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Assessment of the Protective Culture Potential of the *Lactococcus lactis* Ganee-5 strain as a Preservative against Spoilage Bacteria in Tomato Pastes

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ABSTRACT

This study investigated the spoilage patterns and biopreservation of tomato paste by lactic acid bacteria isolated from fermented milk products. All the isolates were screened for hydrogen peroxide, diacetyl, and lactic acid production. Isolate with the highest mean values of evaluating parameters was selected as protective culture for the biopreservation. The isolate was identified as *Lactococus lactis* strain Ganee-5 using molecular techniques, and the sequences were submitted to the Genbank Database to obtain the accession number (MH571417). Antimicrobial properties of the protective culture were evaluated against some selected spoilage bacteria *E. coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 15313), *Salmonella typhimurium* (IFO 12529), and *Staphylococcus aureus* (ATCC 12600), varying zone of inhibitions ranged from 18-25 mm were detected. The potato paste was preserved with *L. lactis* culture, sodium benzoate and control samples while the control samples were left without preservatives. All the experimental set-up was left for 16 days. Physicochemical and nutritional analysis showed that tomato paste with *L. lactis* was preserved closely as much as sodium benzoate ($p < 0.005$). Therefore, *L. lactis* can be adopted for the preservation of the tomato paste to replace chemical preservatives.

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Introduction

Tomato (*Solanum lycopersicum* L) is one of the most important vegetables produced globally, comprising approximately 14 % of world vegetable production [1,2]. It is one of the highly nutritious food ingredient used in the preparation of foods all over the world [3,4]. The following procedures for the treatment of vegetables such as pre and post-harvest methods, use of unsafe water for rinsing the vegetables and sprinkling to keep them fresh are the major predisposing factors responsible for the contaminations [5].

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The high water content in tomatoes makes it susceptible to spoilage by bacteria and fungi during storage, harvesting and transportation [6]. Spoilage accounts for the annual lose of the large proportion of the vegetable produce [7].

Spoilage bacteria or fungi are responsible for the postharvest decay and rapid deterioration which in turn affects the quality and shortens the shelf life of fruits and vegetables [8]. Vegetables have been associated with outbreaks of foodborne disease in many countries with varying magnitude from a few affected persons to many thousands [9].

Spoilage and pathogenic bacteria such as *Bacillus* spp, *Clostridium* spp, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp are responsible for the short shelf-life of food products and food-borne illnesses. In order to achieve improved food safety against such pathogens, food industry makes use of chemical preservatives or physical treatments (e.g. different chemicals and high temperatures). Food preservation is the process of treating food to stop or slow down spoilage, loss of quality, edibility or nutritional value. Food preservation is paramount to food safety and storage and has an impact on food security [10]. There have been growing global safety and health concerns over the use of synthetic chemical and artificial food preservatives and additives such as nitrites and sulphites in foods which have been found to be mutagenic and capable of triggering allergies and intolerances respectively [11]. This has called for the need for natural and safer approaches to food preservation and a lot of effort has been put towards moving away from the use of chemical food preservatives [12].

These preservation techniques have many drawbacks which include the proven toxicity of the chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives. Therefore, there is an intense interest in developing novel antimicrobial agents for use in vegetable foods preservation to avoid spoilage.

Bio-preservation is defined as technique of extending the shelf life of food by using natural or controlled microbiota or antimicrobials [13].

Lactic acid bacteria and their metabolites are envisaged to be potential alternatives to chemical preservatives since they exhibit antimicrobial effects against spoilage and pathogenic bacteria. In addition, lactic acid bacteria are safer to consumers since they have

a generally recognized as safe' (GRAS) status [14]. Consumers' growing awareness of the health risks associated with the use of preservatives has resulted in a demand for the availability of chemical-free food on the market. As a result, there is a need to look for new alternatives that are natural but efficient and do not pose a health risk.

Materials and Methods

Sample Collection

A fermented dairy product ("Fura da nono") was obtained from Fulani herdsmen in Ilorin around Abayawo area, Nigeria. It was collected in sterile universal bottle and kept at temperature of 4 ^{0}C before being taken to the laboratory for microbial analysis. While, ripe and wholesome tomatoes known as Roma tomatoes were purchased from Mandate Market, Ilorin, used in this research. The tomatoes were thoroughly cleaned and blended, then stored before being used.

Physicochemical Parameters of Tomato Paste

pH Determination

The pH meter was calibrated using a buffer solution of pH 4 and 7. A beaker was filled with about 25 ml of tomato paste, pH meter probe was dipped into the beaker and the pH of the sample was taken as described by Akinola *et al.* [15].

Determination of Lycopene Content

Lycopene of the tomato paste was extracted and estimated as described by Suwanaruang [16] using hexane:ethanol:acetone (2:1:1) (v/v). They were vortexed for 1 hour at 30°C in hexane and cooled to room temperature. Lycopene levels in the hexane extracts were calculated according as follow: Lycopene (mg/kg) = Abs 503 nm × 137.4

Determination of β-Carotene content

The β-carotene content was estimated following the method of Owolade *et al*. [17]. The beta-carotene content of Tomato paste samples was determined by analyzing the absorbance at 452 nm using 3% acetone in petroleum ether and 1% chloroform. The standard curve was used to determine the concentration of beta-Carotene in the Tomato paste. The amount of β-Carotene content was expressed in mg/kg of Tomato paste.

Determination of Ascorbic Acid Content

Ascorbic acid content of tomato paste was determined by iodometric titration as described by Dioha *et al*. [18]. Briefly, Ten milliliters of the tomato paste was added into a pre-washed conical flask, which was then filled with 5 milliliters of 10 % potassium iodide (KI) and 2 milliliters of 0.3 M sulphuric acid (H_2SO_4). In addition, 10 mL of 0.01 M potassium iodate was added to the flask ($KI0_3$). A solution of 0.01 M sodium thiosulphate was used to titrate the excess iodine ($Na_2S_2O_3$). A blank titration was performed with 10 mL of distilled water. The amount of ascorbic acid was expressed in mg/L of Tomato paste.

Determination of Titratable Acidity

Titratable acidity was estimated following standard procedure [19] as percentage of citric acid monohydrate.

Total Bacterial Counts

The bacterial load of the tomato paste was carried out using pour plate method as described by Cheesbrough [20]. The total counts were taken as colony forming unit per milliliter (cfu/ml) of the sample [21].

Enumeration and isolation of Lactic acid Bacteria (LAB) from Fermented dairy products ("Fura da nono" and "wara")

Enumeration and isolation of LAB was carried out following method of Wang *et al*. [22]. Fermented dairy products ("Fura da nono" and "wara") were cultivated in De Man Rogosa and Sharpe (MRS) broth at 30 °C for 24 h for enrichment. One milliliter of a sample was mixed with 9 mL sterile physiological saline (0.85% w/v, NaCl) to make an initial dilution. Then subsequently, serially diluted and 1ml of the aliquot was inoculated using pour plate method on molten De Man Rogosa and Sharpe (MRS) agar Cycloheximide at a concentration of 0.01% (v/v) was added to the MRS plates in order to prevent the fungal growth, The plates were packed and sealed in an anaerobic jar then incubated under anaerobic condition at 30 °C for 48-72 h. Colonies with distinct morphological differences (based on color, shape, size, rough or smooth surface) were selected. The catalase activity and Gram reaction of the isolates were assessed. Gram-positive, catalase-negative and non-motile microorganisms were preserved in 10% (w/v) skim milk containing 0.1% (w/v)

sodium glutamate and maintained on agar slant for further characterization and identification.

Evaluation of the LAB Strains for the Production metabolites

The LAB isolates were screened for the production of metabolites following modified method of Ishola and Adebayo-Tayo [23]. The isolates were cultivated in MRS medium and incubated for 8 hours to attain stationary phase. One milliliter of each of the working cultures was transferred into 10 ml of MRS broth in 100 ml conical flasks incubated in an anaerobic jar for 24 hrs at 30 ^{o}C. Ten milliliter Inocula (OD_{600}=1.0) were inoculated into 200-ml conical flasks containing 90 ml of MRS broth and incubated at 30 ^{o}C for 36 hrs. Samples were taken and analyzed for lactic acid, diacetyl, hydrogen peroxide, pH development following methods of Awojobi *et al.*, [24] as follows:

Determination of Hydrogen Peroxide Production

Twenty five milliliter of diluted sulphuric acid was added to the isolates' broth culture. Titration of 0.1 N potassium permanganate was carried out The sample's decolorization was taken as the end point (AOAC, 1990).

Determination of Lactic Acid Production

A titration using 3 drops of phenolphthalein as an indicator was carried out with the NaOH (0.1 N) against a 25 ml broth culture of the isolates. The NaOH was gradually added until the color turned pink. Each milliliter of NaOH equals 90.08 milligrams of lactic acid.

Determination of Diacetyl Production

Twenty five milliliter of the isolate broth culture and 7.5 ml hydroxylamine solution were added to conical flasks for residual titration. HCl of 0.1 N was titrated with the bromophenol blue as an indicator which turned to a greenish end point; 21.5 mg HCl = diacetyl.

Molecular Identification of the LAB Isolate

The isolate was identified using molecular technique. Bacterial DNA was extracted from 18 hours old cultures using the Microbial DNA Isolation Kit (MO BIO, Laboratories,Inc.) according to manufacturer's instructions. The 16S rRNA was amplified by PCR forall the isolates using the primers: 16S forward primer (5'-AGAGTTTGATCCTGGCTCAG-3) and 16S reverse primer (5′ACGGCTACCTTGTTACGACTT-3′). PCR was performed. Each

PCR reaction was run with a negative control (no DNA). The PCR products was electrophoresed on 1.5% agarose gels, stained with 0.4 µg/ml ethidium bromide, and bands visualized with a UV illuminator [25].

Sequence analysis

PCR product was cleaned utilizing ExoSAP- 1T (Affymetrix, Inc., USA). The 5 µl of post-PCR reaction and 2 µl ExoSAP-IT reagents was mixed. The mix was incubated at 37 °C for 15 minutes followed by incubation at 80 °C for 15 minutes. Each purified template was sequenced on both strands using 16s primers. The sequences of ITS 3 and 4 regions, 16s of the tested isolates was edited in order to generate a consensus sequence from forward and reverse sequence in the amplicon using sequence assembly software (DNA BASER). A consensus sequence was analyzed by NCBI BLAST database for bacterial identities [26].

Antimicrobial properties of Bioactive Metabolites Produced by LAB isolates

Production of Bioactive Metabolites

The isolated strain of *Lactococcus* was grown in MRS broth (Hi Media, Mumbai) and maintained anaerobically at 34 °C for 24 h. After incubation, cells were removed from the growth medium by centrifugation (10,000 x g for 30 min, 4 °C) and passed through 0.2 mm filter. The cell-free supernatant was adjusted to pH= 6.0 using 1N NaOH and used as crude metabolite.

Determination of antibacterial effect of the bioactive metabolites

Antibacterial properties of the metabolites was determined by the agar well diffusion method using standard organisms *E.coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 15313), *Salmonella typhimurium* (IFO 12529), *Staphylococcus aureus* (ATCC 12600) as the indicator strains. Nutrient agar was previously streaked with the test organisms on different plates and they were incubated at 37 ºC for 24 hours, after which a cockborer was used to make hole of 6mm on each plate and 5 ml of bioactive metabolites was introduced into the hole and plates were incubated for 24 hours to observe the zone of inhibition. Zone of inhibition was measured in mm using ruler.

Comparative Evaluation of Biopreservatative activities of LAB and Sodium benzoate on Tomato pastes

The tomato pastes were then distributed into different sterile McCartney bottles. Prepared Tomato paste was then pasteurized using High –Temperature-Short time method with water bath pasteurizer at 85 ^{o}C for 15 mins [27]. One milliliter of the inoculum at (OD_{600}=1.0) was aseptically pipetted into 10ml of the pasteurized tomato paste inside McCartney bottles. Similarly chemical preservative (Sodium benzoate) at 0.1% was mixed with 10ml of the pasteurized tomato paste inside McCartney bottles. The treated tomato samples were then stored under ambient conditions (25°C). The physicochemical parameters were monitored as mentioned above for a period of 16 days for the assessment preservative activities of the LAB isolate [24].

Data Analysis

After data collection, the instruments were checked for completeness and consistency. The data was analyzed using statistical package for social sciences (SPSS) and Microsoft excel 2010. Analysis of variance (ANOVA) statistical test was used to determine the mean difference between the treatments conditions used for the research. A 95% confidence level was used and $p \leq 0.05$ or statistical value greater than 1.960 was considered statistically significant. Descriptive statistics like frequencies and percentages were used to present the result. Some results were expressed as mean ± standard deviation, while others were presented in clustered bar chart.

Results and Discusion

The physicochemical and nutritional values obtained were within the range documented in the literature thus the nutritional and bioactive composition of tomatoes such as lycopene, β-carotene and ascorbic acid presents tomatoes as a food of great interest all over the world [28]. Current study evaluated the physicochemical, nutritional properties and bacteriological load of the experimental tomato paste as presented in Table 1.0.

Table 1. Physicochemical characteristics, Nutritional and Bacteriological Load of Tomato paste

Parameters	Values
pH	4.0 ± 0.25
β-Carotene (mg/kg)	12.35 ± 1.60
Lycopene (mg/kg)	290.04 ± 4.56
Ascorbic Acid (mg/L)	12.10 ± 0.33
Titratable Acidity (%)	69.56 ± 2.40
Log Total Bacterial Counts (cfu/ml)	5.0 ± 0.55

Values are means ± SEM (n=3) per treatment

The pH of the sampled tomato paste obtained in this study ranged from 4.8 – 5.0 fallen within the expected range of 3-5 for fruits and vegetable juices [29,30]. The low pH of the tomato has been attributed to the abundance of organic acid largely citric and malic acids [31]. This result seemed to validate the literature information available on the pH values of tomato fruit [32]. Stevens [33] reported that although the pH of ripe tomatoes may exceed 4.6, tomato products are generally classified as acidic foods (pH < 4.6).

pH below 4.5 is a desirable trait, because it effectively inhibits the proliferation of microorganisms [34]. Lycopene is a natural pigment which is responsible for the reddish coloration of tomatoes and other fruits [16]. The average lycopene content of raw tomatoes has been reported at 30 mg kg^{-1} [35]. However, higher values were obtained in this study, this variance in lycopene accumulation could be attributed to the environmental factors (temperature, light, growing season and location), and the agricultural techniques [36,37] (Dumas *et al.,* 2003; Toor *et al.,* 2006). Similarly, the average β-carotene content of raw tomatoes has been reported at 3.9 mg kg^{-1} [38].

The presence of Vitamin C in the fruit and vegetable is highly essential since it plays a crucial role in the body forms and metabolic functions[39], and as such the mean values of ascorbic acid content as evaluated was 46.33 ± 2.98 mg/cm^3 significantly in high proportion. Again, high bacterial load of the tomato paste recorded in the study could be a contamination from either water, equipment used during processing, airborne, soil borne bacteria or tomato flora. Fermented diary product fura do nono used in this study provided a good source for isolating Lactic acid bacteria. The LABs with the highest frequency of

occurrence in the fura do nono was selected for the experiment. The occurrence of this organism has been reported by various researchers [40,41]. This study demonstrated that the selected LAB produced different biomolecules in sufficient quantities such as hydrogen peroxide, diacetyl and lactic acid productions. The ability of this LAB strains to secrete antimicrobial compounds confers the potential ability on them to extend the shelf life of tomato paste and reduce the microbial load [24].

The phylogenetic relationship among the strain GANEE-5 and other related bacteria downloaded from the GenBank database was constructed. The strain was in the phylogenetic branches of the *Lactococcus lactis*. Strain exhibited a maximum identity (100 %) to *Lactococcus lactis* strain NBRC 100933 (NR 113960.1) showed in Fig. 1.0. The bacterial isolate clustered with the members of the genera *Lactococccus*, thus differentiating the bacterial isolate on the genetic basis. Incidentally, Biscola *et al.* [42] had earlier reported that *Lactococcus lactis* 69 habor no virulence genes which implies that the safety and potential technological application of this strain to inhibit undesirable microbial growth is promising.

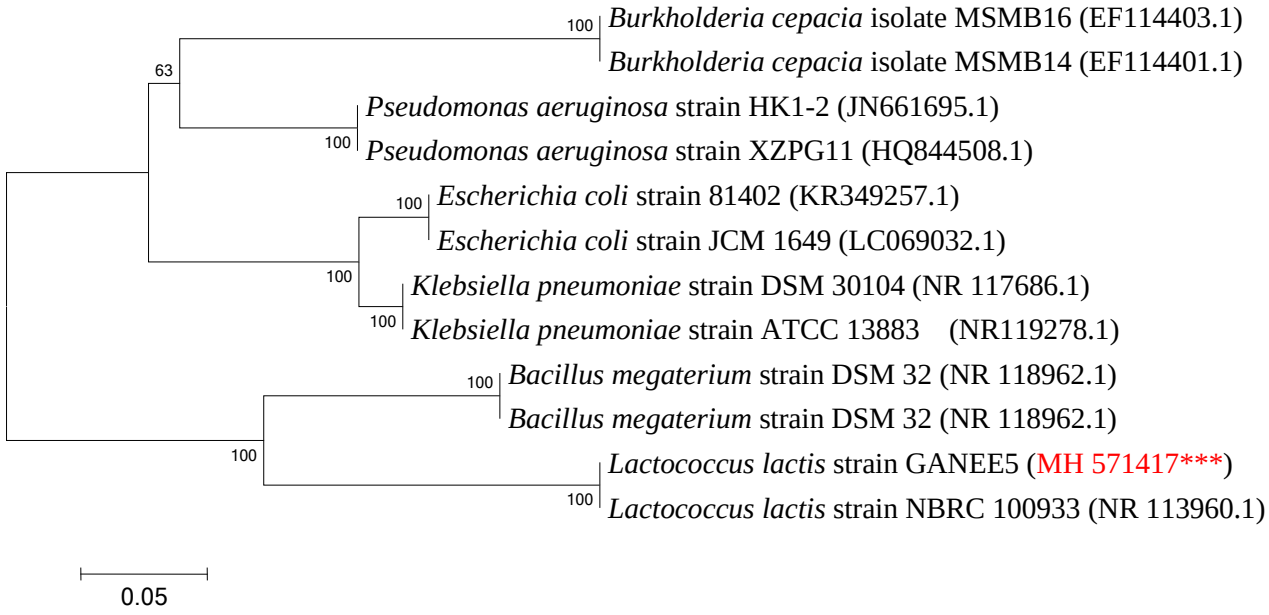

Fig 1. Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16S rRNA gene sequences of native isolates and related sequences obtained from NCBI. Scale bar, 0.05 substitutions per nucleotide position and numbers in parenthesis represent GeneBank accession numbers.

Current study also evaluated the inhibitory effect of the isolated *Lactococcus lactis* sp. lactis Ganee-5 against some selected bacteria. The result showed that the isolate produced inhibitory compound against test organism namely *E.coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 15313), *Salmonella typhimurium* (IFO 12529), *Staphylococcus aureus* (ATCC 12600). The diameter of zone of inhibition ranged from 18-25mm. The mean values of the diameter of the zone of inhibition against *Listeria monocytogene* (ATCC 15313) and *Salmonella typhimorium* (IFO 12529) was 18.72 mm and 25.44 mm respectively. The test organisms' sensitivity to bioactive metabolites varies greatly. The bioactive compound impacts test organisms in varying degrees. The mean values recorded for the zone of inhibition was 25.00 mm and 22.00 mm for both *Staphylococcus aureus* (ATCC 12600) and *E.coli* (ATCC 25922) respectively (Table 2.0). These findings are in line with the work of Sahraoui *et al*. [43] who reported that *Lactococcus lactis ssp. lactis* KJ660075 strain showed a remarkable antibacterial inhibition almost against several food borne pathogens and spoilage bacteria. Also, Yang *et al.* [44] also found that inhibitory effect against several foodborne pathogens e.g. *Listeria innocua, B. cereus, P. fluorescens, Erwinia carotovora* and *Leuconostoc mesenteroides* subsp. *mesenteroides* LAB isolated from cheese and yogurt. Several Authors have reported the inhibitory properties of Lactic acid bacteria against various spoilage and pathogenic bacteria [45,46].

Table 2. Inhibitory effect of the *Lactococcus lactis* strain GANEE5 against selected test organisms

Test organisms	**Zone of inhibition (mm)**
E.coli (ATCC 25922)	22.00 ± 1.60
Listeria monocytogenes (ATCC 15313)	18.00 ± 0.72
Salmonella typhimurium (IFO 12529)	20.00 ±1.10
Staphylococcus aureus (ATCC 12600)	25.00[a] ±1.44

Several Authors have hypothesized different mechanisms for the antagonistic activity of LAB metabolites against the spoilage bacteria. The inhibitory effect of lactic acid is due to undissociated forms of the acids which penetrates the pathogen’s membrane and liberate hydrogen ion in the neutral cytoplasm thus inhibiting vital cell functions [47]. Diacetyl is

known to have a very effective and strong oxidizing effect on organism's cell especially bacteria [48]. In the same way, the antibacterial activity of the LAB was presumed to be associated with synthesis of organic acid, hydrogen peroxide and bacteriocins and this fact may contribute to their colonizing and competitive ability [49]. The authors also claimed the bacteriostasis and death of susceptible bacteria were indeed due to the neutralization of organic acids on the cyctoplasmic membrane, and thus increasing its permeability. This mechanism causes the cell ruptures and eventually kills the bacteria [50].

On the whole, inhibitory activity of LAB has been reported to be due to a combination of many factors such as production of lactic acid which brings about reduction of pH of the fermentation medium [51] and production of inhibitory bioactive compounds such as hydrogen peroxide and bacteriocins which are responsible for most antimicrobial activity [4,23]. Lactic acid bacteria (LAB) play a major part in most fermentation processes, not only because of their ability to improve the flavour and aroma but especially for their preservative effects on food. LAB contributes to preservation by the production of a vast array of antimicrobial compounds and proteins [52,53].

The bio-preservative potential of LAB metabolites has been tested on other food product like suya [54] and chicken meat [55]. A major advantage in the use of lactic acid bacteria and their metabolites is that they are considered as generally recognized as safe (GRAS) and comply often with the recommendations for food products [56]. Unlike some chemical preservatives, LAB metabolites have not been reported to have residual effect on the food product or the consumer's health.

The effect of *Lactococcus lactis* strain Ganee-5 biomolecules and sodium benzoate on the ascorbic acid in preserved tomato paste is presented in Fig. 2. The Ascorbic acid decreased as the number of days of storage increased in the control samples while samples preserved with both sodium benzoate and *Lactococcus lactis spp lactis* as preservatives was stable throughout the 16 days of storage. However, on the 10^{th} days of storage, the ascorbic acid of the control sample decreased and was significantly different to other samples ($p \geq 0.05$). Incidentally the ascorbic acid of both the sodium benzoate and *Lactococcus lactis spp lactis* preserved tomato paste was maintained throughout the storage period. This may be due to

the degradation of tomato paste when exposed to heat, light, or oxygen, as suggested by Akinola et al. [15] on an orange, watermelon, carrot, and ginger juice blend.

The ascorbic acid level in samples decreased with time. This observed diminution in vitamin C content is in agreement with what was reported in literature. Vitamin C content is known to be an important parameter for assessing the nutritional quality of fruits blends as it degrades during storage [57]. This degradation is perhaps due to the high sensitivity of ascorbic acid to oxygen, light and heat - and consequential oxidation in the presence of oxygen by both enzymatic and non-enzymatic catalyst [59]. At 16th day, sample with sodium benzoate gave higher ascorbic acid level (12.10 mg/100 g) than sample with metabolite (10.26mg/100 g); this implies that sodium benzoate preserved ascorbic acid more than metabolite during the storage period. Consequently, vitamin C contents of tomato paste can be enriched using sodium benzoate as preservative, although, the abuse (excess addition) of such preservative has been linked to food poisoning/toxicity, immune depression and cancer in human [60].

The effect of *Lactococcus lactis spp lactis* strain Ganee-5 biomolecules and sodium benzoate on the β-carotene in preserved tomato paste is presented in Fig. 3. The beta carotene content of the preserved tomato paste both with the sodium benzoate and Lactococcus metabolite preservatives showed slightest decrease in β-carotene content in both preservatives significantly not different ($p \geq 0.05$) throughout the storage time. While, there was remarkable decrease in the β-carotene of the control sample. Incidentally, β-carotene was not detected after 16 days of storage. The gradual decrease in beta-carotene as observed in result presented in (Fig 3) may be due to changes in surrounding storage temperature. This gradual decrease in beta-carotene during storage was also observed by Awsi [67].

The effect of *Lactococcus lactis spp lactis* strain Ganee-5 biomolecules and sodium benzoate on the titrat able acidity in preserved tomato paste is presented in Fig.4. The titratable acidity increased as number of days of storage increased and the highest values was recorded in the sodium benzoate preserved samples followed by *Lactococcus lactis spp lactis* strain Ganee-5 biomolecules preserved sample and the lowest in control sample.

The effect of *Lactococcus lactis spp lactis* strain Ganee-5 biomolecules and sodium benzoate on the lycopene in preserved tomato paste is presented in Fig. 4. Result showed that lycopene retention was lowest in control sample whereas treatment with metabolite recorded high lycopene content (Fig. 5), although retention of lycopene was higher in sodium benzoate. Lycopene retention of the control sample ranged from 1.56 mg/kg –14.3 mg/kg on the 12th day. The lycopene content was not detected in day 14-16 of the control samples. Lycopene content in tomato paste preserved with metabolites ranged from 12.22 mg/kg – 14.3 mg/kg while sodium benzoate treated tomato paste recorded ranged from 13.0 mg/kg – 14.3 mg/kg. Lycopene content was decreased during storage period for all samples. The loss of lycopene at all conditions might be due to oxidation which depends on temperature, moisture etc [69].

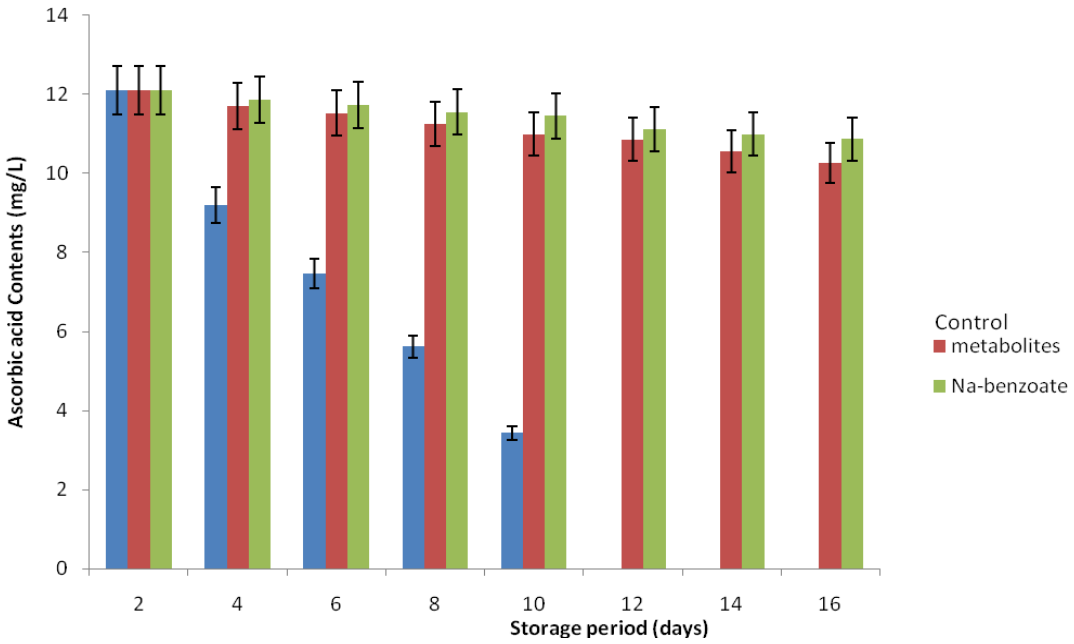

Fig 2. Effect of *Lactococcus lactis* metabolite and sodium benzoate on the ascorbic acid content of tomato paste over a storage time

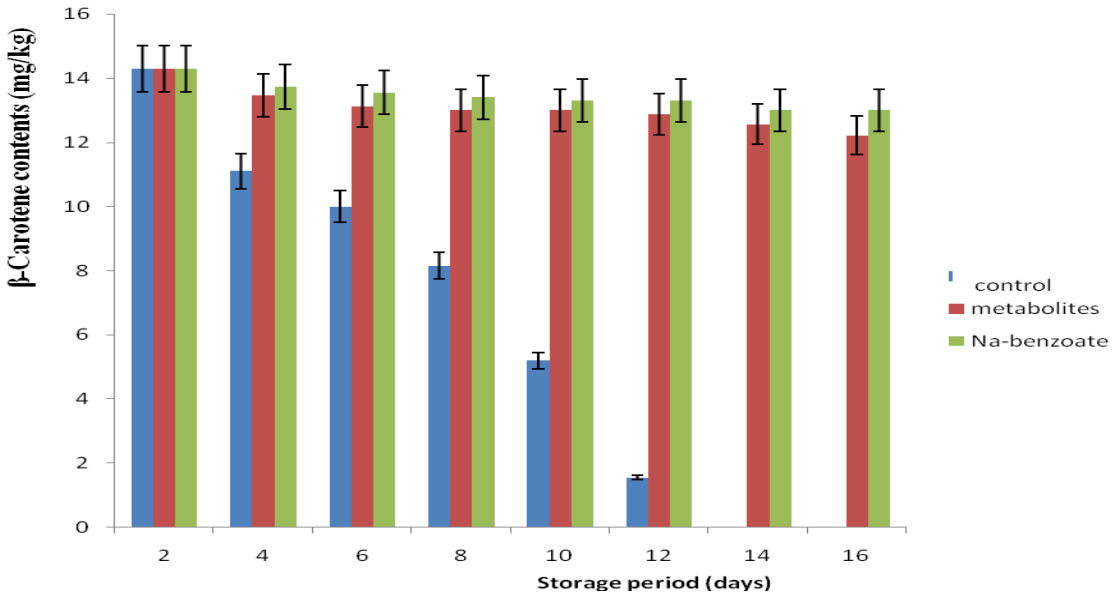

Fig 3. Effect of *Lactococcus lactis* metabolite and sodium benzoate on the β-carotene contents of tomato paste over a storage time

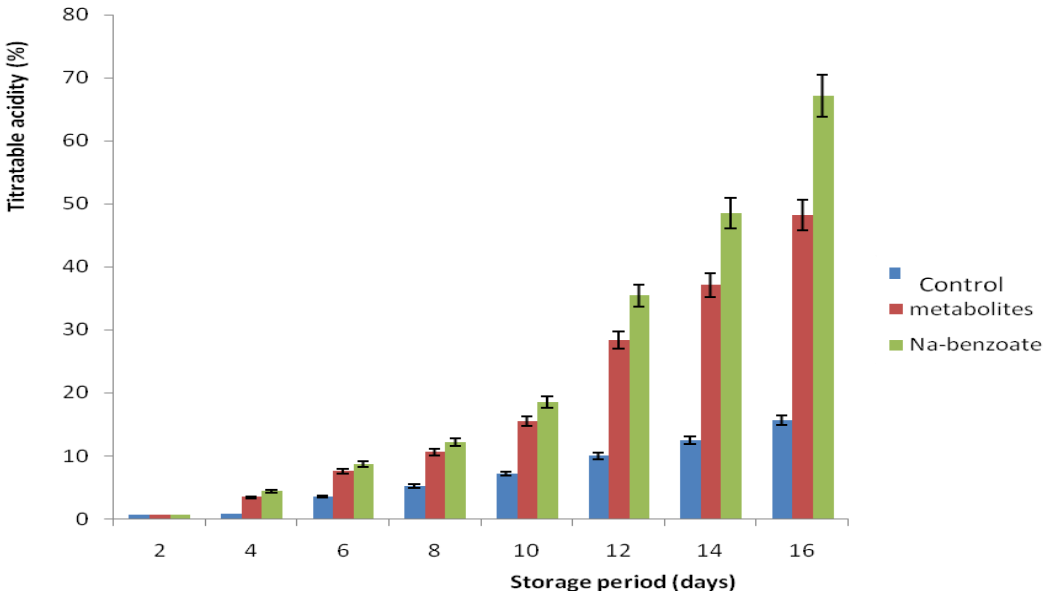

Fig 4. Effect of *Lactococcus lactis* metabolite and sodium benzoate on the titratable acidity content of tomato paste over a storage time

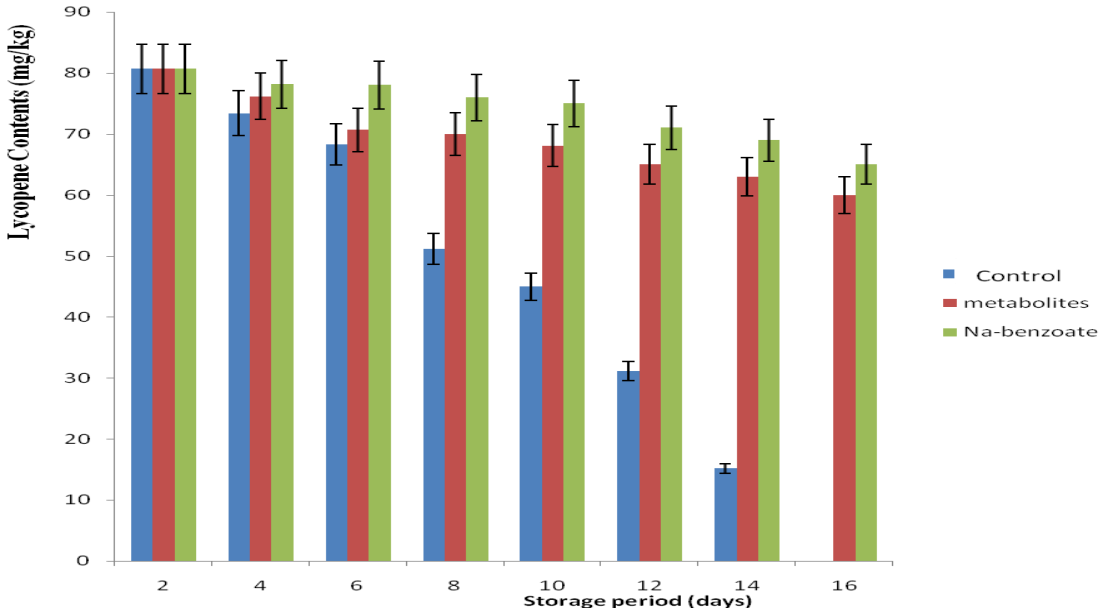

Fig 5. Effect of *Lactococcus lactis* metabolite and sodium benzoate on the Lycopene contents of tomato paste over a storage time

The use of lactic metabolites with biopreservative activity could improve the quality of food and increase its safety by inhibiting the food-borne pathogens and spoilage microorganisms.

Conclusion

This research work confirmed and supported the fact that vital physicochemical property of tomato paste such as ascorbic acid, pH, titratable acidity, β-carotene and lycopene decrease with advancement of storage time. Addition of preservatives (natural and synthetic) was noted not to prevent the loss of ascorbic acid from the result, even though sodium benzoate preserved better than *L.Lactis* metabolite, natural preservative (*L.lactis* metabolites) maybe said to be better than synthetic preservative as no negative impact on humans has been ascribed to its use in tomato paste while use of preservative have been proved to mutagenic. Finally, physicochemical analysis revealed that the tomato paste has good quality characteristics without the addition of preservatives. Therefore, tomato should be consumed fresh. It is therefore recommended that more research needs to be conducted to prove and establish the results reported in this work, especially on the effect of preservatives (natural

and synthetic). Also, storage period should be increased and mineral composition should be analyzed in tomato paste and other vegetables.

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