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BIOPRESERVATIVE EFFECT OF GINGER (*ZINGIBER OFFICINALE*) AND GARLIC POWDER (*ALLIUM SATIVUM*) ON TOMATO PASTE

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ABSTRACT

The study investigated fresh tomato fruits (Roma VF variety) prepared into paste, proportioned into different samples with each receiving different concentrations of ginger, ginger and garlic powder (2 and 4% w/w) and stored over a period of 8 weeks. The total viable (TVC), lactic acid bacteria (LAB) and yeast counts of the samples were enumerated on a weekly basis while the pure microbial isolates were identified. Results showed that TVC and LAB of control sample ranged from 3.42 to 13.45 and from 5.79 to 17.74, respectively, while samples with garlic and ginger had counts ranging from 3.34–4.87 to 3.39–4.86 (log cfu/g), respectively, over the period of storage. The microorganisms were identified as *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacilli acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae*, *Saccharomyces lactis*, *Hansenula anomala*, *Rhodotorula glutinis*, *Rhodotorula flava* and *Rhodotorula rubra*. The study concluded that combined garlic and ginger (2 and 4%) suitably preserved tomato paste for 8 weeks without deterioration at refrigeration temperature (4 ± 1C).

PRACTICAL APPLICATIONS

Health-conscious consumers are wary of potential dangers of consuming chemical preservatives over a period of time. The use of natural products such as ginger and garlic as natural preservatives could significantly improve their utilization in industrial production of tomato paste and even in household production in Africa where refrigeration is greatly hampered by erratic power supply.

INTRODUCTION

Tomato is a savory, typically red, edible fruit produced by the plant *Solanum lycopersicum* (Smith 1994). Tomato fruit is high in water content (95%), low in protein (usually less than 3.5%) and contains digestible carbohydrates largely in the form of reducing sugar while the indigestible carbohydrates are cellulose materials which provide roughage important to normal digestion. There are 19 soluble amino acids in fresh tomato juice and glutamic acid comprises up to 48.45% of the total weight of the amino acids in fresh tomato juice. The phenolic compounds present in the tomato are important antioxidant. Tomato is a good source of vitamins C, provitamin A and minerals. Tomato is also rich in the carotenoid and lycopene. Tomato consumption is believed to benefit the heart among other things. It contains lycopene, one of the most powerful natural antioxidants. In some studies lycopene, especially in cooked tomatoes, has been found to help prevent prostate cancer. Lycopene has also been shown to improve the skin's ability to protect against harmful ultraviolet rays (Redenbaugh *et al.* 1992). Furthermore, tomato is a good source of fiber, which has been shown to lower high cholesterol levels, keep blood sugar levels from getting too high and help prevent colon cancer. Tomato consumption has been associated with decreased risk of breast (Zhang *et al.* 2009) and neck cancers (Freedman *et al.* 2008) and might be strongly protective against neurodegenerative diseases (Rao and Balachandran 2002; Suganuma *et al.* 2002). The demand for tomato has increased over the years but preference for the fresh fruit is high. The wastage in Nigeria and in many developing countries makes its supply inadequate and tomato products (mostly puree) from Europe are still being imported to augment the local production (APMEU 1998).

Deterioration in processed tomato products including paste may be caused by microorganisms, food enzymes or by purely chemical reactions. Tomatoes and their products are ideal medium for the growth of lactic acid bacteria (LAB) particularly *Lactobacillus lycopersici*, *Lactobacillus plantarum* and *Lactococcus lactis* (Gould 1983, 1992).

The inhibition of the growth and activity of microorganisms is one of the main purposes of the use of chemical preservatives (Anu *et al.* 2010). The increasing demand for safe foods, with less chemical additives, has increased the interest in replacing these compounds with natural products, which do not injure the host or the environment. Natural substances such as salt, sugar, vinegar, alcohol and essential oils are used as traditional preservatives. Food additives play a vital role in today's bountiful and nutritious food supply (WHO 2002).

Over the years, much effort has been devoted to the search for new antifungal materials from natural sources for food preservation (Onyeagba *et al.* 2004; Boyraz and Özcan 2005; Haciseferogullari *et al.* 2005). *Alliums* are revered to possess antibacterial and antifungal activities and include the powerful antioxidants, sulfur and other numerous phenolic compounds, which arouse significant interests (Griffiths *et al.* 2002; Benkeblia 2004; Haciseferogullari *et al.* 2005). Crude garlic extracts exhibited activity against both gram-negative (*Escherichia coli*, *Proteus* spp, *Salmonella*, *Serratia*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Klebsiella*) and gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus sanguis*, Group A *Streptococcus*, *Bacillus anthracis*) bacteria (Hughes and Lawson 1991; Farbman *et al.* 1993).

Ajoene, a garlic-derived sulfur-containing compound, demonstrated antimicrobial activity against gram-positive bacteria, such as *Bacillus cereus*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Streptomyces griseus*, *Staphylococcus aureus* and *Lactobacillus plantarum*, and against gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Xanthomonas maltophilia*; ajoene also inhibited yeast growth at concentrations below 20 μg/mL (Naganawa *et al.* 1996; Ankri and Mirelman 1999). Allicin exerted antibacterial activity against *Salmonella typhimurium*, primarily by interfering with RNA synthesis (Feldberg *et al.* 1988). Other studies documented that garlic extracts had fungicidal effects against *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis* and *Trichosporum* (Singh and Agrawal 1988). The growth of *Candida albicans* was inhibited by ajoene at concentrations less than 20 μg/mL (Yoshida *et al.* 1987).

Spices, herbs, essential oils and cocoa are rich in antioxidant properties in the plant itself and *in vitro*, but the serving size is too small to supply antioxidants via the diet. Typical spices high in antioxidants (confirmed *in vitro*) are clove, cinnamon, oregano, turmeric, cumin, parsley, basil, curry powder, mustard seed, ginger, pepper, chili powder, paprika, garlic, coriander, onion and cardamom (Tyler 1994). Herbs and spices containing essential oils in the range of 0.05–0.1% have demonstrated activity against pathogens, such as *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, in food systems (Tajkarimi *et al.* 2010). Shelf-life of unripened cheese was extended by 15 days by treatment with ginger extract (Belewu *et al.* 2005). It was also reported by Indrayan *et al.* (2005) that ginger has a moderately good antimicrobial activity. These spices are well tolerated by most people and generally recognized as safe (Sharma *et al.* 2010; Supreetha *et al.* 2011). This study investigated the effect of the biopreservatives (garlic and/or ginger) on the microbial load types and assessed the shelf life of tomato paste during the storage.

MATERIALS AND METHODS

Sample Preparation

Freshly harvested sound ripe red tomato fruits (Roma VF variety), garlic bulbs and ginger rhizomes were purchased from a local market in Ile-Ife, Nigeria. Fresh tomatoes fruits were sorted for wholesomeness and intense red color and were washed and allowed to drain. The tomatoes were then pulped and screened using a Langsenkamp pulping machine (MOD 18 SN L 295, Indianapolis, Indiana, US) according to the method reported by Oludemi (2003). The slurry was concentrated to tomato paste of 22–24% total solids by boiling in stainless steel pot (Nwanekezi and Onyeali 2005). After concentration, the paste was divided into eight portions labeled A–H. Fresh garlic bulbs and ginger rhizomes were cleaned, peeled and 250 g each was dried in a Gallenkamp hot-air oven (Gallenkamp, UK) at 65C for 12 h and ground with a Marlex Excella grinder (Marlex Appliances PVT, Mumbai, India). The garlic and ginger powders were then sieved through mesh size of 50 to 60 to remove the shafts and residues which were subsequently discarded (Kaewin 2004). The garlic and ginger powder were proportioned into concentrated paste samples at different concentrations as follows:

Sample A: tomato paste; Sample B: tomato paste + 2% ginger; Sample C: tomato paste + 4% ginger; Sample D: tomato paste + 2% garlic; Sample E: tomato paste + 4% garlic; Sample F: tomato paste + 2% garlic + 2% ginger; Sample G: tomato paste + 2% Garlic +4% Ginger and Sample H: tomato paste + 2% ginger + 4% garlic.

Each mixture (250 g) was evenly homogenized, filled up into air-tight plastic containers and stored at refrigeration temperature for further analyses. The filling was up to 90% of the space in the air-tight containers.

Microbiological Evaluation

The total viable count (TVC), (LAB) count and yeast/mould count were determined. Furthermore, the microbes associated with the tomato paste during storage were also isolated and identified.

Enumeration of Microbes

Serial dilution was carried out by mixing 1.0 g of tomato paste sample with 9.0 mL of peptone water to obtain 10^{-1} dilution. From this, subsequent dilutions were made serially until the desired level of dilution was obtained. From each dilution, 1.0 mL was introduced into sterile Petri dish before 20 mL each of molten Nutrient agar was added to obtain TVC. Other plates of diluted samples also received 20 mL of each of de Man Rogosa and Sharpe and potato dextrose agar for LAB count and yeast count, respectively. All the plates were poured in duplicates and incubated in an inverted position in incubators at 37C for 24 and 72 h, respectively, for TVC and LAB count, respectively. Plates were incubated at 28C ± 2C for yeast for 3 days (Banwart 2004). Microbial load was determined by counting distinct colonies with the aid of a colony counter. Plates with 25–250 colonies were reckoned with and the number of colonies on each plate was multiplied with the reciprocal of the dilution factor to obtain the count (Harrigan and McCance 1976; Harrigan 1998; McLandsborough 2005).

Characterization and Identification of Microbes

Pure cultures were obtained from distinct colonies by repeated streaking on fresh agar plates and subjected to microscopic examination and biochemical tests such as oxidase test, catalase test Gram staining, sugar fermentation test and production of carbon dioxide. Relevant bacteria and yeast identification schemes of Gibbs and Shapton (1968), Harrigan and McCance (1976) and Wood and Holzapfel (1995) were employed for identification.

Determination of Titratable Acidity and pH

The total titratable acidity (TTA) and pH were determined weekly for all samples to quantify the acid produced in the samples during storage. Acidity was calculated as citric acid using a conversion factor of 0.064.

pH values of the samples were determined with a pH meter (Corning Scholar 425, UL Laboratories, Shenzhen, China). On each occasion, pH meter was standardized with buffer solutions of pH 4.00 and 7.00 (AOAC 2004). For each, 10% slurry of the sample was made before the determination of the pH.

Lycopene Determination

Tomato sample (1 g ± 0.05) was weighed into a 125-mL flask wrapped with aluminum foil to exclude light. A 50-mL mixture of n-hexane-acetone-ethanol (2:1:1; v : v : v) was added to solubilize all carotenoids (Sadler *et al.* 1990). The flask was stoppered and agitated continuously for 30 min on a magnetic stirrer plate until lycopene was completely extracted. This was confirmed in the colorless pulp fiber. Agitation was continued for another 2 min after adding 10 mL of water. The solution was then allowed to separate using separation funnel into a distinct polar (35 mL) layer and a nonpolar (25 mL) layer containing lycopene. The hexane solution containing lycopene was filtered through 0.22 μm filter paper before measuring the absorbance using spectrophotometer at 472 nm (Sharma and LeManguer 1996). The conversion of absorbance into lycopene concentration was based on its specific extinction coefficient ($E_{1cm}^{1\%}$) of 3,450 in hexane (Sharma and LeManguer 1996; Oludemi 2003) using Beer Lambert equation:

$$A = ECL \qquad \text{(Eq. 1)}$$

$$C = \frac{A}{(EL)} \qquad \text{(Eq. 2)}$$

where absorbance (A) is the product of specific emissivity coefficient, E is the concentration of the absorbing species (C) and L is the path length through the sample.

A is the spectrophotometer readings at 472 nm, ($E_{1cm}^{1\%}$) of 3,450 in hexane, L is 1 cm.

Estimation of Water Activity

This was evaluated by determining the total solid content as well as the water content using the methods of AOAC (2004).

Sensory Evaluation

A 15-member panel was used to evaluate freshly prepared biopreserved tomato paste. The panel was consumers who were familiar with tomato paste quality. Selection was based on interest and availability. The biopreserved tomato paste was then served randomly in coded plates plus a control sample. The panelists were asked to rate the tomato paste in terms of color, appearance, aroma and taste on a 9-point hedonic scale, where 9 represented like extremely and 1 dislike extremely. Overall acceptability of the samples was rated on same scale with 9 = highly acceptable and

1 = highly unacceptable. Data for all parameters were reported as means of 15 judgments. Analysis of variance was computed for each sensory attribute.

STATISTICAL ANALYSIS

Each experiment was conducted in triplicate samples and repeated twice and the means were calculated. Pro-Origin Statistical package (1999–2002) of Origin Lab Corporation (Orig Lab Corporation, Northampton, MA, USA) was used for analysis of variance and means were considered significant at $P < 0.05$.

RESULTS

Microbial Load of Biopreserved Tomato Pastes during Storage

TVC, LAB count and yeast and mould count increased during the period of storage in all the samples.

TVC

The TVC of tomato paste sample without any biopreservative increased from 3.41 to 13.45 log cfu/g from week 2 to week 8 of storage. Tomato paste sample containing 2% ginger was observed to have TVC that ranged from 3.34 to 6.07 log cfu/g during the first 4 weeks of storage and increased to 11.54 log cfu/g at the eighth week of storage. Samples containing 4% ginger had no observable growth for the first 4 weeks of storage. However, an increase in the viable count was observed after the fifth week of storage ranging from 5.54 to 11.65 log cfu/g as shown in Table 1. TVC was effectively suppressed in tomato paste samples containing 2 or 4% garlic or garlic used in combination with 2 or 4% ginger. These samples had TVC of lower than 5.0 log cfu/g during storage.

LAB Count

LAB tomato paste samples presented in Table 2 followed a similar trend with increases in TVC load throughout the 8 weeks of storage. Tomato paste sample with 2% garlic and 4% ginger had the lowest level of LAB after 8 weeks of storage. Samples with 2 and 4% ginger recorded high levels of LAB count similar to the control sample while the samples with 2 and 4% garlic used alone or in combination with 2 or 4% ginger had LAB count ranging from 3.74 to 5.92 log cfu/g after 8 weeks of storage.

Yeast and Mould Count

Yeast count ranged from 13.67 to 14.99 log cfu/g in control sample and in samples preserved with 2 and 4% ginger after

TABLE 1. TOTAL VIABLE COUNT OF BIOPRESERVED TOMATO PASTE IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Tomato paste (TP)	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.42 ± 0.15^{B}	4.66 ± 0.12^{C}	6.07 ± 0.17^{D}	7.94 ± 0.23^{D}	13.00 ± 0.26^{F}	13.08 ± 0.26^{E}	13.45 ± 0.31^{E}
TP + 2% ginger	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.34 ± 0.16^{B}	4.70 ± 0.18^{C}	4.83 ± 0.13^{C}	5.88 ± 0.14^{C}	8.96 ± 0.22^{E}	10.74 ± 0.15^{D}	11.54 ± 0.31^{D}
TP + 4% ginger	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	5.54 ± 0.18^{C}	6.88 ± 0.19^{D}	9.96 ± 0.14^{C}	11.65 ± 0.22^{D}
TP + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.40 ± 0.12^{B}	3.45 ± 0.16^{B}	3.62 ± 0.17^{B}	3.96 ± 0.10^{A}	4.40 ± 0.16^{A}	4.40 ± 0.13^{A}	4.41 ± 0.17^{A}
TP + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.30 ± 0.18^{B}	3.48 ± 0.12^{B}	3.43 ± 0.12^{B}	4.88 ± 0.16^{B}	4.76 ± 0.16^{AB}	4.71 ± 0.11^{BC}	4.54 ± 0.15^{AB}
TP + 2% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.34 ± 0.12^{B}	3.41 ± 0.13^{B}	3.64 ± 0.14^{B}	4.68 ± 0.13^{B}	4.80 ± 0.17^{BC}	4.81 ± 0.15^{BC}	4.87 ± 0.11^{C}
TP + 4% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.39 ± 0.13^{B}	3.40 ± 0.10^{B}	3.68 ± 0.10^{B}	4.63 ± 0.13^{B}	4.60 ± 0.15^{AB}	4.62 ± 0.12^{AB}	4.69 ± 0.11^{ABC}
TP + 2% ginger + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.58 ± 0.16^{B}	3.49 ± 0.14^{B}	3.49 ± 0.13^{B}	4.54 ± 0.15^{B}	4.57 ± 0.14^{AB}	4.67 ± 0.13^{AB}	4.83 ± 0.16^{BC}

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

TABLE 2. LACTIC ACID BACTERIA COUNT OF BIOPRESERVED TOMATO PASTE IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Tomato paste (TP)	0.00 ± 0.00^{A}	5.79 ± 0.24^{C}	6.40 ± 0.21^{D}	7.40 ± 0.13^{D}	8.54 ± 0.25^{E}	10.76 ± 0.34^{F}	11.67 ± 0.32^{E}	14.54 ± 0.12^{D}	17.74 ± 0.59^{D}
TP + 2% ginger	0.00 ± 0.00^{A}	3.82 ± 0.13^{B}	5.30 ± 0.25^{C}	6.93 ± 0.13^{C}	8.65 ± 0.31^{E}	9.54 ± 0.31^{E}	11.92 ± 0.33^{E}	14.60 ± 0.39^{D}	14.96 ± 0.71^{D}
TP + 4% ginger	0.00 ± 0.00^{A}	3.71 ± 0.15^{B}	6.43 ± 0.21^{D}	6.98 ± 0.13^{C}	7.99 ± 0.23^{D}	9.65 ± 0.29^{E}	11.98 ± 0.37^{E}	14.08 ± 0.44^{D}	14.78 ± 0.55^{D}
TP + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.41 ± 0.11^{B}	4.34 ± 0.22^{C}	4.88 ± 0.26^{B}	4.93 ± 0.22^{B}
TP + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.52 ± 0.18^{B}	3.57 ± 0.13^{B}	4.40 ± 0.22^{C}	4.83 ± 0.23^{D}	5.59 ± 0.23^{D}	5.79 ± 0.29^{C}	5.92 ± 0.25^{C}
TP + 2% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.39 ± 0.13^{B}	3.54 ± 0.13^{B}	3.64 ± 0.23^{B}	3.60 ± 0.19^{C}	3.68 ± 0.13^{B}	4.76 ± 0.17^{B}	4.79 ± 0.26^{B}
TP + 4% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.65 ± 0.13^{C}	4.58 ± 0.14^{C}	4.72 ± 0.21^{B}	4.86 ± 0.21^{B}
TP + 2% ginger + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.67 ± 0.22^{A}	3.74 ± 0.11^{A}

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

TABLE 3. YEAST COUNT OF BIOPRESERVED TOMATO PASTE IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Tomato paste (TP)	0.00 ± 0.00^{A}	4.77 ± 0.22^{C}	5.65 ± 0.19^{C}	7.92 ± 0.34^{D}	8.91 ± 0.11^{C}	10.79 ± 0.33^{D}	11.67 ± 0.37^{CD}	12.88 ± 0.34^{B}	13.67 ± 0.45^{C}
TP + 2% ginger	0.00 ± 0.00^{A}	4.51 ± 0.19^{C}	5.78 ± 0.23^{C}	7.62 ± 0.29^{D}	8.93 ± 0.24^{C}	9.91 ± 0.32^{C}	11.93 ± 0.29^{D}	12.64 ± 0.43^{B}	14.98 ± 0.64^{D}
TP + 4% ginger	0.00 ± 0.00^{A}	5.32 ± 0.18^{D}	6.32 ± 0.20^{D}	6.68 ± 0.18^{C}	9.69 ± 0.23^{D}	10.78 ± 0.28^{D}	10.99 ± 0.29^{C}	12.51 ± 0.27^{B}	13.99 ± 0.33^{CD}
TP + 2% garlic	0.00 ± 0.00^{A}	3.65 ± 0.15^{B}	3.59 ± 0.22^{B}	3.62 ± 0.17^{B}	3.64 ± 0.11^{B}	3.57 ± 0.21^{B}	3.61 ± 0.27^{B}	3.66 ± 0.18^{A}	4.54 ± 0.12^{B}
TP + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.44 ± 0220^{B}	3.94 ± 0.14^{A}	4.98 ± 0.23^{B}
TP + 2% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.43 ± 0.19^{B}	3.62 ± 0.26^{B}	3.72 ± 0.11^{B}	3.81 ± 0.15^{A}	3.82 ± 0.17^{A}
TP + 4% ginger + 2% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.39 ± 0.23^{B}	3.54 ± 0.21^{A}	3.87 ± 0.22^{A}
TP + 2% ginger + 4% garlic	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	3.91 ± 0.19^{A}	4.60 ± 0.25^{B}

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

TABLE 4. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM TOMATO PASTE

	Isolates					
Test	B1	B2	B3	B4	B5	B6
Morphology	Rods	Rods	Cocci	Rods	Rods	Rods
Color of growth	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+
Catalase test	–	–	–	–	–	–
Growth at:						
15°C	+	+		–	–	–
45°C	–	–		+	+	+
Production of CO_2	–	+	+	–	+	
Dextran production	–	–	+	–	–	–
Fermentation						
Glucose	+	+	+	–	+	–
Lactose	+	–	–	–	+	+
Arabinose	+	+	–	–	+	–
Trehalose	+	–	+	+	+	+
Salicin	+	–	–	–	–	+
Galactose	+	+	–	–	+	+
Sucrose	+	+	–	+	+	+
Raffinose	+	+	–	–	–	+
Probable identity of organism	***Lactobacillus plantarum***	***Lactobacillus brevis***	***Leuconostoc mensenteroides***	***Lactobacillus delbrueckii***	***Lactobacillus fermentum***	***Lactobacillus acidophilus***

8 weeks of storage (Table 3). Tomato paste samples preserved with 2 or 4% garlic used alone or in combination with 2 or 4% ginger had yeast counts that ranged from 3.82 to 4.60 log cfu/g after 8 weeks of storage.

Identity of Bacteria Isolated in Biopreserved Tomato Paste Samples

The bacterial isolates from the tomato paste samples preserved with garlic and/or ginger were identified to be gram-positive, nonspore forming and catalase negative. The results of biochemical tests and the identification keys reveal that the isolates were *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacilli acidophilus* and *Leuconostoc mesenteroides* as shown in Table 4.

Identity of Yeasts in Biopreserved Tomato Paste

Yeasts isolated from the tomato paste samples were identified as *Saccharomyces cerevisiae*, *Saccharomyces lactis*, *Hansenula anomala*, *Rhodotorula glutinis*, *Rhodotorula flava* and *Rhodotorula rubra* as presented in Table 5.

Titratable Acidity and pH of Biopreserved Tomato Paste Samples

The TTA of tomato paste with 2% ginger as presented in Fig. 1 was fairly stable for the first 4 weeks and increased in most samples until the eighth week of storage. For paste with 4% ginger, a similar trend was observed for the first week and slightly increased at eighth week of storage. In tomato paste with only 2% garlic an increased throughout the storage period was observed while a similar trend was also observed during the first 3 weeks of storage paste with 4% garlic and then decreased to a stable value for the remaining period of storage. For tomato paste samples that contain the combination of 2% garlic and 2% ginger as shown in Fig. 2, the TTA increased during the first 5 weeks of storage followed by a decrease to stable value for the remaining period of storage. Similar trends were observed in samples containing 2% garlic and 4% ginger, as well as in 2% ginger and 4% garlic samples. Increases in TTA were more pronounced in samples with 4% ginger, 2% garlic and in sample with 2% garlic and 2% ginger after 8 weeks of storage at ambient temperature.

On the other hand, tomato paste with 4% garlic, sample with 2% garlic and 4% ginger and sample with 2% ginger and 4% garlic exhibited no increase in TTA after 8 weeks of storage.

The pH of tomato paste with only 2% ginger (Fig. 2) was stable for first two weeks and decreased from the third to sixth week. In samples containing 4% ginger, the pH value was stable from the first 3 weeks and then decreased to a stable value for the remaining period of storage. The pH samples containing only 2% and 4% garlic decreased throughout the storage period, respectively. The

TABLE 5. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF YEAST ISOLATES FROM TOMATO PASTE

	Isolates					
Test	Y1	Y2	Y3	Y4	Y5	Y6
Morphology:						
Pellicle	–	+	–	–	–	–
Colour	White		Pink	Red	Cream	Red
Shape	Ovoid	Cylindrical	Ovoid	Ovoid		Ovoid
Reproduction	Budding	Budding	Budding	Budding	Budding	Budding
Fermentation						
Glucose	+	+	–	–	+	–
Sucrose	+	+	–	–	+	–
Maltose	+	+	–	–	–	–
Galactose	+	+	–	–	+	–
Raffinose	+	+	–	–	+	–
Lactose	–	–	–	–	+	–
Sugar assimilation						
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Raffinose	–	–	–	–	+	–
Lactose	–	–	–	+	+	–
Nitrate assimilation	–	+	–	–	–	–
Identity of Organisms	***Saccharomyces cerevisiae***	***Hansenula anomala***	***Rhodotorula glutinis***	***Rhodotorula flava***	***Saccharomyces lactis***	***Rhodotorula rubra***

pH of tomato paste samples with 2% garlic and 2% ginger (Fig. 2) both decreased throughout the storage period while a similar trend was recorded for samples containing 2% garlic and 4% ginger as well as 2% ginger and 4% garlic samples.

Lycopene Concentration of Tomato Paste with Garlic and/or Ginger

The lycopene concentration of tomato paste as shown in Table 6 reduced upon addition of ginger, garlic and ginger-

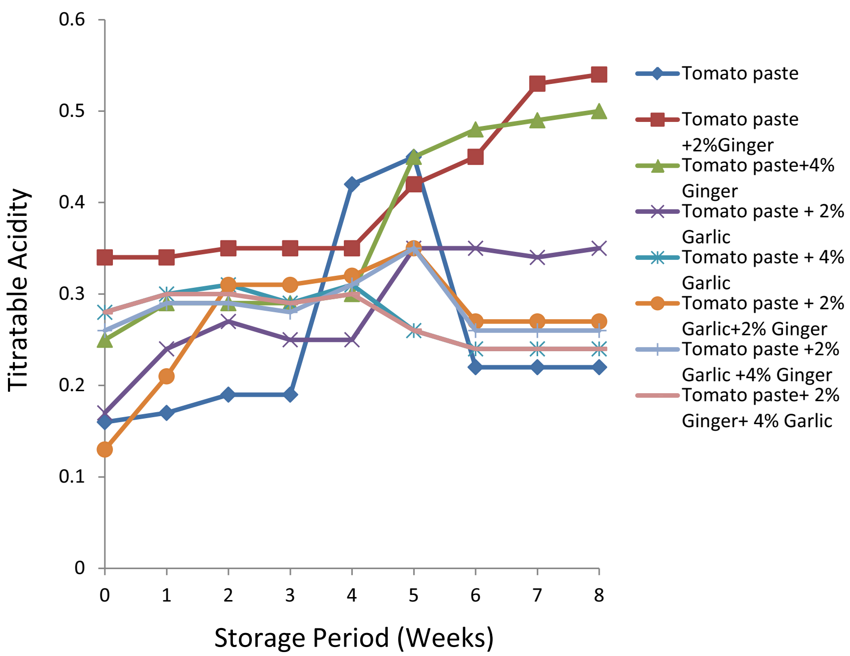

FIG. 1. TITRATABLE ACIDITY OF BIOPRESERVED TOMATO PASTE GARLIC AND/OR GINGER

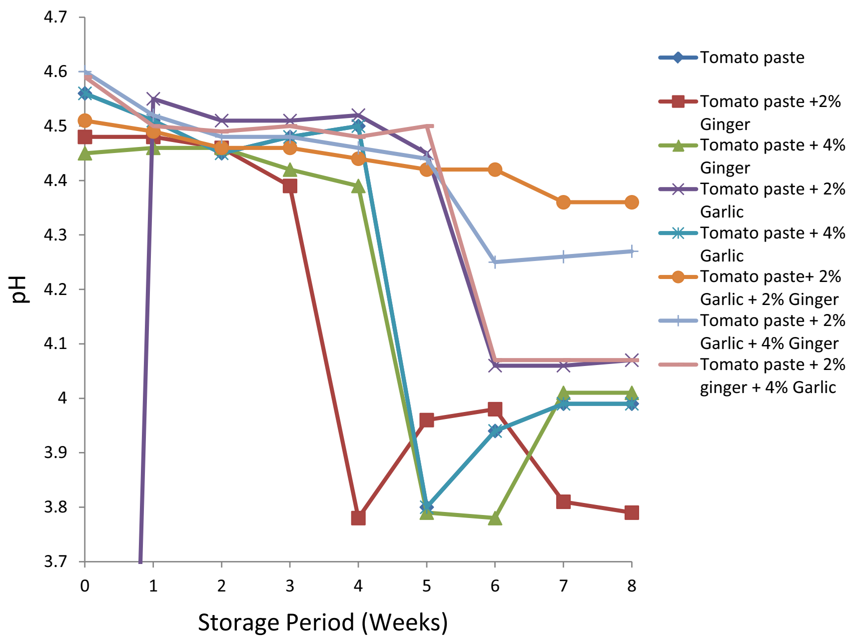

FIG. 2. pH OF BIOPRESERVED TOMATO PASTE WITH GARLIC AND/OR GINGER

garlic blends. Samples without biopreservatives and samples containing only ginger at 2 and 4% exhibited decreases in lycopene content during storage with the greatest reduction (35.06%) recorded for samples without preservatives. However, increases from 5.87 to 9.02 mg/100 g for tomato paste containing 2% garlic and 2% ginger, 5.25 to 8.62 mg/100 g for tomato paste samples containing 2% garlic and 4% ginger and 7.60 to 9.08 mg/100 g for tomato paste samples containing 2% ginger and 4% garlic were observed during storage. For tomato paste samples containing only 2% garlic and 4% garlic, the lycopene concentration showed similar trends from 6.97 to 8.89 mg/100 g and 7.14 to 9.06 mg/100 g, respectively.

Water Activity during Storage

The water content of the control and biopreserved samples ranged from 92.94 to 88.46% over the period of storage (Table 7). There was an increase in water content of control sample from an initial level of 91.89% to 92.85% after 8 weeks of storage. Other samples treated with 2 and 4% ginger also exhibited marginal increases in water content over a similar period of storage. Samples containing between 2 and 4% garlic either alone or in combination with ginger had lower water content after storage for 8 weeks. In all cases, the decrease or increase in water was not up to 5% over the initial water content. Table 7 gives the results of changes in total solids of the control and treated tomato paste samples. The range of the total solids was 7.05–11.54%. Control sample and samples treated with 2–4% ginger exhibited marginal decrease in total solids over the storage period. Samples containing 2–4% garlic either wholly or partly showed increase in total solids during storage. Garlic powder evidently increased total solids.

TABLE 6. LYCOPENE CONTENT OF TOMATO PASTE IN STORAGE (MG/100G OF SAMPLE)

Sample	Week 0	Week 2	Week 4	Week 6	Week 8
Tomato paste (TP)	9.07 ± 0.06^{a}	8.92 ± 0.03^{a}	8.47 ± 0.08^{b}	7.88 ± 0.04^{c}	5.89 ± 0.16^{d}
TP + 2% ginger	9.14 ± 0.04^{a}	9.07 ± 0.06^{a}	8.80 ± 0.14^{b}	8.67 ± 0.03^{bc}	8.55 ± 0.07^{b}
TP + 4% ginger	8.96 ± 0.03^{b}	8.89 ± 0.04^{b}	8.52 ± 0.06^{b}	8.44 ± 0.04^{b}	8.43 ± 0.10^{c}
TP + 2% garlic	6.98 ± 0.07^{d}	7.68 ± 0.28^{c}	8.39 ± 0.08^{b}	8.52 ± 0.04^{b}	8.89 ± 0.71^{a}
TP + 4% garlic	7.14 ± 0.06^{c}	7.87 ± 0.04^{c}	8.57 ± 0.04^{ab}	8.81 ± 0.01^{ab}	9.06 ± 0.01^{a}
TP + 2% ginger + 2% garlic	5.87 ± 0.09^{e}	6.22 ± 0.03^{d}	7.24 ± 0.06^{c}	7.71 ± 0.01^{b}	9.02 ± 0.14^{a}
TP + 4% ginger + 2% garlic	5.25 ± 0.07^{f}	5.45 ± 0.03^{e}	6.64 ± 0.05^{d}	7.74 ± 0.04^{c}	8.62 ± 0.11^{a}
TP + 2% ginger + 4% garlic	7.60 ± 0.04^{c}	7.77 ± 0.07^{c}	8.54 ± 0.18^{b}	8.55 ± 0.08^{b}	9.08 ± 0.07^{a}

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

TABLE 7. MOISTURE CONTENT AND TOTAL SOLIDS OF TOMATO PASTE IN STORAGE (%)

Storage period (Week)	Characteristic	Tomato paste	Tomato paste + 2% ginger	Tomato paste + 4% ginger	Tomato paste + 2% garlic	Tomato paste + 4% garlic	Tomato paste + 2% garlic + 2% ginger	Tomato paste + 2% garlic + 4% ginger	Tomato paste + 2% ginger + 4% garlic
0	Moisture content	91.89 ± 0.02^{d}	92.66 ± 0.02^{d}	92.78 ± 0.02^{ab}	92.81 ± 0.02^{a}	91.78 ± 0.02^{a}	91.67 ± 0.02^{a}	92.59 ± 0.02^{a}	92.05 ± 0.02^{a}
2	Moisture content	92.03 ± 0.02^{c}	92.74 ± 0.02^{bc}	92.76 ± 0.02^{ab}	90.15 ± 0.02^{b}	89.95 ± 0.02^{b}	90.72 ± 0.02^{b}	91.55 ± 0.02^{b}	91.37 ± 0.02^{b}
4	Moisture content	92.65 ± 0.01^{b}	92.79 ± 0.01^{bc}	92.85 ± 0.01^{ab}	89.04 ± 0.01^{c}	88.84 ± 0.01^{c}	90.63 ± 0.01^{c}	89.79 ± 0.01^{c}	88.79 ± 0.01^{c}
6	Moisture content	92.83 ± 0.02^{a}	92.82 ± 0.02^{b}	92.92 ± 0.02^{ab}	88.80 ± 0.02^{d}	88.73 ± 0.02^{d}	89.72 ± 0.02^{d}	89.73 ± 0.02^{c}	88.54 ± 0.02^{d}
8	Moisture content	92.85 ± 0.01^{a}	92.94 ± 0.01^{a}	92.97 ± 0.01^{a}	88.64 ± 0.01^{e}	88.49 ± 0.01^{e}	89.75 ± 0.01^{d}	89.66 ± 0.01^{c}	88.46 ± 0.01^{d}
0	Total solids	8.11 ± 0.02^{a}	7.34 ± 0.02^{c}	7.22 ± 0.02^{c}	7.19 ± 0.02^{c}	8.22 ± 0.02^{c}	8.33 ± 0.02^{c}	7.41 ± 0.02^{e}	7.95 ± 0.02^{b}
2	Total solids	7.97 ± 0.02^{b}	7.26 ± 0.02^{b}	7.24 ± 0.02^{b}	9.85 ± 0.02^{b}	10.05 ± 0.02^{b}	9.28 ± 0.02^{b}	8.45 ± 0.02^{d}	8.63 ± 0.02^{b}
4	Total solids	7.35 ± 0.01^{c}	7.21 ± 0.01^{a}	7.15 ± 0.01^{a}	11.16 ± 0.01^{a}	11.16 ± 0.01^{a}	9.37 ± 0.01^{b}	10.21 ± 0.01^{c}	11.21 ± 0.01^{a}
6	Total solids	7.17 ± 0.02^{cd}	7.19 ± 0.02^{a}	7.08 ± 0.02^{a}	11.20 ± 0.02^{a}	11.27 ± 0.02^{a}	10.28 ± 0.02^{a}	10.27 ± 0.02^{b}	11.46 ± 0.02^{a}
8	Total solids	7.05 ± 0.03^{d}	7.06 ± 0.03^{a}	7.03 ± 0.03^{a}	11.36 ± 0.03^{a}	11.51 ± 0.03^{a}	10.25 ± 0.03^{a}	10.34 ± 0.03^{a}	11.54 ± 0.03^{a}

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

DISCUSSION

Microbial Load of Biopreserved Tomato Pastes during Storage

In comparison with control sample, samples treated with 2 and 4% ginger had slightly lower TVC, LAB, yeast and mould count after storage of 8 weeks probably because of the antimicrobial gingerols and shogaol components of ginger (Wilkinson 2003; Atai *et al.* 2009). Similar observations have been reported when ginger extracts were added to fermented meat sausage (Al-Jalay *et al.* 1987) and meat products (Ziauddin *et al.* 1995). It was also reported that ginger extract treatment extended the shelf life of West African soft cheese for 15 days (Belewu *et al.* 2005).

The no or low microbial growth on tomato paste with garlic during storage may be due to the antimicrobial properties of *Allium* extracts which comprises of allicin, thiosulfonates and other compounds (Ankri and Mirelman 1999; Harris *et al.* 2001). It was also reported by Yadav *et al.* (2002) that aqueous garlic extract at 4% was an effective preservative in extending shelf life of minced chicken meat during refrigerated storage. Furthermore, chicken gizzard snacks treated with 4% garlic extract remained microbiologically safe up to 14 days of refrigerated storage (Yadav and Singh 2004). Ajayi (2013) reported that canned tomato paste should not exceed log 6.3 in its microbial load. It can be said that the garlic when used at 2% or more either alone or in combination with ginger effectively preserved the tomato paste for the duration of 8 weeks. The uses of garlic and ginger in foods have been recognized as safe and with no limitation other than in accordance with good manufacturing practices (FAO/WHO 2014).

Identity of Bacteria and Yeast Isolates in Biopreserved Tomato Pastes during Storage

LAB associated with biopreserved tomato paste samples investigated in this study agrees with the findings of the previous studies. *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Leuconostoc mesenteroides* had been isolated from tomatoes naturally fermented under partial anaerobic conditions (Beltrán-Edeza and Hernández-Sánchez 1989; Stratiotis and Dicks 2002) from ketchup, sauerkraut and kimchi, which have garlic and ginger as ingredient, and pickled vegetable (Doyle 2007). *Leuconostoc mesenteroides*, *Lactobacillus brevis* and *Lactobacillus plantarum* were also found to be the major acid producers in vegetable fermentation (Sven-Olof 2008). They are obligately heterofermentative and occasionally strains can exhibit pseudocatalase activity especially if grown under glucose limitation (Wood and Holzapfel 1995;

TABLE 8. SENSORY EVALUATION OF TOMATO PASTE SAMPLES

Samples	Color	Appearance	Aroma	Taste	Overall acceptability	Rank
Control	7.38[a]	7.08[c]	5.85[d]	6.77[a]	7.08[a]	1
4% garlic	7.38[a]	7.31[a]	5.92[c]	4.92[d]	6.53[b]	2
2% ginger	7.08[b]	6.76[d]	6.08[a]	5.69[b]	6.38[c]	3
2% garlic	7.08[b]	7.15[b]	6.00[b]	4.84[e]	6.23[d]	4
2% ginger + 2% garlic	6.08[c]	5.77[e]	5.38[e]	4.84[e]	6.00[e]	5
4% ginger + 4% garlic	5.85[d]	5.31[g]	5.31[f]	5.00[c]	5.30[f]	6
2% ginger + 4% garlic	5.46[e]	5.26[h]	4.92[g]	4.61[g]	5.00[g]	7
4% ginger + 2% garlic	5.20[f]	5.38[f]	5.38[e]	4.69[f]	4.77[h]	8

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$).

Fu and Mathews 1999). *Lactobacillus delbrueckii* was found capable of utilizing tomato juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment (Yoon *et al.* 2004).

Titratable Acidity and pH of Biopreserved Tomato Paste Samples in Storage

In agreement with the findings of this study, it was reported that the TTA of tomato paste stored at different temperatures exhibited a gradual increase throughout the storage period. However, rise in acidity was more at higher temperature than at lower temperature. Highest increase (18.39%) was recorded in samples stored at 25C followed by 6C storage conditions (10.34%) while least increase (7.47%) occurred in samples stored at −10C (Muhammad *et al.* 2010). Codex (2007) recommends pH of less than 4.6.

Increase in TTA of tomato paste may be due to organic acids produced by LAB and it may also be due to the oxidation of alcohol and aldehyde during processing. It has been reported that the amount of organic acids produced by LAB is influenced by storage temperature, the higher the temperature, the greater the increase in acidity (Gould 1992). This is possible because of the increase in microbial growth and consequent increase in acid production (Prescott *et al.* 2002). The increase in TTA may also be due to the utilization of the reducing sugars leading to production of organic acid. The increase in acidity usually exhibits an inhibitory effect on the growth of pathogens (Molin 2007). The range of TTA recorded for the control sample and samples treated with ginger and garlic were within the less than 7% recommended for tomato paste (WFP 2011).

Lycopene Concentration of Tomato Paste with Garlic and/or Ginger

The study has shown that garlic and ginger had a synergistic effect on lycopene content of biopreserved tomato paste. Application of the two on tomato paste is advantageous as it increases the much-desired lycopene (Olaniran *et al.* 2013). The increase in lycopene concentration during storage may be due to the gradual increase in total soluble solid as a result of the slight decrease in total soluble solid of the tomato paste (Nguyen and Schwartz 1998). It may also be due to the *trans* and *cis* isomerization in the presence of fat and re-isomerization during storage (Shi *et al.* 2002). Changes in lycopene content in tomato paste during storage have been reported (Anguelova and Warthesen 2000; Takeoka *et al.* 2001; Dewanto *et al.* 2002; Hackett *et al.* 2004; Seybold *et al.* 2004; Goula *et al.* 2006; Toor and Savage 2006). Dietary intake of moderate amounts of lycopene (5–20 mg) has been associated with increase cellular defenses and help to prevent oxidative damage to cellular components, cardiovascular disease, cancers of the prostate and gastrointestinal tract (Gann *et al.* 1999; Agarwal and Rao 2000; Kaur and Kapoor 2002; Wong *et al.* 2006).

Water Activity of the Tomato Paste Samples

Water activity of the tomato paste samples (untreated and treated) was fairly stable over the period of storage. Addition of these biopreservatives (ginger/and or garlic) used at 2–4% did not produce detectable unpleasant effect on the water activity during 8 weeks of study (Table 7).

Sensory Assessment of the Tomato Samples

As presented in Table 8, the tasters found the color of tomato paste treated with 4% garlic comparable with control sample. In terms of other sensory quality parameters evaluated, the same sample was found agreeable. However, other tomato paste samples especially the ones treated with a combination of garlic and ginger was rated low in comparison with the control tomato paste.

CONCLUSION

These studies have shown that garlic and ginger added at 2–4% w/w could be used as effective biopreservatives in tomato paste for not less than 8 weeks. The combination of garlic and ginger as biopreservatives was effective in reducing bacteria and yeast counts. Garlic at 2% and 4% alone were more effective against LAB and yeast load than ginger at the same concentration.

Tomato paste treated with 4% garlic was effectively preserved against microbial deterioration, lowered chemical characteristics and was even found acceptable to the tasters. In some countries in Africa, where power is in limited supply, the addition of 2–4% garlic and or ginger to tomato paste can be of assistance in enhancing its shelf life particularly when tomato is in season.

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