

Research Article



# The effects of preservatives on tomato (Lycopersicon esculentum mill.) paste at varying environmental conditions

#### **Abstract**

Studies were carried out on United Trading Company (UTC variety) tomato paste production to determine the effects of different preservatives on the shelf-life stability of the tomato paste produced aseptically in the laboratory. The experimental tomato fruits were purchased from two local markets in Jos North during the peak of harvest in October 2014. Among the experimental tomato fruits purchased, 6 were spoiled and 44 were looking healthy. The 6 spoiled fruits were used for the isolation of fungi species causing rot in the fruits using standard mycological methods. The 44 healthy tomato fruits used for the production of the paste were weighed, washed with tap water, boiled for 20 minutes (100°C). The epicarp of the boiled tomatoes was peeled and the peeled tomatoes were blended and boiled (to evaporate the water to minimal level) for 1 hour to make a paste. The paste was then cooled for 4 hours on a table. After cooling, 10g of the paste was weighed each in bijou bottles which were 48 in number. Each 10g of the paste weighed was given different treatment using  $T_1(0.01g)$  salt concentration),  $T_2(0.02g)$  salt concentration),  $T_3(0.03g \text{ salt concentration}), T_4(0.02g \text{ sodium benzoate}), T_5(\text{candle wax used in sealing the})$ bottles) and finally T<sub>6</sub>(10g of the paste without any preservative to serve as control). The experimental bottles were divided into 2 sets with each set containing 24 bottles. One set was kept in the refrigerator (4°C) while the other set was kept at an ambient temperature of 25°C±1°C. The experimental pastes (treated and untreated) were evaluated for mycological quality at 1-month interval for 4 months. For every interval, 6 bottles of the paste carrying all treatments and control in each environmental condition were evaluated for mycological quality. After every evaluation, the contents of the paste were discarded. Fungi species isolated in the spoiled/untreated tomato paste include Aspergillus niger, Schizosacchomyces pombe, Brettanomyces intermidius and Fusarium sp. Treated tomato paste using candle wax and 0.03g salt concentration produced the best results under both environmental conditions and thus recommended for usage by producers of tomato paste.

Keywords: preservatives, tomato, paste, fungi, environmental conditions, SDA

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**Abbreviations:** SDA, saboroud dextrose agar; CFU, colony forming unit

# Introduction

Botanically, tomato (*Lycopersicon esculentum* Mill) is a fruit, a berry, consisting of the ovary, together with its seeds, of a flowering plant. However, the tomato has much lower sugar content than other edible fruits, and is therefore not as sweet. It belongs to the *Solanaceae* family widely grown throughout the warm temperate and tropical region of the world, ranking next to potatoes and sweet potatoes in importance. Numerous varieties of tomatoes are widely grown in temperate climates across the world, with greenhouses allowing its production throughout the year and in cooler areas. The plants typically grow to 1–3 meters (3–10ft) in height and have a weak stem that often sprawls over the ground and vines over other plants. It is a perennial in its native habitat, and grown as annual in temperate climates. An average common tomato weighs approximately 100g.

Tomatoes have formed a very important aspect of human diet in Nigeria. They are used for a variety of dishes and are also processed in the form of various products such as tomato juice, puree, cocktail, paste, ketchup, sauce, jelly, soups, powder and tomato chutneys. Tomatoes are rich in vitamin C and antioxidants mainly lycopene,

carotenes, organic acids and phenolics. In recent years, there has been a global trend towards the use of phytochemicals from natural resources such as vegetables, fruits, oilseeds and herbs, as antioxidants and functional ingredients.<sup>1</sup> Consumption of beta-carotene and lycopene has been related to lower incidence of cardiovascular disease and prostate, gastrointestinal and epithelial cell cancer.<sup>2,3</sup>

Tomato is a high water-containing perishable and is very much susceptible to microbial spoilage.<sup>4</sup> Over the years, various methods have been developed for the preservation of tomatoes such as canning,<sup>5</sup> sun-drying and the use of chemical preservatives such as sodium benzoate and sodium metabisulfite<sup>6,7</sup> for the preservation of tomato juice, paste, etc. In Jos, Plateau State, UTC tomato is produced throughout the year but during the peak (December), farmers spend a lot of money on the production but usually sell at a loss. The study therefore seeks methods to preserve the tomato paste naturally without preservatives, since synthetic chemicals are known to be associated with cancer.

#### Statement of problem

Farmers of tomato in Jos, Plateau State and Nigeria as a whole suffer losses at the peak of harvest of tomato due to an abundance of the product and a lack of method of preservation. The product however, becomes scarce during the raining season (from April) as





a result of different diseases attacking the plant and the fruit. Such losses can be curtailed if the product is well-preserved for food security and better profit.

# **Materials and methods**

# Sample collection

Healthy tomato fruits were purchased from Farin Gada and Terminus markets of Jos North LGA, Plateau State. Out of a total of 50 fruits purchased, 6 were found to have deteriorated; they were put in a sterile polythene bag and transported to the laboratory in the University of Jos. These fruits became the experimental samples.

#### Preparation of culture medium

Exactly 16.25g of Saboroud Dextrose Agar (SDA) was weighed and diluted in 250ml of distilled water. The medium was diluted in 500ml conical flask and autoclaved at 121rpm for 15 minutes. The medium was allowed to cool for 30 minutes after which 1g of chloramphenicol was added into the medium and shaken vigorously and allowing it to cool. This was done to suppress the growth of bacteria. Exactly 15ml of the medium (SDA) was poured into 16 sterile Petri-dishes and allowed to solidify before culturing the spoiled tomatoes.

# **Culturing of spoiled tomatoes**

Six (6) spoiled fruits were used in the isolation and identification of fungal isolates that attack ripe tomato fruits. The 6 rotten tomatoes were washed with clean water and their surfaces sterilized with 70% ethyl alcohol. A sterile scapel was used to cut 3mm x 3mm sections of the tomato tissue. The diseased portion where the fungi are more likely to be active was cut into slices. These slices were transferred aseptically on prepared Saboroud Dextrose Agar (SDA) on 5 Petri-dishes and incubated for 4 days at an ambient temperature of 25°C±1°C. The set up was observed for 7 days to isolate the fungi associated with the fruit. Pure cultures of the isolate were made after series of inoculations into sterile Saboroud Dextrose Agar.

# Isolation and identification of fungi species

The wet mount of the isolate in lactophenol in cotton blue were examined microscopically and identified based on their colonial morphology, mycelia structure, spores and associated structures. Morphological structures were identified with the aid of a light microscope.

### Isolation and identification of yeast species

Gram staining was done for the identification of the yeast isolates. This examination was done by adding a drop of distilled water to prepare the smear. A portion of mycelia mat from the yeast colony was transferred into the drop of mounting fluid with the help of flame and needle. The yeast propagates were gently spread so that the yeast cell mixed with the stain of crystal violet, glucose iodine, alcohol, water and saphranin. The slides were prepared and observed under low and high power objectives of a light microscope.

# Production of tomato paste

Healthy fruits were washed with tap water and rinsed to remove dirt. A knife was employed to cut the bottom of each fruit horizontally

and vertically in a plus-like sign. This was done to enable the skin of the tomato fruit to peel so that the internal tissue will peel off after cooking the tomato. Water was boiled at 100°C and the cut tomatoes were immersed into it. The tomato fruits were gently added and boiled for 10 minutes until the skin split and peeled off. The boiled tomatoes were blended with the aid of sterile blender to produce a paste. The paste was then transferred to a clean pot and boiled for 20 minutes until a thick consistency was achieved. The paste was allowed to drain completely with the aid of a sieve in order to reduce the moisture content.

#### Preservatives used

Two (2) different preservatives (sodium benzoate and salt) were used to treat the tomato paste which was subjected to ambient temperature of 25°C±1°C and 4°C respectively for a period of 4 months. A total of 48 bottles were used and 10g of the tomato paste were weighed and each of these treatment were added to the tomato paste which were represented as:  $T_1(0.01g \text{ salt conc.})$ ,  $T_2(0.02 \text{ salt conc.})$ ,  $T_3(0.03 \text{ salt conc.})$ ,  $T_4(0.02 \text{ sodium benzoate})$ ,  $T_5(\text{candle wax})$ ,  $T_6(\text{Control})$ ,  $R_1(25^\circ\text{C}\pm1^\circ\text{C})$ , and  $R_2(4^\circ\text{C})$ . 10g of untreated tomato paste ( $T_5$ ) was sealed in a bottle with candle wax to prevent the entry of fungi spores.

#### Procedure for serial dilution

1ml of tomato paste was weighed with the aid of a weighing balance and was added into the first universal bottle containing 9ml of distilled water and shaken vigorously for 1 minute. 1ml of the content from the bottle was taken and injected into the second universal bottle containing 9ml of distilled water and shaken vigorously for 1 minute. The same procedure was repeated for 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> bottle. It was shaken after each injection of 1ml from the previous bottle. The whole process was carried for 7dilutions of the tomato paste samples and repeated for another 7 universal bottles containing 9ml of distilled water each. After the serial dilution was carried out on the tomato paste samples. 1ml of the mixture from the 7<sup>th</sup> bottle was taken with a syringe and spread on each of the 3 plates containing 15ml of solidified SDA medium. 1ml of the mixture from the 7<sup>th</sup> dilution in the universal bottles were taken and spread on each of the Petri-dishes containing 15ml of solidified SDA medium.

#### Incubation

The 6 plates were labeled according to the sample inoculated in it. The incubator was properly cleaned up with cotton wool soaked in ethanol before placing the labeled Petri-dishes in the incubator and set at ambient temperature of 25±1°C.

# Colony count and determination of colony forming units (CFU/g) of the fungi

The grid membrane method was used for colony count which involves the ruling of lines horizontally and vertically to form grids behind the Petri-dishes and after 24 hours, the first colony count was taken. Tiny colonies formed were counted according to where they were located between the rows and columns of the grid. The colony forming unit (CFU/g) of fungi for each site was computed using the formula: colony forming units (CFU)=Average number of colony in 3 replicates.

#### **Results and discussion**

Results for the isolation of fungal isolates from Farin Gada and Terminus markets showed that *Aspergillus niger* was found present, while *Penicillium* sp. was found to be present in only Terminus market. *Schizosaccharomyces pombe* and *Bretianomyces intermedius* was found present in both Farin Gada market and Terminus market respectively (Table 1).

**Table 1** Fungi associated with tomato (*Lycopersicon esculentum*) fruit of Farin Gada and Terminus markets after 7 days of inoculation

Fungal Isolates	FGM <sup>1</sup>	FGM <sup>2</sup>	FGM <sup>3</sup>	TM¹	TM <sup>2</sup>	TM <sup>3</sup>
Aspergillus niger	+	_	+	_	_	+
Penicillium sp	-	-	-	+	-	_
Schizosaccharomyces pombe	+	+	-	+	-	+
Bretianomyces intermedius	+	+	-	+	-	+

Key: +, Present; -, Absent; FGM, farin gada market; TM, terminus market

Results of the treated paste kept at 25±1°C and 4°C within the period of 4 months showed that at 25±1°C, there was presence of growth on the T.R. treatment throughout the period of preservation which showed 100% occurrence, whereas, at 4°C there was no growth recorded within the period of 4 months and there was 0% frequency of occurrence. At 25±1°C, presence of growth was recorded in both the 3<sup>rd</sup> and 4<sup>th</sup> month of preservation which showed 50% frequency of occurrence on T<sub>2</sub>R<sub>1</sub> treatment, no growth was recorded and there was 0% frequency of occurrence within the period of 4 months. T.R. treatment had no record of growth within the period of 4 months occurrence likewise T<sub>2</sub>R<sub>2</sub>. T<sub>4</sub>R<sub>4</sub> treatment however, showed presence of growth within the 4 months period of preservation which also had 100% frequency of growth occurrence, whereas T<sub>4</sub>R<sub>2</sub> treatment had no record in the 3rd and 4th month which had 50% frequency of occurrence. T<sub>5</sub>R<sub>1</sub> and T<sub>5</sub>R<sub>2</sub> treatments had no record of growth which showed 0% frequency of occurrence, but in T<sub>6</sub>R<sub>1</sub> and T<sub>6</sub>R<sub>2</sub> had record of growth throughout the period of preservation which showed 100% frequency of occurrence as seen in Table 2.

Table 2 Colonization by fungi species in treated and untreated tomato paste (Lycopersicon esculentum) paste after 120 days (4 months) of storage at 25±1°C and 4°C

Treatment	l <b>M</b> onth	2Months	3Months	4 Months	Total	% of occurrence
T <sub>I</sub> R <sub>I</sub>	+	+	+	+	4	100
$T_1R_2$	_	_	+	+	2	50
$T_2R_1$	_	_	+	+	2	50
$T_2R_2$	_	_	+	+	2	50
$T_3R_1$	_	_	_	_	0	0
$T_3R_2$	_	_	-	_	0	0
$T_4R_1$	+	+	+	+	4	100
$T_4R_2$	_	_	+	+	2	50
$T_5R_1$	_	_	-	_	0	0
$T_5R_2$	_	_	-	_	0	0
T <sub>6</sub> R <sub>1</sub>	+	+	+	+	4	100
T <sub>6</sub> R <sub>2</sub>	+	+	+	+	4	100

 $\textbf{Key}: T_{i}, 0.01 \text{ salt conc}; T_{2}, 0.02 \text{ salt conc}; T_{3}, 0.03 \text{ salt conc}; T_{4}, 0.02g \text{ sodium benzoate}; T_{5}, Candle \text{ wax}; T_{6}, Control; R_{i}, 25\pm1^{\circ}\text{C} \text{ (room temp.)}; R_{2}, 4^{\circ}\text{C} \text{ (refrigerator)}; R_{2}, 4^{\circ}\text{C} \text{ (refrigerator)}; R_{3}, 25\pm1^{\circ}\text{C} \text{ (room temp.)}; R_{4}, 25\pm1^{\circ}\text{C} \text{ (room temp.)}; R_{5}, 25\pm1^{\circ}\text{C} \text{$ 

Results of the occurrence of fungi species isolated in treated and untreated tomato paste showed that *Aspergillus niger* was found present in  $T_1R_1$  treatment. *Penicillium* sp was found present in  $T_6R_1$ 

treatment, *Schizosaccharomyces pombe* was found in  $T_4R_1$  treatment and *Bretianomyces intermedius* was found in  $T_6R_2$  treatment. *Fusarium* sp was absent in all treatments (Table 3).

Table 3 Occurrence of fungi species isolated in treated and untreated tomato (Lycopersicon esculentum) paste after 30 days (1 month) of storage at 25±1°C and 4°C

Treatment	Aspergillus niger	Schizosaccharomyces pombe	Bretanomyces intermedius	Fusarium sp	Penicillium sp
T <sub>I</sub> R <sub>I</sub>	+	_	=	-	_
$T_1R_2$	-	_	-	-	_
$T_2R_1$	_	_	_	-	_
$T_2R_2$	_	_	_	_	_
$T_3R_1$	_	_	_	_	_
$T_3R_2$	_	_	_	-	_
T₄R₁	_	+	_	_	_
$T_4R_2$	_	_	_	-	_
$T_{s}R_{i}$	_	_	_	_	_
$T_5R_2$	_	_	_	-	_
$T_{6}R_{1}$	_	_	_	_	+
T <sub>6</sub> R <sub>5</sub>	_	_	+	_	_

**Key**  $T_1$ , 0.01 salt conc;  $T_2$ , 0.02 salt conc;  $T_3$ , 0.03 salt conc;  $T_4$ , 0.02g sodium benzoate;  $T_5$ , Candle wax;  $T_6$ , Control;  $R_1$ , 25±1°C (room temp.);  $R_2$ , 4°C (refrigerator)

Results of the occurrence of fungi species isolated from treated and untreated tomato paste after 60 days revealed the presence of *Schizosaccharomyces pombe* in  $T_6R_2$  treatment. *Bretianomyces intermedius* was found present in  $T_4R_1$  treatment and *Fusarium* sp was present in  $T_6R_1$  treatment as seen in Table 4.

Results of the occurrence of fungi species in treated and untreated tomato paste showed that *Penicillium* sp was present  $T_1R_1$  treatment, *Schizosaccharomyces pombe* was present in  $T_6R_2$  treatment.

Bretanomyces intermedius was also found in  $T_4R_1$  treatment. Fusarium sp was found present in  $T_kR_1$  treatment as seen in Table 5.

Results of the occurrence of fungi species isolated in treated and untreated tomato paste after 120 days(4 months) of storage at 25±1°C and 4°C revealed Aspergillus niger present in  $T_1R_1$  and  $T_6R_1$  treatments. Penicillium sp was found in  $T_2R_1$  treatment and Schizosaccharomyces pombe was found present in  $T_4R_1$  and  $T_6R_2$  treatments. Fusarium sp was found present in  $T_4R_1$  treatment as seen in Table 6.

Table 4 Occurrence of fungi species isolated in treated and untreated tomato paste after 60 days (2 months) of storage at 25±1°C and 4°C

Treatment	Aspergillus niger	Penicillium sp	Schizosaccharomyces pombe	Bretanomyces intermedius	Fusarium sp
$T_{i}R_{i}$	_	+	_	_	_
$T_1R_2$	_	_	_	_	_
$T_2R_1$	_	_	_	_	-
$T_2R_2$	_	_	_	_	-
$T_3R_1$	_	_	_	_	-
$T_3R_2$	_	_	_	_	-
$T_4R_1$	_	_	_	+	-
$T_4R_2$	-	-	_	_	_
T <sub>5</sub> R <sub>1</sub>	-	-	_	_	_
$T_5R_2$	-	-	_	_	_
T <sub>6</sub> R <sub>1</sub>	_	_	_	_	+
T <sub>4</sub> R <sub>2</sub>	_	_	+	_	_

**Key:**  $T_1$ , 0.01 salt conc;  $T_2$ , 0.02 salt conc;  $T_3$ , 0.03 salt conc;  $T_4$ , 0.02g sodium benzoate;  $T_5$ , Candle wax;  $T_6$ , Control;  $R_1$ ,  $5\pm 1$  °C (room temp.);  $R_2$ , 4°C (refrigerator)

Table 5 Occurrence of fungi species isolated in treated and untreated tomato paste after 90 days (4 months) of storage at 25±1°C and 4°C

Treatment	Aspergillus niger	Penicillium crysogenum	Schizosaccharomyces pombe	Bretanomyces intermedius	Fusarium sp
$T_{i}R_{i}$	_	+	_	_	_
$T_1R_2$	_	_	+	_	-
$T_2R_1$	_	_	_	+	_
$T_2R_2$	_	_	_	_	_
T <sub>3</sub> R <sub>1</sub>	_	_	_	-	_
$T_3R_2$	_	_	_	-	_
T₄R <sub>I</sub>	_	_	+	+	_
$T_4R_2$	_	_	_	-	-
T <sub>s</sub> R <sub>1</sub>	_	_	_	_	_
T <sub>5</sub> R <sub>2</sub>	_	_	_	_	_
T <sub>6</sub> R <sub>1</sub>	_	_	_	_	_
T <sub>6</sub> R <sub>2</sub>	+	_	_	_	_

Key: $T_1$ , 0.01 salt conc; $T_2$ , 0.02 salt conc; $T_3$ , 0.03 salt conc; $T_4$ , 0.02g sodium benzoate; $T_5$ , Candle wax; $T_6$ , Control;  $R_1$ , 25±1°C (room temp.); R2, 4°C (refrigerator)

Table 6 Occurrence of fungi species isolated in treated and untreated tomato paste after 120 days (4 months) of storage at 25±1°C and 4°C

Treatment	Aspergillus Niger	Penicillium Crysogenum	Schizosaccharomyces Pombe	Bretanomyces Intermedius	Fusarium Sp
T,R,	+	- ′ •	_	_	_
T,R,	_	-	+	_	_
T <sub>2</sub> R <sub>1</sub>	_	+	_	-	-
T,R,	_	-	_	_	_
T¸R¸	_	_	_	_	_
T,R,	_	-	_	_	_
T₄R₁	_	_	+	_	_
T₄R,	_	_	_	_	+
T¸Rį	_	_	_	-	-
T¸R,	_	_	_	_	_
T <sub>6</sub> R <sub>1</sub>	+	_	_	_	_
T¸R¸	_	_	+	_	_

**Key:**  $T_1$ , 0.01 salt conc;  $T_2$ , 0.02 salt conc;  $T_3$ , 0.03 salt conc;  $T_4$ , 0.02g sodium benzoate;  $T_5$ , Candle wax;  $T_6$ , Control;  $R_1$ , 25±1°C (room temp.);  $R_2$ , 4°C (regenerator)

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# **Discussion**

This research revealed that farmers of tomato can produce paste from their tomatoes during the peak period for up to four (4) months. From the results obtained using treated and untreated tomato paste kept at  $25\pm1^{\circ}\mathrm{C}$  and  $4^{\circ}\mathrm{C}$  within the period of 4 months,  $T_3R_1(0.03g$  salt conc.) which was kept at  $25\pm1^{\circ}\mathrm{C}$ ,  $T_3R_2(0.03g$  salt conc.) kept at  $4^{\circ}\mathrm{C}$  were able to preserve the paste within the period of 4 months.  $T_5R_1(\mathrm{candle\ wax})$  kept at  $4^{\circ}\mathrm{C}$  also had great effect on the tomato paste within the 4 months period.  $T_1R_1(0.01g\ \mathrm{salt\ conc.})$  kept at  $25\pm1^{\circ}\mathrm{C}$ ,  $T_4R_1(0.02g\ \mathrm{sodium\ benzoate})$  had no effect on the tomato paste within the 4 months period. The untreated tomato paste represented as  $T_6R_1$  kept at  $25\pm1^{\circ}\mathrm{C}$  and  $T_6R_2$  untreated tomato paste kept at  $4^{\circ}\mathrm{C}$  were attacked by fungi throughout the period of storage. The following treatments were able to preserve the tomato paste for a period of 2 months:  $T_1R_2(0.01g\ \mathrm{salt\ conc.})$  kept at  $4^{\circ}\mathrm{C}$ ,  $T_2R_2(0.02g\ \mathrm{salt\ conc.})$  kept at  $25\pm1^{\circ}\mathrm{C}$  and  $T_4R_3(0.02g\ \mathrm{sodium\ benzoate})$  kept at  $4^{\circ}\mathrm{C}$ .

This study revealed that Aspergillus niger, Penicillium sp, Scizosaccharomyces pombe, Bretanomyces intermedius, Fusarium sp were present in the affected tomato paste kept at 25±1°C and 4°C. The most occurring organism in the tomato paste was yeast. Bretanomyces intermedius was the most prevalent. This organism is known to cause spoilage in beer, wine, soft drinks, pickles and ales. Schizosaccharomyces pombe is only distantly related to the species, it is osmophilic and resistant to some chemical preservatives. The ambient temperature of 25±1°C had no much effect on the tomato paste compared to the refrigerated tomato paste kept at 4°C. This showed that temperature variation contributed to the spoilage of the tomato paste. A similar work was reported by Anon<sup>8</sup> who suggested initial contamination of raw materials was one of the variables that affected the microbial profile and shelf-life stability of canned tomato products.

UTC producers are hereby advised to use big bottles to make paste of their tomato during its peak period, use candle wax to seal the bottles or salt concentration of 0.03g for 10g to preserve their products for up to 4 months until when it is scarce, then they can sell their products at a profit instead of allowing them to waste.

# **Recommendation**

From the result of this study, farmers and individuals are advised to preserve tomato in form of paste using this method of preservation, especially in developing countries like Nigeria where food scarcity is on the increase. As a result of fungi spoilage, this method can be used in preserving tomato paste in order to maintain its natural taste, colour and quality. Industries can be created by individuals or group of people in order to sustain the availability of tomato paste.

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#### **Conflict of interest**

The authors whose names are listed in this research certify that they have no affiliations with any organization or entity with any financial interest and as such there is no conflict of interest among the authors of this research.

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