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Biopreservative Efficacy of Some Common Spices Sold in Dakingari Market on Hot- Break Locally Processed Tomato Paste

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Abstract:
The research investigated the biopreservative efficacy of some common spices sold in dakingari market as substitutes for chemical preservatives on hot-break locally processed tomato paste (Platinum 7 F1 variety). After concentration, the paste was divided into thirty one (31) portions labeled A–O2. The spices were added to tomato paste in the following proportion of ginger; garlic; onion; pepper; ginger and garlic; ginger and onion; ginger and pepper; garlic and onion; garlic and pepper; onion and pepper; ginger, garlic and onion; ginger, garlic and pepper; garlic, onion and pepper; onion, pepper and ginger; ginger, garlic, onion and pepper 3 and 5 percent. Each mixture (450g) was evenly homogenized, filled up into air-tight Mayonnaise jars and stored at room temperature over a period of 9 weeks.
Pure cultures were obtained from distinct colonies by repeating streaking on fresh agar plates and subjected to microscopic examination and biochemical tests such as Gram staining. Microbial examination and yeast counts of the samples were enumerated on a weekly basis while the pure microbial isolates were identified. Results showed that those without fungi growth are; Sample G (ginger and onion), Sample O (onion, pepper and ginger), Sample D (onion), Sample E (pepper), Sample M (ginger, garlic and pepper) and Sample L (ginger, garlic and onion), Sample I (garlic and onion) (3 and 5%). The microorganisms were identified as organism: *Rhizopus orazae, Penicillium citrinum, Aspergillus micronesiensis, Aspergillus flavus, Aspergillus niger , Phoma multristrata, Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc mensenteroides, Lactobacillus acidophilus, Lactobacillus fermentum.*
The study concluded only Sample D (onion) and Sample N (garlic, onion and pepper) that has no bacteria growth (3 and 5%).The study concluded that combined samples with combination of garlic or ginger (3 and 5%) suitably preserved tomato paste for 9 weeks without deterioration. The use of natural products such as ginger, onion and garlic as natural preservatives could significantly improve tomato utilization in household production in Dakingari and Nigeria at large where refrigeration is greatly hampered by erratic power supply. Further investigation for other biopreservatives is recommended to ascertain the safety consumption of tomato paste of the people in the study area and the need to look into more new natural bio-preservatives which can promote human health besides acting as food preservative.

Keywords: Tomato paste, Biopreservative, Spices, Food, Dakingari.

1.0 Introduction

Tomato (*Solanum lycopersicum*) is the most consumed vegetable crop around the world with global production of 182 million tonnes (FAO, 2018). It is characterized by its unique colour, flavour, taste, antioxidants content, and other valuable nutrients. Tomato is an important part of the human diet that can be consumed either fresh or processed; it is mainly used as an indispensable component in various types of foods (Banat *et al.*, 2002). 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). With the ever increasing consumption of tomato paste, it has attracted the attention of many researchers to investigate bioavailability in terms of human health (Baysal et al., 1990).Because of their healthy micronutrients and accessibility throughout the year, demand for tomato and tomato products is increasing for years (Abushita et al., 1997; Campbell et al., 2007). In recent years, interest towards phenolic compounds has increased, especially for flavonoids which have many biological effects such as anti-inflammatory, anti-allergic and antibacterial beside its disease preventive effects (Hvattum, 2002). Consumption of tomato and tomato products may decrease the risk of some types of cancer and chronic diseases (Balestrieri et al., 2004; Karakaya and Yılmaz, 2007), may prevent liver damage, which results from alcohol consumption and also age related molecular degeneration (Tapiero et al., 2004).

Nigeria ranks as the 16th largest tomato producing nation in the world and has the comparative advantage and potential to lead the world in tomato production and exports (FAO, 2010). The production of tomatoes in Nigeria in 2010 was about 1.8 million metric tonnes, which accounts for about 68.4% of West Africa, 10.8% of Africa's total output and 1.28% of world output (FAO, 2010). Unfortunately, the country still experiences deficiency in critical inputs, lack of improved technology, low yield and productivity, high postharvest losses and lack of processing and marketing infrastructure. The demand for tomato and its by-products far outweighs the supply. Nigeria has a large market for processed tomato products. Apart from the Nigerian market, the advantage of the trade liberalization in the West African market could be used to enhance the sale of processed tomato products in this region. At present, a significant percentage of processed tomato products used in Nigeria are imported, resulting in unnecessary pressure on foreign exchange reserve (Ugonna et al., 2015).

Food is so important for the survival, food preservation is the oldest technique used by human beings to avoid spoilage. Preservation is mainly done for some reasons like, to preserve the natural characteristics of food, to preserve the appearance. Food preservatives are categorized as natural, chemical and artificial. But still there are certain preservatives especially chemical preservatives in foods that are harmful if taken more than the prescribed limits. It will cause severe problems like allergies, skin rashes, asthma, kidney and liver damage, cancer, genetic defects etc. Biopreservatives are commonly used in food products to satisfy the increasing demand of consumers with increasing advancement in food and technology. Thus, as a result food industry is using naturally produced preservatives to increase the shelf life of product without any new technology. Biopreservatives are the new alternatives derived from natural sources to preserve and enhance the keeping quality of food and well suited for food application with increasing demand of consumers for chemical free foods. To extend the shelf life of products by use of natural preservatives or controlled microbiota and/or antimicrobial compounds

obtained from microbes is referred to as bio-preservation. The natural food preservatives preserve the food by lowering the pH value, altering water activity (aw) and settling the redox potential of the product (Alzoreky and.Nakahara , 2003).

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). The perishable nature of tomatoes and the insufficient storage facilities may increase the postharvest losses, which can be avoided by processing the surplus produce (Abano *et al.*, 2012). Therefore, keeping the nutritional value and sensory quality of tomatoes by improving processing conditions is of vital importance. Paste, dried vegetables, canned foods, tomato juice and similar products provide a variety of products for consumers in the food industry and make it possible to consume these sensible products out of the normal production period (Buyukbay et al., 2009). The foods with chemical preservatives are now being neglected by the cosumers and people they prefer products which are generally recognized as safe (GRAS) that are naturally biopreservative and medicinal.

1.1. Study Area

Dakingari town is the headquarter of Suru Local Government area of Kebbi State. Suru Local Government has a total population of 202,400 and Dakingari town has about 12600 residents acording to 2016 census. The area is located between longitude $4^{0}03'$ 51" E and latitude $11^{0}38'$ 51" N. The famous Hausa and Fulani tribes of Northern Nigeria dominate the town. The town is homogenous with predominantly Islamic belief. Rainfall starts late May – June and ends early (October – November), and 7 – 8 months dry season (from October to May). Thus, the rainfall is erratic in nature. Over 50 percent of the inhabitants practice one form of agriculture or another (Fishing, crop farming, and animal husbandry etc.)The area is intensively cultivated and has a high agricultural potential.

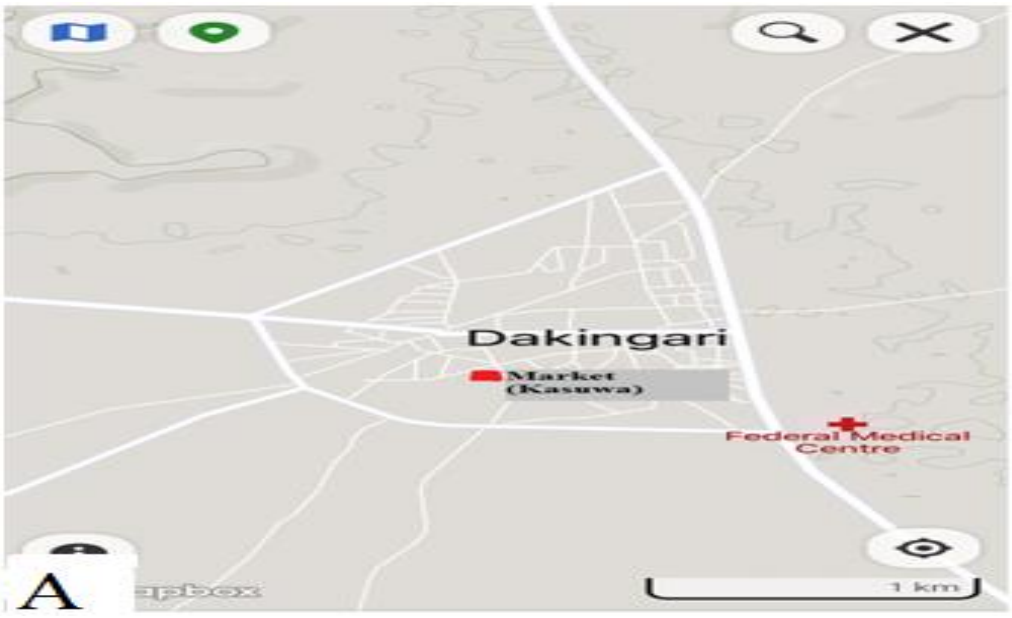

Figure 1: Map of Dakingari (Extracted from Google Map) indicating sampling point **(A)**

2.0 Materials and Methods

The sample for this research is freshly harvested sound ripe tomato, ginger rhizomes, garlic bulbs, onion bulbs and red pepper fruits were purchase from a local market in Dakingari, Kebbi state Nigeria.

2.1. Sampling

Fresh tomatoes fruits was sorted for wholesomeness, intense red colour and to be washed and allowed to drain. Blender was used to grind till smooth.The slurry was concentrated to tomato paste of 20–25% total solids by cooking in stainless steel pot (cook till water is totally dried). After concentration, the paste was divided into thirty one (31) portions labeled A–O2. Fresh garlic bulbs, ginger rhizomes, onion bulbs and red pepper were cleaned, peeled and 450g each was dried in hot-air oven (Gallenkamp, UK) at 65C for 12 h and grind with an Industrial Blender grinder. The ginger, garlic, onion and pepper powders will be sieve through mesh size of 50 to 60 to remove the shafts and residues which will be subsequently discard. The spices were added to tomato paste in the following proportion of ginger; garlic; onion; pepper; ginger and garlic; ginger and onion; ginger and pepper; garlic and onion; garlic and pepper; onion and pepper; ginger, garlic and onion; ginger, garlic and pepper; garlic, onion and pepper; onion, pepper and ginger; ginger, garlic, onion and pepper 3 and 5 percent. Each mixture of 450g (The samples are shown in figure 2.2 A) was evenly homogenized, filled up into air-tight Mayonnaise jars and stored at room temperature over a period of 9 weeks. The samples are shown in figure 2.1 (A).

2.2. Microbiological Evaluation

The total colony count and yeast/mould count will be determined. Furthermore, the microbes associated with the tomato paste during storage will also be isolated and identified.

2.3. Enumeration of Microbes

Serial dilution will be carried out by mixing 5.0 g of tomato paste sample (The samples are shown in figure 2.2 B) with 5.0 ml of distill water to obtain 1−1 dilution. From this, subsequent dilutions was be made serially until the desired level of dilution is 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 (The samples are shown in figure 2.1 B). Other plates of diluted samples also received 20 ml of each of potato dextrose agar for fungi colony count, respectively. All the plates will be poured in duplicates (3 and 5 mls of added spices) and incubate in an inverted position in incubators at 37C for 24 and 72 hrs, respectively, for bacterial and fungal colony count, respectively. Microbial load was determined by counting distinct colonies with the aid of a colony counter. Plates with 1–50 colonies were reckoned with.

2.4. Characterization and Identification of Microbes

Pure cultures will be obtained from distinct colonies by repeating streaking on fresh agar plates and subjected to microscopic examination and biochemical tests.

Plate 2.1: Plate **(A):** Fresh tomato variety (Platinum F1) sold in Dakingari market, Plate **(B)** : Fresh tomato paste with added spices (450g), Plate **(C)** : petridishes inoculated for microbial culturing from preserved samples.

Plate 2.2: Plate **(A):** Mass Measurement of tomato paste concentrates combine with spices (450.00g), Plate **(B):** Mass of tomato paste sample for Serial dilution Serial dilution (5.00g).

3.0 Results Analysis and Discussion

Table 3.1 Morphological Characteristics of Fungal Isolates from Tomato Past

S/N	Sample Code	No. of Colonies Count	Colony Colour	Organism Morphology	Identity of Organisms
1	A	10,2,4	Black,Green and milk	Mycelia	*Rhizopus orazae, Penicillium citrinum, Aspergillus micronesiensis*
2	B	30	Green	Hypae	*Aspergillus flavus*
3	C	1,1,1	Black and greenish blue	Hypae	*Aspergillus flavus, , Aspergillus niger Penicillium citrinum*
4	D	-	-	-	-
5	E	-	-	-	-
6	F	2	Black	Hypae	*Phoma multristrata*
7	G	-	-	-	-
8	H	1	Black	Mycelia	*Rhizopus orazae*
9	I	2	Black	Mycelia	*Phoma multristrata*
10	J	2,1,1	Black, White and Milk	Hypae	*Phoma multristrata, Penicillium citrinum, Aspergillus spp*.
11	K	1,1	Red and Green	Oval shape,	Un identified spp.

				Mycelia	*Aspergillus flavus*
12	L	-	-	-	-
13	M	-	-	-	-
14	N	5,1,1	Black	Hypae	*Rhizopus orazae, Aspergillus flavus,* *Penicillium citrinum*
15	O	-	-	-	-
	P	1, 2,1	Black and Green	Mycelia	*Aspergillus flavus,Penicillium citrinum,* *Aspergillus niger*
17	A2	1	milk	Mycelia	*Rhizopus orazae, Penicillium citrinum,* *Aspergillus micronesiensis*
18	B2	3	Green	Hypae	*Aspergillus flavus*
19	C2	1,1,1	Black and Greenish blue	Hypae	*Aspergillus flavus, Aspergillus niger* *Penicillium citrinum*

20	D2	-	-	-
-				
21	E2	-	-	-
-				
22	F2	1	Black	Hypae
Phoma multristrata				
23	G2	-	-	-
-				
24	H2	-	-	-
-				
25	I2	1	Black	Mycelia
Phoma multristrata				
26	J2	1	Black	Hypae
Phoma multristrata,				
27	K2	2	Red and Green	Oval shape,
Un identified spp.				
28	L2	-	-	-
29	M2	-	-	-
30	N2	2	Black	Hypae
Rhizopus orazae				

31	O2	-	-	-
-				

Table 3.1 shows the morphological characteristics of fungal isolates from tomato paste. Serial dilution will be carried out by mixing 5.00 g of tomato paste sample with 5.00 ml of distill water to obtain 1−1 dilution. From this, subsequent dilutions will be made serially until the desired level of dilution is obtained. From each dilution, 1.0 ml will be introduced into sterile Petri dish before 20 ml each of molten potato dextrose agar for fungal growth. Plates will be incubated at 28C ± 2C for fungi for 3 days. Microbial load was determined by counting distinct colonies with the aid of a colony counter. Pure cultures were obtained from distinct colonies by repeating streaking on fresh agar plates and subjected to microscopic examination.

Fungal colony counts of the samples were enumerated on a weekly basis while the pure microbial isolates were identified using microbial atlas. Results showed that those without fungi growth are; Sample O (onion, pepper and ginger) which is in conformity with the statement of Tyler 1994, that 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. *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) and which is similar to Sample D (onion) that shows strong antifungal properties. 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) and this is similar with the findings in this study where Sample G (ginger and onion), Sample E (pepper), Sample M (ginger, garlic and pepper) and Sample L (ginger, garlic and onion), Sample I (garlic and onion) (3 and 5%) also shows strong antifungal properties. Ajoene, a garlic-derived sulfur-containing compound, demonstrated antimicrobial activity .Ajoene also inhibited yeast growth at concentrations below 20 µg/mL (Naganawa *et al*. 1996; Ankri and Mirelman 1999; Singh and Agrawal 1988; Harris *et al*. 2001). Other studies documented that garlic extracts had fungicidal effects against *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis* and *Trichosporum*. 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 . This is similar to the findings in this study that concluded that combined samples with combination of garlic or ginger (3 and 5%) suitably preserved tomato paste for 9 weeks without deterioration . The fungal species identified are: *Rhizopus orazae*, *Penicillium citrinum, Aspergillus micronesiensis, Aspergillus flavus, Aspergillus niger , Phoma multristrata.*

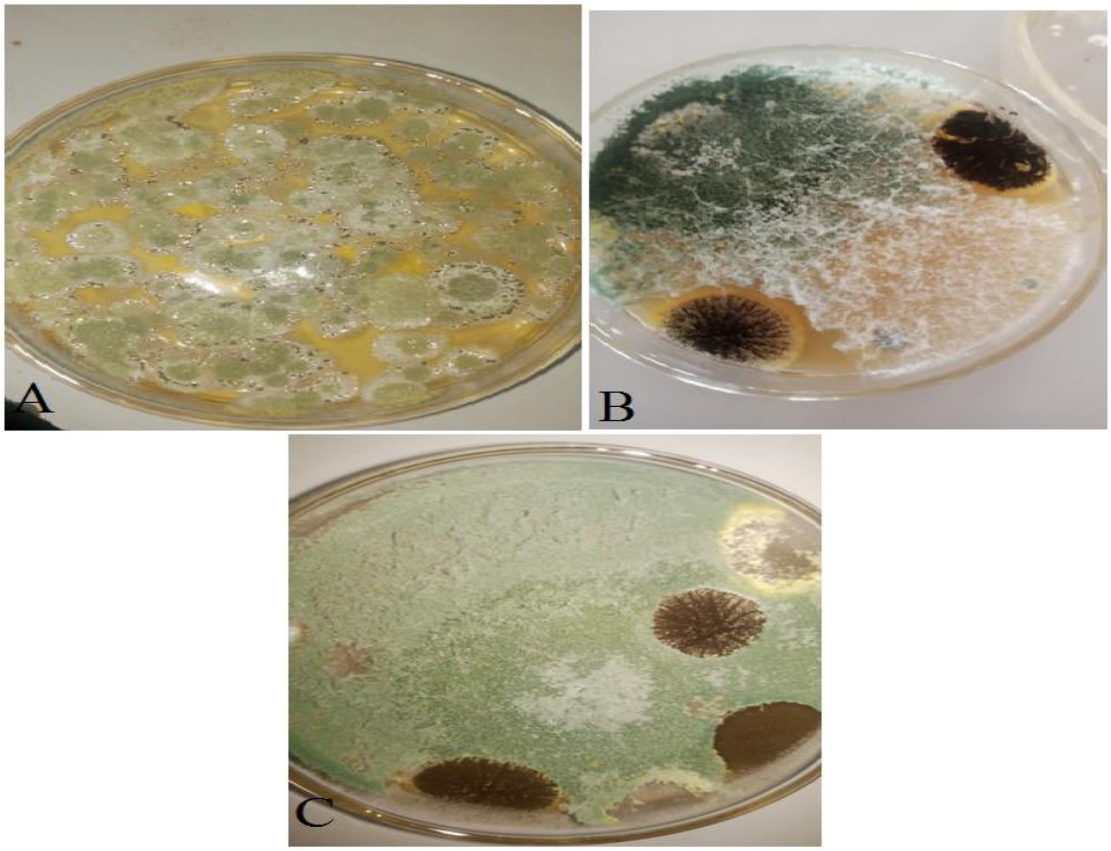

Plate 3.1: Plate **A (sample B); Plate B (sample C); Plate C (sample P)**

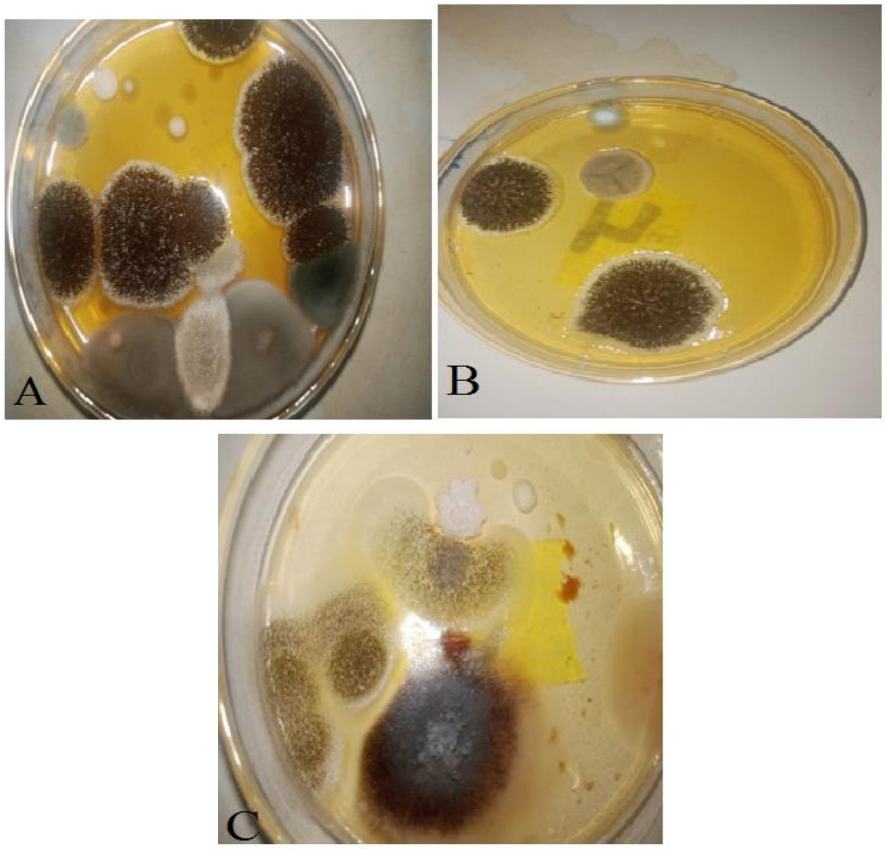

Plate 3.2: Plate **A (sample N); Plate B (sample J); Plate C (sample A)**

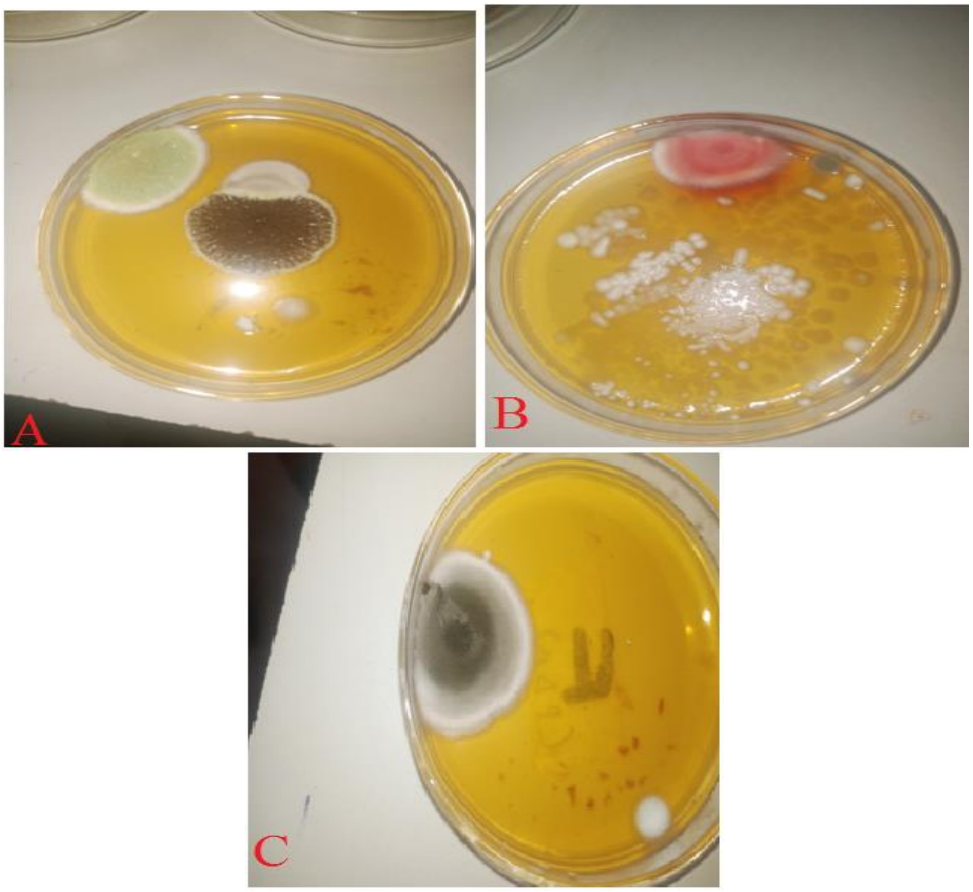

Plate 3.3: Plate **A (sample H); Plate B (sample K); Plate C (sample F)**

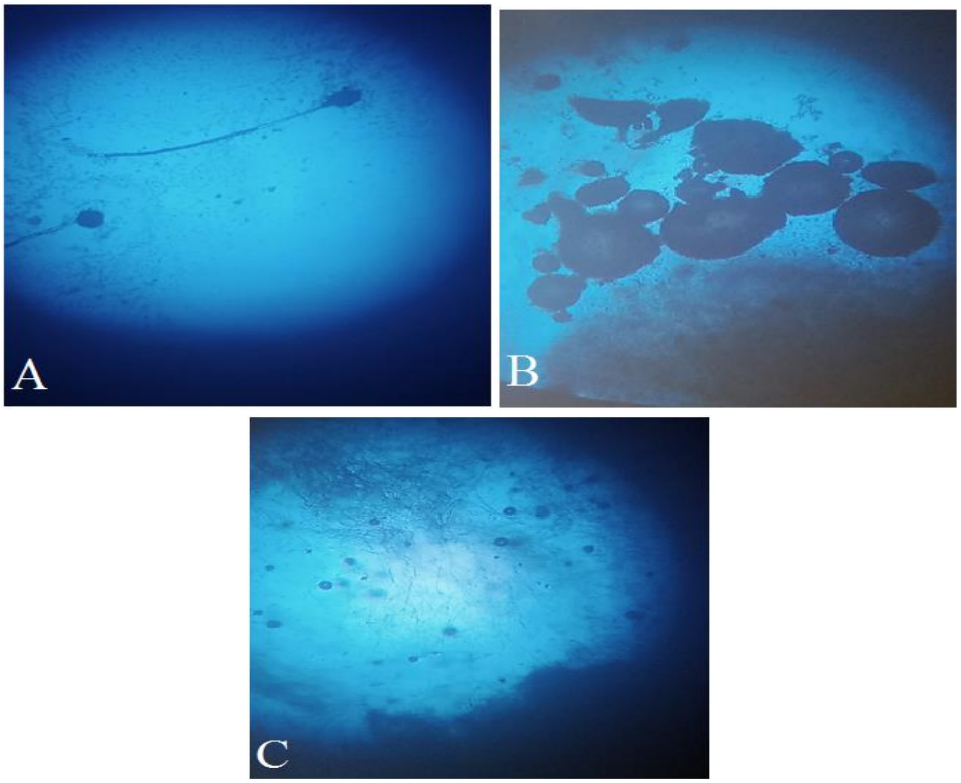

Plate 3.4: Plate **A (sample N mycelium); Plate B (sample N spores); Plate C (sample N spores and hypae)**

Table 3.2 Morphological and Biochemical Characteristics of Bacteria Isolates from Tomato Paste

TEST	ISOLATES				
	SPP. 1	SPP.2	SPP.3	SPP.4	SPP.5
Morphology	Cocci	Rods	Rods	Rods	Rods
Color of growth	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+
Fermentation					
Glucose	+	–	+	+	+
Lactose	–	–	+	+	–
Arabinose	–	–	+	+	+
Trehalose	–	+	+	+	+
Salicin	–	–	–	–	–
Galactose	+	–	–	+	+
Sucrose	–	+	+	+	+
Raffinose	+	–	–	–	–
Probable identity of					

Organism		***Lactobacillus***		
Lactobacillus	***Leuconostoc***	***Lactobacillus***	***Lactobacillus***	
		plantarum		***brevis***
mensenteroides	***acidophilus***	***fermentum***		

Table 3.2 shows the Morphological and Biochemical Characteristics of fungal isolates from tomato paste. Serial dilution will be carried out by mixing 5.00 g of tomato paste sample with 5.00 ml of distill water to obtain 1−1 dilution. From this, subsequent dilutions will be made serially until the desired level of dilution is obtained. From each dilution, 1.0 ml will be introduced into sterile Petri dish before 20 ml each of molten Nutrient agar will be added to obtain TVC. Other plates of diluted samples also will receive 20 mL of each of All the plates will be poured in duplicates and incubate in an inverted position in incubators at 37C for 24 and 72 hrs for bacterial for 3 days. Microbial load was determined by counting distinct colonies with the aid of a colony counter. Pure cultures were obtained from distinct colonies by repeating streaking on fresh agar plates and subjected to microscopic examination.Bacterial colony counts of the samples were enumerated on a weekly basis while the pure microbial isolates were identified using microbial atlas. Pure cultures will be obtained from distinct colonies by repeating streaking on fresh agar plates and subjected to microscopic examination and biochemical tests such as fermentation test and Gram staining.

LAB associated with biopreserved tomato paste samples investigated in this study agrees with the findings of (Beltrán-Edeza and Hernández-Sánchez 1989; Stratiotis and Dicks 2002; Sven-Olof 2008). *Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus fermentum* and *Leuconostoc mesenteroides* had been isolated from tomatoes naturally fermented under partial anaerobic conditions *Leuconostoc mesenteroides*, *Lactobacillus brevis* and *Lactobacillus plantarum* were also found to be the major acid producers in vegetable fermentation.

These studies have shown that all the concentrates with either ginger or garlic and the combination of two at 3% and 5% are found to be effective against microbial activities especially bacteria which is in conformity with the similar findings of (Abiola F. Olaniran, Sumbo H. Abiose and Adekanmi H. Adeniran,2015) that gagarlic 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.

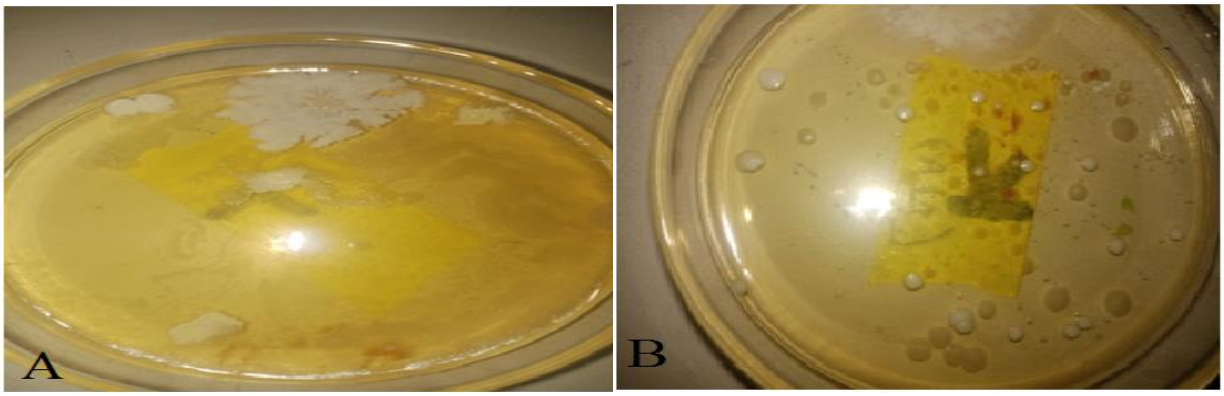

Plate 3.5: Plate **A (Bacterial colony, Rod shape); Plate B (Bacterial colony, cocci shape)**

CONCLUSION

The research work was carried out successfully. Results showed that those without fungi growth are; Sample G (ginger and onion), Sample O (onion, pepper and ginger), Sample D (onion), Sample E (pepper), Sample M (ginger, garlic and pepper). Sample L (ginger, garlic and onion) and Sample I (garlic and onion) (3 and 5%). The study also concluded that only Sample D (onion) and Sample N (garlic, onion and pepper) that has no bacteria growthand arefound to have antifungal effect in the preservation of tomato (3 and 5%), but pepper has no or little preservative efficacy with rapid deterioration at 3 and 5%. In Dakingari and Nigeria atlarge where power is in limited supply, the addition of 3–5% garlic, onion and or ginger to tomato paste can be of assistance in enhancing its shelf life particularly when tomato is in season. The natural bio-preservatives use for this study especially garlic, onion and ginger especially at 5% can promote food safety which in turn promote promote human health.

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