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Studies on Growth Variables, Metabolites and Safety Effect of Lactobacillus Acidophilus, Streptococcus Salivarious Subsp. Thermophilus and Lactobacillus Delbrueckii Subsp. Bulgaricus (As Starter Culture) on Cocos Typical Based Extract During Time-Monitoring Bioprocessing (Fermentation)

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ABSTRACT

The role of Lactic Acid Bacteria (LAB) is vital during bio-processing (fermentation) which could result to desirable nutritional, sensory and safety quality product. This study investigated on growth kinetics variables, metabolites and safety effect of Lactobacillus acidophilus, Streptococcus salivarious subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (as starter culture) on Cocos typical based extract during time-monitoring bio-processing. This was done anaerobically in the bioreactor with optimized timing of 0-72 h at 37 °C, but samples were drawn every 12 h intervals. Specific LAB growth rate was calculated using Monod equation while specific consumption rate of substrates and generation rate of metabolite in the fermenting samples were modeled using Luedeking-Piret equation. The total LAB count (6.36 to 10.54 log10 cfu/ml) and lactic acid concentration (0.07 to 1.74%) increased but pH (6.48 to 4.03), total sugar (20.96 to 10.88%), total soluble sugar (3.01 to 0.17%), total solid (5.98 to 2.81%) and fructo-oligosaccharide (100.05 to 34.31 mg/kg) decreased with increasing fermentation period. At 12 h, higher specific growth rate with the lowest doubling time of 0.22 h^{-1} and 3.15 h respectively. Both specific fructose and sucrose rate consumed by the lactic acid bacteria were higher at 72 h. Similarly, at 72 h, more lactic acid was produced and the least concentration was observed at 36 h of fermetation period. Zone of inhibition as antibacterial effect of each samples against Escherichia coli, Salmonella typhi and Staphylococcus aureus ranged from 10.11-19.33 mm, 9.13-19.83 mm and 9.89-21.17 mm respectively. It was deduced accordingly that the Cocos typical extracts (at 24 h and 36 h) served as good carbon sources for Lactobacillus acidophilus, Streptococcus salivarious subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus) and the production of useful metabolites that could guarantee the prevention of pathogens was enhanced.

Keywords: Cocos typical extract; bio-processing (fermentation); growth kinetics; antibacterial activity, Lactic Acid Bacteria (LAB)

INTRODUCTION

Plant milk are becoming more demanding because they aid consumers' health and they are used to replace animal-based milk (with exorbitant cost in Nigeria) (Amapu *et al*., 2024; Alma and Judith, 2024; Adebo and Sobowale, 2025). As a result, focus has been on milk extract from plants such as fruits, nuts and legumes (Akusu and Emelike, 2018; Fatemeh, 2014; Okoroafor *et al.*, 2025). Within the last few decade, more researchers have interest to study the growth kinetics variables of Lactic Acid Bacteria (LAB) in fermented plant-sourced foods (Kpikpi *et al*., 2009). Most of them focused on the enhancement of flavor, texture, composition and safety qualities that *Cocos typical* extract that LAB could impart (Zipori *et al.*, 2024; Pua *et al*., 2022). However, Lactic

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Acid Bacteria ingested (via this food) must be in right proportion, before they can render health benefits beyond inherent general nutrition (Coşansu *et al.*, 2021; Domínguez-Murillo and Urías-Silvas, 2024).

Optimum desirable quality of LAB fermented foods (in terms of nutritional, sensory and safety values are exhibited at specific fermentation time and temperature (Undugoda and Nilmini, 2019). Their activity and growth vary under various proportions, temperature, fermentation time, pH and water activity. According to Shetty *et al*., (2017). these parameters could be determined or illustrated mathematically. This study focused more on the optimization of fermentation time and specific temperature for LAB-processed *Cocos typical* and how these affect other advantageous parameters. This could limit cost of industrial plant mass and ensure correct conditions as well as consistent outputs (Barao *et al*., 2018). This further can predict the amount of desirable metabolites produced at specific condition or the quantity of consumed carbon sources during fermentation (Zhang *et*. *al*., 2018). The production of lactic acid is time and temperature dependent. The microorganisms involved are known to have optimum growth at 37 ^{o}C, according to Olusola (2014).This work centered on several fermentation period, in order to establish the optimum time the vital metabolites or improved properties could widely be generated in the food.

Growth of LAB is stimulated by prebiotics, which are indigestible food ingredients that stimulate the growth and maintain LAB microbiota. Prebiotics in *Cocos typical* extract are in form of fruto-oligosaccharide. Fructo-oligosaccharide in *Cocos typica*l extract are thereby significant part of the food nutrient that selectively stimulate the growth and activity of LAB (Panitantum, 2004). Meanwhile, above normal body required level, non-digestible fruto-oligosaccharide in *Cocos typica*l causes Small Intestinal Bacterial Overgrowth (SIBO) (Leena, 2007). This challenge disrupts digestion, causes intense physical discomfort and damages the small intestine (Leena, 2007). The effect of bioprocessing on non-digestible fruto-oligosaccharides need to be studied and linked with growth rate of LAB, with the fact to enhance safety and quality of *Cocos typical* extract (Leena, 2007).

LITERATURE REVIEW

Existence of traditional bioprocessed (fermented) dairy and plant milk analogues are significant in Nigeria (Yuliana *et al*., 2010). Such food products could be inform of yogurt, cheese and others (Ladokun and Oni, 2014). Probiotic LAB are known to exert medical and nutritional quality, which could be delivered to humans via various plant extracts and related products (Han *et al*., 2021). Certain minimum number of probiotics needed daily in the guts, for an average individual, is 10^{6}–10^{7} CFU/g (Han *et al.*, 2022). According to Leena, (2007), twelve gramme of LAB per day or less is usually well tolerated. These beneficial LAB that persist in the gut ecosystem could decrease intestinal pH and suppress proteolytic bacteria. They likewise slow aging process in consumers (Vernazza *et al.*, 2006; Gordon, 2008).

More so, proportions of LAB in foods are time and temperature dependent (Amapu *et al*., 2024). Prebiotics act as lubricant stimulant to bowels and have effect on LAB growth, gastrointestinal flora, stool characteristics and mineral (like calcium, magnessium and iron) absorption in adolescents and postmenopausal women. They act as biomarkers of immune function and increase feacal flora. They ensure exhibition of inhibitory effect on precancerous colon and reduce fasting glucose and apolipoprotein B levels in type-2 diabetic patients (Sharon *et al.*, 2009).

Aside from the nutritional and health benefits, Anyogu *et al*. (2021) and Ajibola *et al*. (2023) emphasized on the safety value of fermentation. Most life-threatening diseases are consequences of unsafe food consumed. Enhancing the quality and safety of food consumed is a major concern in the world today. As highlighted by Coşansu *et al*. (2021), LAB could cure issues like food allergies, bowel disorder or gastrointestinal infections, lactose intolerance, genitourinary infections, food allergies, in humans, most especially among the children. Moreover, one of the important metabolites that contribute to antibacterial properties of foods, during LAB fermentation is lactic acid (Barão *et al*., 2019). This could prevent cancer of the large intestine (Mrudula *et al*., 2024). Pathogens that cause bacteria infections could be rendered inactive in the presence of this metabolite, during production and storage (Asli, 2011). Nevertheless, small intestinal LAB overgrowth resulting from over produced number of LAB leads to the production of toxins, enzymes, and intestinal gases (H_2, CH_4 and CO_2), which interrupt with the health of consumers (Thammarutwasik, 2009).

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The current study was directed towards examining the growth, substrates and metabolites kinetics of *Lactobacillus acidophilus, Streptococcus salivarious* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* starter cultures during the time-monitoring bioprocessing (fermentation) of *Cocos typical* based extract. The effect of these LAB on non-digestible fructo-oligosaccharides was assessed and the antagonistic behavior of the extract was screened against common pathogens (*Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*).

MATERIALS AND METHODS

Microorganisms utiized

Matured 12 month old western tall *Cocos typical* was purchased from the farm from Sapo, Badagry, in Lagos State. The commercial starter cultures, *Lactobacillus acidophilus, Streptococcus salivarious* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* of Direct Vat type (DVS) Yo mix, were gotten from Kuto market, Ogun State, Nigeria.

Laboratory fermentation of Cocos typical extract

Immediately after the drilling out of water in broken-shelled, de-husked *Cocos typical*, homogenizing of 4 kg of comminute meat with 4500 ml of distilled water was done, according to modified method of Olusola (2014). Thermal treatment of the mixture was then employed at 65 ºC for 30 min and cooled at 4 ºC in ice bath. Viability of LAB from starter cultures (*Lactobacillus acidophilus, Streptococcus salivarious* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) from the Direct Vat type (DVS) Yo mix was verified by sub-culturing on De Mann Rogosa Sharoe (MRS) Agar at 37 ºC for 24 h of incubation. Identification was by the method of Ayodeji *et al*. (2017) at 1500 bp of 16S RNA with primer 27F (3'-GAGTTTGATCCTGGCTCAG-5') and 1492R (3'-TACCTTGTTACGACTT-5'). Probiotics test of the cultures was confirmed using Tambekar and Bhutada (2010). Thereafter, six flasks containing the extract were inoculated with a milliliter of 10^5 MacFarland of the mixture of 3% (v/v) of *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. b*ulgaricus* at 37 ºC for 72 h. Fermented samples from each flask were drawn and refrigerated at 12 h interval, one after the other (with label A, B, C, D, E, F and G). Measurement of pH were executed, using AOAC (2000).

Total lactic acid bacteria count

This was done using MRS agar with 0.01% sodium azide prepared according to the manufacturer's specification. This medium was sterilized in an autoclave at 121 ºC for 15 min. Each sample (1 ml) was dissolved in sterile de-ionized peptone water and serially diluted. An appropriate dilution of 1 ml was inoculated on nutrient and MRS agar plates and the plates were incubated anaerobically using anaerobic jars for 48 h at 37 °C. The total Lactic Acid Bacteria count was done for each sample in log10 cfu/ml (AOAC, 2000).

Total lactic acid concentration

This was determined through AOAC (2005) by titrating 1.0 ml of sample against 0.1N NaOH using phenolphthalein as the indicator. The appearance of a pink colour marked the end point of titration. The measurements were done in duplicate. The titratable acidity (expressed as percent lactic acid) was determined using:

1 ml 0.1N NaOH = 0.009008 ml = lactic acid (Acid Factor);

TA= [Normality of Base x Volume of Base x Acid Factor x100]/Volume of Sample.

Estimation of total solids, total soluble solids and total sugar

This is the amount of solids remaining after heating the sample at 105 °C to constant weight. Conversely, the moisture content is a measure of the amount of water (and other components volatilized at 105 °C) present in the sample. This was determined in gravimeter using the method described by Bradley Jr (2003). A measured weight of each test sample was put in a previously weighed dish and evaporated to dryness over a steam bath. It

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was then dried in an oven at 105 °C for an hour. It was cooled in desiccators and then reweighed by difference. The dry weight of the samples was obtained and expressed as a percentage of the sample weight.

The total soluble solids were determined using a digital hand held refractometer and the total soluble solid content was expressed as Brix at 25 °C. The amount of residue remaining from a 0.2 μm filtered liquor sample after heating the sample at 105 °C to constant weight was calculated, according to by Bradley Jr (2003).

Total soluble solids (%)= {(Weight-dry pan plus dry sample – Weight-dry pan)/ Weight sample as received} x 100

Moisture (%) = 100 – [{(Weight-dry pan plus dry sample – Weight-dry pan)/ Weight sample as received} x 100]

The total sugar contents indicator was determined as described by AOAC (1999), using the principle of ethanolic extraction of sugar from samples by the organic solvent - petroleum ether using absorbance method. One gram of each sample was weighed into 200 ml volumetric flask. Cotton wool was placed over the sample in the volumetric flask to prevent splashing. Fifteen milliliters of 85% ethanol was added to each flask. The solutions were voltexed and filtered through Whatman's No. 1 filter paper. Four separating funnels were set up. Chloroform solution of 10 ml was added to each filtrate. Further extraction was carried out in the separating funnels. Each filtrate formed a layer on the chloroform.

The chloroform layer was discarded and 10 ml of petroleum either was added to each filtrate to remove the lipid content present. The petroleum ether layer below was used to determine the total sugar contents. Five milliliters of petroleum ether was weighed out inside a boiling flask. Distilled water of 0.5 ml was added to obtain a diluted extract. Phenol (0.5 ml of 5% (w/v)) was added to each of the content in each flask, along with 2.5 ml of concentrated sulphuric acid (Conc. H_2S04), for colour development. Each of the flask content was then heated and allowed to cool after which the absorbance was read off at 490 nm wavelength using Spectronic 20D – Spectrophotometer.

Estimation of glucose, fructose and sucrose concentration

This was determined using the phenol-sulphuric acid method (Shetty *et al*., 2017). Each sample of 0.1 g was weighed and homogenized with 600 ml of distilled water, in a beaker. Filtrate of the mixture was transferred into a 1 L volumetric flask. Two millimeters of aliquot of carbohydrate solution was pipetted with the addition of 1 ml of 5% aqueous phenol solution and 5 ml of concentrated H_2S04, in a test tube. The solution was cool for 10 min, vortexed for 30 s and placed in a water-bath at room temperature for 20 min for color development. At absorbance of 490 nm, the solution was quantified using a UV visible spectrophotometer. It was blanked with 2 ml distilled water, 1 ml aqueous phenol (5%) and 5 ml conc. H_2S04. Then, extrapolation was done to get the concentration of the carbohydrate standard graph. A standard graph of absorbance against the known concentrations of the standards for all the carbohydrate was plotted. Finally, the concentration of the sugar was calculated using:

$$\text{Sucrose/glucose/fructose (mg/100g)} = \frac{\text{conc. (mg/l) x volume of sample x dilution factor}}{\text{Sample weight x 1000}}$$

Estimation of fructo-oligosaccharide

Each sample of 10 g was weighed into a beaker and 30 ml of boiled deionised water (hot) were added, stirred on hot plate at 80 degree Celsius for 10 min and allowed to cool. Carrez reagent 1 of 5 ml were added with 5 ml of carrez reagent 11, mixed and filtered into a 50 ml standard flask and made up to mark of 50 ml using distilled water. Absorbance at 480 nm was read using equal volume of carrez 1 and carrez 11 reagents as blank. Also standard fructose standard graph was prepared or from an existing fructose standard graph, extrapolated to obtain the concentration of fructo-oligosaccharide in each sample (Petkova *et al.*, 2011).

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Modelling study of growth kinetics of *Lactobacillus acidophilus, Streptococcus salivarious subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in Cocos typical extract biomass

The specific LAB growth rate was calculated using Monod equation (Monod, 1942) while specific consumption rate of glucose, fructose, sucrose and generation rate of lactic acids in samples were modeled using Luedeking-Piret equation. During the log phase when cell utilizes nutrients and grow to increase biomass, the growth behaves similar to autocatalytic reaction. At this phase, growth rate is proportional to cell mass (x) of that period. At this time, t, the rate of cell mass increase (dx/dt) is equal to the specific growth rate (μ) and the cell concentration.

$td = 0.693/ \mu$……(i)

$\mu = 2.303(\log_{10}X – \log_{10}X_0)/t – t_0$…… (ii)

Monod equation for Exponential growth (Shuler, 2003): Cells + Substrate → More cells + Products.

From Luedeking- Piret type kinetics, the specific consumption rate of glucose (v; g glucose/g cell per h) and the specific production rate of lactic acid (π; g lactic acid/g cell per h or percentage) can be calculated from the differentials of glucose concentration (ΔS; g glucose/l) and lactic acid concentration (ΔP; g lactic acid/l) at each time as the following equations:

$V = -(1/x)(\Delta S/\Delta t)$…….(iii)

$\Pi = (1/x)(\Delta P/\Delta t)$……...(vii)

***Lactobacillus acidophilus, Streptococcus salivarious subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* antimicrobial Assay**

Salmonella typhi, Escherichia coli 0157:H7 and *Staphylococcus aureus* were isolated from spoilt fruits in the microbiology laboratory of the Department of Food Science and Technology, Federal University of Agriculture Abeokuta, Ogun State Nigeria. These pathogens were isolated from spoilt *Cocos typical* fruits streaked on *Salmonella-Shigella* Agar, MacConkey Agar and Mannitol Salt. Pure culture of the isolate were subjected to series of biochemical tests and identified genotypically with 16S RNA primer. The sequence and condition of the PCR were described in Appendix 1.

Then, 24 h incubated culture of *Salmonella typhi, Escherichia coli 0157:H7,* and *Staphylococcus aureus* each was prepared and inoculated in 10 ml of sterile water, homogenized and standardized by 10^5 MacFarland-standardized. These were spread on Muellen Hinton Agar plates. Wells of 7 mm in diameter were cut into these agar plates using sterile cork borer and 50 μl of the bioprocessed Cocos typical extract were placed into each well. One well was filled with fermented *Cocos typical* extract which serve as negative control. The culture plates were incubated at 37°C for 24 h and the zones of inhibition was measured in diameter (mm). Meanwhile, paper discs were impregnated with streptomycin and imipenem antibiotics were used as control according to Olateru *et al*. (2020). Antimicrobial tests were done in triplicate.

Other Statistical Analysis

Triplicate values of data were subjected to one-way analysis of variance (ANOVA SPSS 21.0 version) and means were separated using Duncan multiple range test at 95% confidence level.

RESULTS AND DISCUSSION

Results of the total Lactic Acid Bacteria count, pH, lactic acid concentration produced, as well as total solid, soluble solid and sugar of the fermented *Cocos typical* extract were presented in Table 1. There was significant difference ($p<0.05$) in all the parameters evaluated. A log phase was observed in total LAB count and the lactic acid produced ranged from 6.36 to 10.54 log10 cfu/ml and 0.07% to 1.74% respectively. The pH, and total solid,

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soluble solid with sugar decreased from 6.48 to 4.03, 20.96 to 10.88%, 5.98 to 2.81%, 3.01 to 0.17% respectively as fermentation time increased.

Metabolic activities of *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during the processing accounted for increase in total LAB count and lactic acid produced. This also is responsible for the decrease observed in the pH, total solid, total soluble solid and total sugar of the samples.

This is in support to Pato *et al*. (2021), who reported *L. acidophilus* to have imparted better taste and aroma in *Cocos spp* extract from Indonesia, due to higher pH and lactic acid values (Yuliana *et al.*, 2010). The pH values of samples were observed to be in right amount that consumers' gastrointestinal tracts could tolerate. In this regard, results from this study fall at best favourable pH even at 72 h of fermentation, so they could deliver desired beneficial probiotic LAB (Guler-Akin and Akın, 2007; Obi and Ikenebomeh, 2007).

During fermentation, there was gradual release of soluble mineral element leached out through the soft texture of samples and dissolution of the bound sugars into the fermented milky extract. This, however, were utilized by the LAB. Trend in this outcome is seen with the study of Shetty *et al*., (2017). So, reduction or depletion of sugars occurs, as viable LAB count increase with fermentation time.

From Table 2, monosaccharides found in the fermented *Cocos typical* extract with LAB were glucose and fructose while the dissaccharide was sucrose. It was observed that as fermentation period and LAB growth increased, there was reduced trend in the glucose, fructose and sucrose concentration from 1.69 to 0.79 g/100g, 2.17 to 1.02 g/100g and 1.08 to 0.51 g/100g respectively.

The amount of fructo-oligosaccharide (prebiotics) progressively reduced as fermentation period increased. It falls rapidly from 100.05 to 34.31 mg/kg between 0-72 h of fermentation. The results showed no significant difference ($p<0.05$) existed in the samples' fructo-oligosaccharide. As growth of the fermenting organisms upto 72 h, there was tremendous reduction in fructo-oligosaccharide content. This was also presented in Table 2.

Reduction of glucose, fructose and sucrose occurred due to hydrolysed breaking down of complex carbohydrates into simpler form by the metabolic action of LAB. Ngoc *et al.*, 2013) described the action of enzyme invertase on the sugar during bioprocessing.

Combination of three LAB could also be factor that speedy the rate of decomposition process of complex sugars giving higher rate of reduction of glucose, fructose and sucrose, as LAB population increase with time. This result corroborate with the earlier report of Tuitemwong and Tuitemwong (2003) and Adelodun and Abiodun (2012). The relationship of fruto-oligosacharide (prebiotics) amount per fermentation time and Total LAB count showed inversely proportion

Table 1: Growth Attributes of *Lactobacillus acidophilus, Streptococcus salivarious* subsp. thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus in the Fermentation of Cocos typical Milk.

Sample	Fermentation Period (h)	Total LAB Count (log_{10} cfu/ml)	pH	Lactic Acid Concentration (%)	Total Solid (%)	Total Soluble Solid (%)	Total Sugar (%)
A	0	6.36 ± 0.52^{b}	6.48 ± 0.02^{c}	0.07 ± 0.02^{a}	20.96 ± 0.35^{f}	5.98 ± 0.03^{f}	3.01 ± 0.26^{f}
B	12	7.49 ± 0.32^{bc}	4.56 ± 0.23^{b}	0.31 ± 0.06^{b}	19.09 ± 0.11^{e}	6.33 ± 0.03^{g}	2.45 ± 0.05^{e}
C	24	7.79 ± 0.27^{d}	4.31 ± 0.05^{ab}	0.59 ± 0.05^{c}	16.20 ± 0.47^{d}	3.82 ± 0.04^{e}	2.01 ± 0.01^{d}
D	36	8.08 ± 0.15^{a}	4.24 ± 0.05^{a}	0.62 ± 0.00^{c}	15.62 ± 0.02^{d}	3.39 ± 0.00^{d}	1.06 ± 0.00^{c}
E	48	8.87 ± 0.30^{e}	4.17 ± 0.03^{a}	0.74 ± 0.00^{d}	14.21 ± 0.02^{c}	3.11 ± 0.01^{c}	0.52 ± 0.04^{b}
F	60	9.90 ± 0.11^{e}	4.09 ± 0.02^{a}	0.89 ± 0.00^{e}	12.37 ± 0.08^{b}	2.66 ± 0.03^{a}	0.37 ± 0.01^{ab}
G	72	10.54 ± 0.59^{c}	4.03 ± 0.01^{a}	1.74 ± 0.01^{f}	10.88 ± 0.59^{a}	2.81 ± 0.02^{b}	0.17 ± 0.01^{a}

Mean of samples with different superscript letter are significantly different ($p<0.05$); Mean of triplicate values±standard error

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Table 2: Relationship between Lactobacillus acidophilus, Streptococcus salivarious Subsp. thermophilus and Lactobacillus delbrueckii Subsp. Bulgaricus with reducing sugar and fructo-oligosaccharide of the Cocos typical milky extract during time-monitoring fermentation

Sample	Fermentation Period (h)	Total LAB Count (log10cfu/ml)	Glucose (g/100g)	Fructose (g/100g)	Sucrose (g/100g)	Fructo-oligosaccharide, FOS (mg/kg)
A	0	6.36 ± 0.52^{b}	1.69 ± 0.34^{a}	2.17 ± 0.08^{a}	1.08 ± 0.22^{ab}	100.05 ± 0.02^{f}
B	12	7.49 ± 0.32^{bc}	1.52 ± 0.11^{b}	1.97 ± 0.23^{b}	0.98 ± 0.15^{cd}	85.43 ± 0.12^{e}
C	24	7.79 ± 0.27^{d}	1.46 ± 0.14^{b}	1.88 ± 0.51^{bc}	0.93 ± 0.30^{c}	60.3 ± 0.06^{d}
D	36	8.08 ± 0.15^{a}	1.27 ± 0.07^{c}	1.71 ± 0.01^{c}	0.85 ± 0.16^{d}	60.05 ± 0.05^{d}
E	48	8.87 ± 0.30^{e}	1.28 ± 0.10^{d}	1.63 ± 0.26^{d}	0.83 ± 0.55^{d}	59.26 ± 0.03^{c}
F	60	9.90 ± 0.11^{e}	0.81 ± 0.12^{e}	1.40 ± 0.44^{e}	0.66 ± 0.13^{e}	34.61 ± 0.20^{b}
G	72	10.54 ± 0.59^{c}	0.79 ± 0.04^{f}	1.02 ± 0.02^{f}	0.51 ± 0.49^{f}	34.31 ± 0.02^{a}

Mean of samples with different superscript letter are significantly different ($p<0.05$); Mean of triplicate values±standard error to one another. The beneficial LAB whose growth is affected by the prebiotics in bio-processed product, which must be present in adequate amount at the time of consumption in order to render it being effective as (Siró, 2011). Therefore, the fermented *Cocos typical* extracts can efficiently deliver probiotic bacteria due to the proportion of fruto-oligosacharide in them and prevent the risk of Small Intestinal Bacterial Overgrowth (SIBO). Reduction rate of fruto-oligosaccharide as fermentation time increased with higher LAB count, rendered bioprocessed *Cocos typical* extract to be safe for individual, including the infants.

Growth rate with residual doubling time, glucose, fructose and sucrose at a specific fermentation period (from Monod parameters) for *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* was given in Table 3. The fermentation period of 12 h had higher specific growth rate with lowest doubling time of 0.22 h^{-1} and 3.15 h respectively. Fermentation period at 24 h and 36 h gave the least values of 12.00 and 12.59 respectively, and more doubling time of 0.06 h^{-1}. From this study, evidences showed that specific growth rate increased but reduction in doubling time of LAB was observed. This was in line with the reports of Phuc (2011) and Dubey (2012). The lowest specific glucose rate consumed by the lactic acid bacteria was at 48 h of bio-processing with -0.4 gh^{-1}, meanwhile, more consumption was observed at 60 h of fermentation period with 218.28 gh^{-1}. This reflection of the utilization of the extracts' nutrients by the LAB with accumulation of lactic acid concentration was observed in the reports of Van-Neil *et al.,* (2002) and Otles and Cagindi (2003). The speedy up by environmental factors such as pH, medium composition, aeration and so on may also contribute to this (Fadela *et al.,* 2009). Both specific fructose and sucrose rate consumed by LAB were at 24 h of fermentation was at least value but highest outcome was seen at 72 h. Similarly, at 72 h, more lactic acid was produced and few amount was observed at 36 h.

The growth kinetics reflect that *Cocos typical* extract support the growth of *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus.* This is based on material balance, limit substrates (specific glucose, fructose and sucrose consumed) and metabolite (specific lactic acid produced), distributed between cells, at different fermentation time. This vitality and number of probiotic bacteria are critical factors for their beneficial functions in the host, according to (Coşansu *et al*., 2021). Temperature and fermentation time played vital role in the proliferation of the LAB, and impacted direct desirable characteristics of the fermented Cocos typical milky extract. This is in agreement with the research of Barão *et al*. (2019). After 36 h, it was observed that the tread of growth rates, doubling time, specific glucose, fructose and sucrose rate, and lactic acid rate produced, begin to change, unfavourably. This revealed these samples could render the best quality composition to consumers, at 36 h of bio-processing. This is in support with the outcome of study of Yuliana *et al*. (2010).

Zone of inhibition quantified was shown in Table 4. Ranged results were seen in *L. bulgaricus* (10.11-19.33 mm), *S. thermophilus* (9.13-19.83 mm) and *L. acidophilus* (9.89-21.17 mm). Within the zone of inhibition, streptomycin and imipenem had values ranging from 13.49-16.41 mm, with significant difference. Present of ntimicrobial properties of LAB (in all samples) against commonly found pathogens (*E. coli, S. typhi* and *S.*

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aureus), might be due to production of organic acids (lactic acid), peptides (bacteriocins), carbon dioxide, hydrogen peroxide, ethanol and diacety (Farnworth, 2005; Sarkar, 2007). Although, antimicrobial properties of LAB is said to be strain specific but collective activities of more than one strain ensure more efficient antimicrobial action. The increase in the medium chain fatty acid as supported by Jay (1982) and this could exert antimicrobial activities (Akinpelu *et al.*, 2015). The mechanism(s) of antimicrobial activity in probiotic LAB strains appears to be multifactorial, due to bacteriocins and/or lactic acid produced (Gauri *et al*., 2013). This was reflected in the result of mixed cultured strains of LAB used in this study. It was observed that short-chain acids (lactic) produce during fermentation which increase with time had inhibitory effect. Combination of three different probiotic strains in this study, achieve stronger inhibitory effect on the growth of the pathogenic bacteria. This is in line with the report of Denkova (2013). Other inhibitory agents found in LAB that could control major pathogenic bacteria in Nigerian bioprocessed food are acidocin (in *L .acidophilus*) and bacteriocin (Amin, 2011). These agents including lactic acid, weaken the outer plasma membrane and sequestrate magnesium ions by chelating effect, causing inhibition of pathogens. Some antimicrobial activities in LAB such as those

Table 3: Growth Kinetics of Lactobacillus acidophilus, Streptococcus salivarious Subsp. thermophilus and Lactobacillus delbrueckii Subsp. bulgaricus during fermentation of Cocos typical milky extract using Monod parameters

Sample	Fermentation Period (h)	Specific Growth Rate, μ (h^{-1})	Doubling Time, td (h)	Specific Glucose Rate Consumed, V (g/h)	Specific Fructose Rate Consumed, P (g/h)	Specific Sucrose Rate Consumed, Y (g/h)	Specific Lactic Acid Rate Produced, π (g/h)
A	0	–	–	–	–	–	–
B	12	0.22 ± 0.01^{c}	3.15 ± 0.15^{a}	0.32 ± 0.03^{c}	0.37 ± 0.01^{a}	0.19 ± 0.012^{b}	6.86 ± 0.08^{a}
C	24	0.06 ± 0.01^{a}	12.00 ± 0.78^{c}	0.12 ± 0.03^{b}	0.18 ± 0.01^{b}	0.10 ± 0.01^{a}	14.14 ± 0.60^{b}
D	36	0.06 ± 0.01^{a}	12.59 ± 0.32^{c}	0.72 ± 0.01^{d}	0.64 ± 0.01^{c}	0.29 ± 0.01^{c}	28.11 ± 0.01^{c}
E	48	0.15 ± 0.05^{bc}	4.57 ± 0.17^{b}	-0.40 ± 0.06^{a}	3.10 ± 0.12^{d}	0.97 ± 0.02^{c}	357.50 ± 0.80^{d}
F	60	0.20 ± 0.02^{c}	3.51 ± 0.20^{a}	218.22 ± 0.058^{e}	129.54 ± 0.02^{e}	69.18 ± 0.01^{d}	4964.26 ± 0.02^{e}
G	72	0.12 ± 0.01^{ab}	5.59 ± 0.01^{b}	22.85 ± 0.02^{f}	593.98 ± 0.02^{f}	304.02 ± 0.01^{e}	36735.12 ± 0.01^{f}

Table 4: Lactobacillus acidophilus, Streptococcus salivarious subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus antimicrobial Assay of the fermented Cocos typical milky extract on different indicators

Antimicrobial in Cocos typical extract and antibiotics as control	Zone of Inhibition (mm) of the Tested Pathogens		
	Escherichia coli	Salmonella typhimurium	Staphylococcus aureus
A	10.11 ± 0.17^{c}	9.13 ± 0.17^{a}	9.89 ± 0.17^{b}
B	13.33 ± 0.44^{b}	13.50 ± 0.76^{b}	12.67 ± 0.88^{b}
C	14.67 ± 0.60^{c}	15.17 ± 0.60^{c}	15.00 ± 0.00^{c}
D	16.50 ± 0.29^{d}	16.67 ± 0.17^{d}	17.00 ± 0.29^{d}
E	18.00 ± 0.29^{e}	17.50 ± 0.29^{de}	17.67 ± 0.17^{d}
F	18.67 ± 0.17^{ef}	18.33 ± 0.17^{e}	19.67 ± 0.44^{e}
G	19.33 ± 0.17^{f}	19.83 ± 0.60^{f}	21.17 ± 0.44^{f}
Streptomycin	15.67 ± 0.34^{e}	13.49 ± 0.29^{d}	16.25 ± 0.55^{c}
Imipenem	15.98 ± 0.29^{d}	15.34 ± 0.18^{e}	16.41 ± 0.28^{d}

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Mean of samples with different superscript letter are significantly different ($p<0.05$); Mean of triplicate values±standard error associated with molecules frequently exported by bacteria, (the hemolysins or hydrolytic enzymes), have effectiveness in diarrhoea caused by *Salmonella* among children (Amit and Vipan, 2013).

Many researchers had confirmed that the component of fatty acid (such as capric, lauric, miristic, oleic, palmitic and miristic acids) had effective activity against Candida spp, Aspergillus niger, Bacillus cereus, Bacillus subtilis, Micrococcus lysodeikticus, Penicilium citrinum, Peudomonas aeroginosa, Streptococcus pneumonia (also Palmitic and Myristic Acid), Saccharomyces cerevisiae, Streptococcus group A and others (Bergsson et al, 2001; Sheehan et al, 1999; Ogbolu et al, 2007).

CONCLUSIONS

Cocos typical extract served as good carbon source for *Lactobacillus acidophilus, Streptococcus salivarious* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus.* As they feed on the macromolecules, there was increase in their growth and in produced metabolites. Therefore, this study established that fermented *Cocos typical* can render probiotic LAB, which exert health benefit above nutritional value. In addition, this study proved that as at 72 h of fermentation, viability was still enhanced in *Cocos typical* extract, exhibiting log phase of microbial growth. Meanwhile, stability of desirable or nutritious compositions and the safety of this product was be maintained at 36 h of fermentation.

It could also be inferred that *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* impart inhibitory effect on pathogens (*E. coli, S. typhimurium and S. aureus*) and greatly reduced the non-digestible frutooligosaccharide, in the samples.

It was deduced accordingly that the bioprocessed *Cocos typical* extracts (at 24 h and 36 h of fermentation with *L. acidophilus, S. salivarious* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) were safe for consumption and they could be recommended for the vulnerable group, the pregnant women, vegetarian, infants and children.

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Non-Ethical Approval

Author declares that this research work did not involve the use of human data or human tissue and It is thereby affirmed that during the course of researching, no harm nor discomfort to the participant occurred.

Conflict of Interest Disclosure

Not available

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Appendix 1 Supporting information

Supplementary data associated with this article can be found in the online version at DOI:10.1016/j.microb.2025.100286.

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