICS OF *STREPTOCOCCUS SALIVARIOUS* SUBSP*. THERMOPHILUS* AND *LACTOBACILLUS DELBRUECKII* SUBSP*. BULGARICUS* STARTER CULTURES DURING FERMENTATION OF ACHA (*DIGITARIA EXILIS*, STAPF) BASED MILK

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ABSTRACT
This study determined the growth kinetic (maximal specific growth rate (h-1), generation time (h), and viability (CFU/ml) of yogurt starter cultures; *Streptococcus salivarious* subsp*. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in acha (*Digitaria exilis,* Stapf) based milk was carried out. Acha-based milk was extracted, batch pasteurized in an Erlenmeyer flask, and inoculation with 3% yogurt cultures (Direct Vat type (DVS) Yo mix consisting of *Streptococcus salivrarious* subsp. *thermophilu*s and *Lactobacillus delbrueckii* subsp. *bulgaricus i*n ratio 1:1 The result showed that *Streptococcus thermophilus* counts increased from 4.00 x 10^6 to 2.40 x 10^8 (CFU /ml) at an extended time of 6 h, while *Lactobacillus bulgaricus* counts increased from 5.00 x 10^6 (CFU /ml) to a maximum count of 1.42 x 10^8 (CFU /ml) at 5 h during acha milk fermentation. Comparatively, *Streptococcus thermophilus count* increased from 6.80 x 10^7 to 1.70 x 10^8 (CFU /ml), while that of *Lactobacillus bulgaricus increased* from 1.20 x 10^7 to 9.60 x 10^7 (CFU /ml) after 6 h of fermentation in the dairy milk. The maximum specific growth rate of 0.406 h1 and doubling time (td) of 102 min was registered for *Lact obacillus bulgaricus,* while *Streptococcus thermophilus* exhibited higher growth with a specific growth rate of 0.416 h-1 at a doubling time of 100 min. The acidifying activity of the lactic acid bacteria showed a lower rate of 0.619 h-1 in the acha milk compared with 1.136 h-1 observed in the dairy milk. The curdling time for the acha milk occurred 1.5 hours after that of the control. These findings confirmed the possibility of producing yogurt from 'acha' based milk as an alternative to conventional cow milk-based yogurt.

Keywords: Growth, kinetics, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* 'Acha' and Yogurt.

INTRODUCTION
Plant-based milk alternatives constitute a food system that is produced to surrogate conventional milk. The animal milk analogue is the fastest-growing among the functional and specialty beverages (Sethi ***et al.,*** 2016; Shaikh, 2016). By 2032, the projection is that the demand for the plant-based milk market will witness over 13.5 % CAGR and worth USD 10 billion inputs (Global Market Insights, 2022). Due to this, the Plant-Based Food Association (PBFA), launches new drives towards plant-based 'dairy' products in "support for consumers' health (PBFA 2022). In addition, the COP26 UN Global Climate Summit, for a more sustainable, equitable, and resilient food system has currently advocated for a shift toward the plant-based food system (PBFA 2024).

Acha (***Digitaria*** spp.) is a traditionally treasured staple cereal cultivated on the Plateau, Nigeria, It is a pleasurable consumed cereal and gained wide acceptance due to its high nutritional content and energy value. The grain is a rich source of essential amino acids with a higher load of sulfur-containing methionine and cysteine that could complement diets that lack animal or protein-based foods (Wonang *et al.,* (2017). Although plant-based milk lacks nutritional balance compared with bovine milk, however, they contain functionally active components that promote health (Sethi *et al***.,*** 2016).

So far, efforts towards the development of plant-based yogurt have centered mostly on assessing the composition, safety, and consumers' acceptability (Favaro-Trindade *et al***.,*** 2001; Isanga and Zhang, 2007; Zipori *et al*., 2024). In most reports, the plant-based dairy analogues are challenged with not meeting sensory properties in terms of flavours and texture properties (McClements *et al*., 2019; Pua *et al*., 2022). Advanced research revealed that plant-based products can sustain viable lactic acid bacteria incorporated as sensory biomodulators and probiotic carriers (Peyer et al., 2016; Rasika *et al*., 2021; Shori and Zahrani, 2021). Maintaining the appropriate number and mutual proportions of the conventional *S. thermophilus and L. bulgaricus* is however crucial in yogurt processing (Undugoda and Nilmini, 2019). This is because their optimal number and proportion are required to create the desired yogurt characteristics (Tamime and Robinson, 1999). It is plausible, therefore, that the adaptation of starter cultures to rapid acidification, for example, could curdle plant proteins and mitigate the problem of unpleasant flavor associated with most milk analogs (Zipori et al., 2024). Notably, the traditional yogurt starter cultures are also not archetypally adapted for the production of plant-based yogurt. To understand this gap, a concerted effort is necessary to enhance the conventional yogurt starter cultures to plant milk fermentation. It is, therefore, necessary to determine the growth kinetics of the conventional yogurt bacteria in the 'acha milk' to optimize their potential in acha-based "yogurt" production.

MATERIALS AND METHODS

Sample Collection

Ten kilograms of dehulled 'Acha' (*Digitaria exilis*, Stapf) were purchased locally in Jos, Nigeria, and transported to the Bacteriology and Chemistry Laboratory of the National Veterinary Research Institute (NVRI) Vom for further analysis.

Starter Culture Collection

Direct Vat Set type (DVS) Yo-mix, containing strains of *Lactobacillus bulgaricus* and *S. thermophilus*, was purchased from a grocery shop in Jos, Nigeria.

Sample Preparation

The dehulled Acha was cleaned and destoned by manual washing with clean tap water. It was then drained, spread on a 70% alcohol-disinfected tray, and oven-dried at 55°C for 12 hours. The dried Acha was then milled using a laboratory disc miller and manually sieved with a domestic fine sieve. The resultant flour was stored at 4±2°C in a sealed plastic container until used.

Processing and Fermentation of Acha Milk

Acha-based milk was extracted by homogenizing a ratio of 1:8 (g/ml) flour to sterile distilled water in a 500 ml container using a blender at maximum speed for 5 min (Poulter and Caygil, 2006). The acha flour water extract was filtered through a double-layer cheesecloth. The resultant filtrate was then pasteurized by holding the solution in an Erlenmeyer flask at 70°C for 3 minutes using a water bath. After pasteurization, the solution was rapidly cooled using an ice bath and re-homogenized for 5 minutes with soya oil at 30 ml per liter. Dairy milk was used as control milk and prepared by reconstituting dairy powdered milk to a 10% total solid content to match the consistency of acha-based milk for comparison. Triplicate batches of 500 ml each of pasteurized acha-based milk in an Erlenmeyer flask were raised to an inoculation temperature of 45°C using a water bath. Both the Acha-based milk and the dairy milk were then inoculated with 3% (9g) Direct Vat Set type (DVS) Yo-mix (strains of *Lactobacillus bulgaricus* and *S. thermophilus* in a 1:1 ratio) commercial yogurt cultures. The inoculated milk samples were incubated at 43°C for 6 hours, and fermentation was arrested by holding at 4 ± 2°C until evaluated.

Physicochemical Analysis

pH of Acha Yoghurt

The pH of the milk during fermentation was determined at hourly intervals according to the method of the International Dairy Federation (I.D.F) (1991).

Titratable Acidity

The titratable acidity of the milk was measured according to the method of the International Dairy Federation (I.D.F) (1991).
Titratable acidity = V X 0.9/m.
International Dairy Federation. (IDF, 2002) Fermented milk: Proceedings of the IDF seminar on aroma and texture of fermented milk, held in Kolding, Denmark. 301: 280-315.
Where V = volume (ml) of 0.1M sodium hydroxide solution required to titrate a sample of milk during fermentation to a pH of 8.3, m = mass (g) of the tested milk and 0.9 is the conversion factor for lactic acid.

Maximal Specific Growth Rate

The maximal specific growth rate (μmax) for lactic acid bacteria cultures was calculated using the equation adopted by Soro-Yao *et al*., (2014).

$$\mu\ max = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

Where X_2 and X_1 are the cell number (cfu/g) for corresponding time (h) t_2 and t_1 of exponential phase respectively.

Doubling Time

The doubling time (td) was calculated using the formula:
td = ln 2 / μmax,
where μmax is the maximal specific growth rate (μmh-1).

Maximal Acidification Rate

The maximum pH obtained for the fermenting milk at the exponential phase was used to calculate the maximal acidification rate.

$$pH\ max/t = \frac{pH_1 - pH_2}{t_2 - t_1}$$

where pH_1 and pH_2 are maximum pH values at exponential phase at time t_1 and t_2 respectively.

Enumeration of Yoghurt Starter Cultures

Enumeration of the yogurt cultures was carried out hourly as described by Mohammed et al. (2007) and APHA (1992). De Mann Rogosa Sharpe (MRS) medium (Merck, Darmstadt, Germany) was used for the enumeration of L. bulgaricus. Modified M-17 agar (Merck) selective for *S. thermophilus* was formulated as recommended by APHA (1992).

RESULTS AND DISCUSSION

The co-culture fermentation of *S. thermophilus* and *L. bulgaricus* in acha-based milk resulted in a gradual decrease in pH from 6.49 to 5.25, accompanied by a progressive increase in titratable acidity from 0.365 to 1.188 ± 0.015 (% lactic acid) over a 6-hour fermentation period. The pH decrease with a corresponding increase in titratable acidity are consistent with the acidification process commonly associated with *L. bulgaricus* and *S. thermophilus* co-culture fermentation of milk sugars (Hussain et al., 2009). Comparatively, the pH observed during acha-based milk fermentation is above the pH range of 4.6–4.9 recommended for plant-based yogurt (Dhakal *et al.*, 2023). The higher pH value obtained in the acha yogurt may impact the desired yogurt characteristics negatively (Favaro-Trindade *et al*., 2001). Additionally, the titratable acidity observed in the acha 'yogurt' is slightly higher than the 0.24 % lactic acid reported in soy yogurt (Favaro-Trindade *et al*., 2001) but falls within the range reported for whole and skimmed dairy yogurt (Salvador and Fiszman, 2004). The acidifying activity of *S. thermophilus* and *L. bulgaricus* co-cultured in acha and dairy milk is presented in Table 1. A lower acidification rate of 0.206 h^{-1} during acha milk fermentation was observed compared to 0.619 h^{-1} in control milk. The level of acidity is significantly ($P < 0.05$) lower in acha milk by 66.72 % than the proportion observed in the cow-based yogurt, suggesting that the

yogurt cultures exhibited acidification activity in acha milk at a relatively lower speed compared to cow milk. Moreover, the acidification capacity of the yogurt starter showed *S. thermophilus* exhibited higher acidification capacity compared to *Lb. delbrueckii subsp. bulgaricus*, in line with reports of Kayacan *et al*. (2022).
The growth dynamics of *S. thermophilus* and *L. bulgaricus* during batch fermentation of acha milk are presented in Figure 1. This trajectory revealed that *S. thermophilus* counts increased from 4.00 x 10^6 (cfu/ml) to a maximum growth of 2.40 x 10^8 (CFU/ml) over an extended time of 6 hours. However, maximum counts of *L. bulgaricus* (1.42 x 10^8 CFU/ml) were attained at a reduced fermentation time of 5 hours. The findings of this work imply that the growth of lactic acid bacteria in acha milk competes favorably with that of dairy yogurt. However, the growth profile of the starter cultures in the acha milk was characterized by a longer lag time and lower viable count, meeting the standard requirements to sustain 10^6 yogurt bacteria per mL (Mohammed *et al*., 2007; Rathore *et al*., 2012) and 7 log CFU /ml probiotic to benefit its health effects (Ouwehand and Salminen, 1998). The population of the two bacteria strains in acha-based milk varied (P<0.05), with *S. thermophilus* having a higher cell density than *L. bulgaricus* at the end of the fermentation. This variation in growth is consistent with the behavior observed between *S. thermophilus* and *L. bulgaricus* co-cultured in dairy milk fermentation (Dorato et al., 2004; Mahdian and Tehrani, 2007) and has been attributed to proto-cooperation where metabolites produced stimulate growth and response to the composition of the milk (Hickey *et al.,* 1986).

The growth of starter bacteria in the dairy milk is shown to markedly increased with a maximal specific growth rate of 0.291 h^{-1} and doubling time (td) of 47.46 minutes for *L. bulgaricus*. Comparatively, *S. thermophilus* exhibited a higher doubling time (54.00 minutes) with a specific growth rate of 0.017 h^{-1}. However, in the acha milk, the *L. bulgaricus* population was significantly higher with a kinetic growth rate of 0.406 h^{-1} and a doubling time of 60.42 minutes. This growth rate is similar to that reported for *S. thermophilus* and *Lb. delbrueckii subsp. bulgaricus* (Kayacan *et al*., 2022). The shorter doubling time exhibited by *S. thermophilus* in the milk, is consistent with earlier report that the biomass increase of *S. thermophilus* should be higher in a mixed culture than *L. bulgaricus* (Raizi and Ziar, 2008). It can be inferred from the reduced specific growth rate and higher doubling time observed by the bacteria in acha milk could be responsible for the longer set-time. The resultant effect in the growth reduction of yogurt starter cultures during fermentation is the typically poor yogurt characteristics. This is always associated with poor nutrient availability and dispersion in products (Barragán *et al.,* 2020). In addition, this reduced growth might impair acidity and aroma production that are typical with yoghurt characteristics (Eltham and Mustafa, 2007). Since both *S. thermophilus* and *L. bulgaricus* are responsible for the production of substantial number of acids and aroma compounds respectively (Tamime and Robinson, 1999; Eltham and Mustafa, 2007).

Conclusion

The yogurt starter cultures exhibited similar growth pattern in acha-based milk to the conventional milk. Comparatively, the growth kinetic of *S. thermophilus* and *L. bulgaricus* occurred at slower rate in acha based milk. This finding suggests the plausibility of using the acha-based milk as a good substrate for production of yogurt competing with the conventional. An optimization of fermentable sugars, inoculums size adjustment and protein enrichment interplay could serve as key areas to enhance the growth kinetics of starter bacteria in the development of acha-based yogurt.

Table 1. Total Titratable Acidity pH and Acidifying Rate of Acha and Dairy Milks during Co-culture fermentation with *S. thermophilus* and *L. bulgaricus*

Fermentation Time (hr.)	pH	Acidifying rate (h^{-1})	pH	Acidifying rate (h^{-1})	Titratable Acidity (% lactic acid)	
	Acha milk		Dairy milk		Acha Milk	Dairy milk
0	6.49±0.12	0.206 ±0.011^a	6.60±0.05	0.619± 0.009^b	0.365±0.004	0.225±0.010
1	6.30±0.28		6.67±0.43		0.423±0.012	0.162±0.029
2	6.40±0.26		5.70±0.03		0.720±0.007	0.360±0.045
3	6.07±0.09		5.30±0.12		0.990±0.010	0.270±0.004
4	5.85±0.29		4.99±0.09		0.990±0.006	0.459±0.002
5	5.60±1.04		4.90±0.10		1.269±0.003	0.396±0.004
6	5.25±0.47		4.86±0.05		1.188±0.015	0.657±0.011

Values are means ± SD and superscripts with different letters significantly varied (p < 0.05)

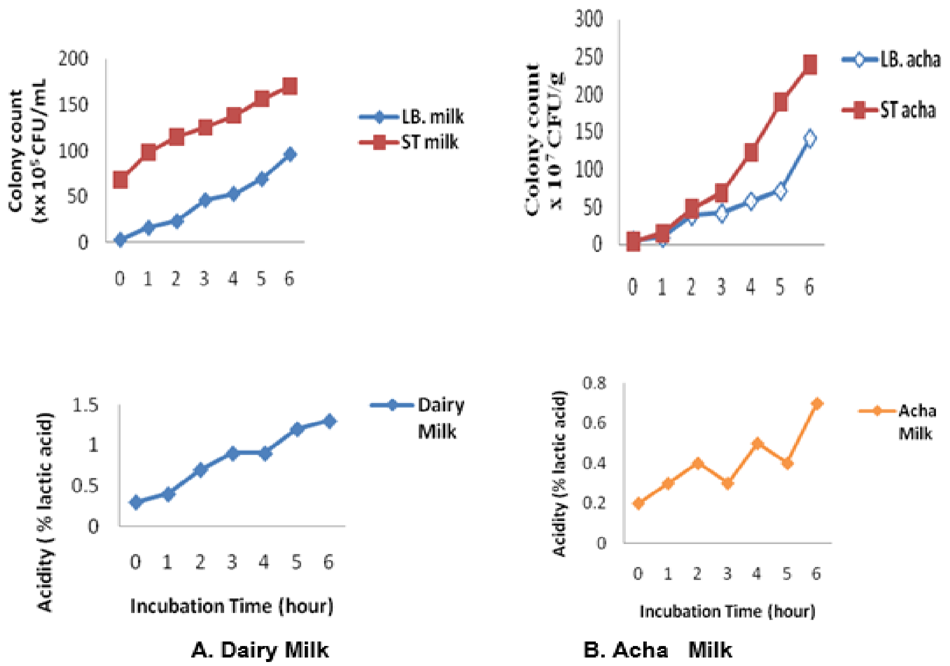

Figure 1. Growth and Acidification profile of *S. thermophilus* (ST) and *L. bulgaricus* (LB) during Dairy Milk (A) and Acha Milk (B) Fermentation

Table 2. Maximal Specific Growth Rate and Doubling Time (td) of *S. thermophilus* and *L. bulgaricus* during Acha Fermentation

	Specific growth rate (h^{-1})		Doubling time (min)	
Lactic acid bacteria	Dairy milk	Acha milk	Dairy milk	Acha milk
Streptococcus thermophilus	0.107±0.004	0.415±0.008	6.429±0.001	1.668±0.004
L. bulgaricus	0.291±0.002	0.406±0.003	0.791±0.002	1.707±0.005

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