Tomato paste production from *Solanum lycopersicum*, microorganisms associated and shelf-life extension through biopreservation methods

RESEARCH ARTICLE

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ABSTRACT

Tomatoes are major ingredients in hundreds of dishes worldwide. *Solanum lycopersicum* is a well-utilised plant species in Africa. This research investigated the production of tomato paste, the control of microorganisms associated with paste spoilage, and the extension of the paste's shelf life through the application of biological materials. Fresh tomato fruits were purchased, and standard techniques were followed in the production of the paste and the monitoring of spoilage. The preservation of the paste was carried out through the application of 1ml, 1ml, and 10ml of lemon juice, vinegar, and vegetable oil, respectively. The control treatment received 1ml of distilled water. Microorganisms associated with the paste were isolated and identified using standard microbiological methods. Proximate analysis of the tomato paste was conducted. The results revealed that, at room temperature, spoilage of ripened tomato fruits began on the fourth day, with total deterioration occurring by day 28. *Klebsiella aerogenes* and *Rhizopus stolonifer* were identified as associated microorganisms. Proximate analysis established that tomato paste preserved with vegetable oil maintained its nutritional content most effectively. The crude protein values were 1.93% (day 1) and 2.3% (day 28), and carbohydrate values were 4.28% (day 1) and 5.71% (day 28). Vinegar and lemon juice were not effective in the preservation of tomato paste, evidenced by the early appearance of bubbles in the paste on day 3 of paste production, signifying degradation, and massive discolouration by day 7. For effective biopreservation and extension of tomato paste shelf life, the vegetable oil preservation technique should be used.

Methodology

Standard microbiological techniques and proximate analysis for tomato paste preservation study.

Key Variables

Lemon juice, vinegar, vegetable oil preservatives tested against microbial spoilage.

Main Finding

Vegetable oil provided the best biopreservation with optimal nutritional content maintenance.

Keywords: Tomato paste; Microorganisms; proximate analysis; shelf life; biopreservation

INTRODUCTION

A global staple, tomatoes are consumed at over 200 million metric tonnes annually, driven by high demand for both fresh and processed forms (AMRC, n.d.; Data Bridge Market Research, 2023). Tomatoes rank second in production in the Philippines (after aubergine) and fifth in Indonesia. In these countries, small business owners and farmers prioritize tomato production due to its high monetary value, which significantly contributes to their income (FAO, 2023; Philippine Statistics Authority, 2022; Ogunbayo, 2025).

Tomatoes are popular for home gardens and are essential in various dishes and products like ketchup and chutney. The edible, often red, berry-type fruit of the nightshade plant, *Solanum lycopersicum* (tomato plant), is consumed raw, in salads, sauces, and drinks (FAO, 2022; Padmanabhan et al., 2016).

01

Nigeria's Tomato Production

Nigeria produces approximately 3.9 million metric tonnes annually, making tomato farming a fast-growing sector with immense opportunities for entrepreneurs and agribusiness investors.

02

Post-Harvest Challenges

Despite substantial output, the country faces massive post-harvest losses of up to 45% due to poor storage and transportation infrastructure.

03

Economic Impact

Nigeria still spends over $360 million annually on importing processed tomato products, highlighting the need for improved local processing.

In Nigeria, tomato farming is a fast-growing sector for entrepreneurs and agribusiness investors (Ogunbayo, 2025), with annual production of approximately 3.9 million metric tonnes in 2023 (FAO, 2023). Despite this, the country experiences massive post-harvest losses, with up to 45% of harvested tomatoes spoiling due to poor storage and transportation (Ogunbayo, 2025). Nigeria also spends over $360 million annually importing processed tomato products (PricewaterhouseCoopers Nigeria's Agribusiness Report).

Tomato paste is a thick, semi-solid concentrate made by cooking tomatoes for hours to reduce moisture, straining seeds and skin, and cooking again (Aldrich et al., 2010). In contrast, tomato purée is a liquid with a consistency between crushed tomatoes and paste, made from briefly boiled and strained tomatoes (Padmanabhan et al., 2016).

Research Justification

The increasing consumer preference for natural and minimally processed foods has led to a growing interest in biopreservation techniques as alternatives to conventional chemical preservatives. Recent research highlights consumer concerns regarding the potential health risks and environmental impact associated with synthetic additives (Chen & Lee, 2023; Silva et al., 2024). Recent comprehensive reviews have demonstrated the growing global interest in biopreservation technologies as sustainable alternatives to chemical preservation methods (Muthuvelu et al., 2023). Biopreservation methods offer a promising approach to extend the shelf life of food products while addressing these concerns, minimising health risks, and promoting sustainable practices (Wang & Kim, 2023; Garcia et al., 2024).

Despite the recognised potential of biopreservation, there is a noticeable research gap concerning the application of readily available and natural substances like vegetable oil as a biopreservative for tomato paste. While some studies explore various biopreservation agents, specific investigations into the efficacy of vegetable oil in preventing microbial spoilage and maintaining the nutritional integrity of tomato paste are limited.

Research Objectives

- To produce tomato paste from *Solanum lycopersicum* under controlled conditions.
- To isolate and identify microorganisms associated with the spoilage of tomato paste.
- To investigate the shelf life of tomato paste when preserved with biological materials.
- To extend the shelf life of tomato paste through the application of selected biopreservation methods, specifically focusing on lemon juice, vinegar, and vegetable oil.

Research Hypotheses

It is hypothesised that the application of biological materials, particularly vegetable oil, will significantly extend the shelf life of tomato paste by inhibiting microbial growth and preserving its nutritional quality, thereby offering a superior biopreservation method compared to lemon juice and vinegar.

LITERATURE REVIEW

Economic Importance and Cultivation

Tomatoes are considered to be one of the most economically important crops of all those that exist in the world. Economically speaking, tomatoes are worth a tremendous amount of money because they give more yields (Hammerschmidt & Franklin, 2005; Ogunbayo, 2025). They can also be grown both indoors in greenhouses and outdoors, although tomatoes that are grown outside tend to have higher nutrient contents than those grown in greenhouses. Tomatoes have many advantages over growing other types of crops, such as: Their high yield which results in their high economic value, their very high nutritional value with high levels of pro-vitamin A and C. As well as being ranked first on their nutritional contribution to a human's diet, and their short duration crop. They are very well suited for different cropping systems that are used on grains, pulses, cereals and oilseeds (Hammerschmidt & Franklin, 2005; Bjarmadottir, 2023).

Nutritional Characteristics of Tomato

While most often associated with lycopene, a carotenoid phytonutrient widely recognised for its antioxidant properties, tomatoes provide a unique variety of phytonutrients. Included are additional carotenoids (including beta-carotene, lutein and zeaxanthin); flavonoids (including naringenin, chalconaringenin, rutin, kaempferol and quercetin); hydroxycinnamic acids (including caffeic, ferulic and coumaric acid); glycosides (including esculeoside A); and fatty acid derivatives, including 9-oxo-octadecadienoic acid (Aldrick & Salandanan, 2010).

Tomatoes are also an excellent source of vitamin C, biotin, molybdenum and vitamin K. They are also a very good source of copper, potassium, manganese, dietary fibre, vitamin A (in the form of beta-carotene), vitamin B6, folate, niacin, vitamin E and phosphorus. Additionally, they are a good source of chromium, pantothenic acid, protein, choline, zinc and iron (Anthon et al., 2011; Bjarmadottir, 2023).

Cardiovascular Health

The highest average intakes of lycopene were linked to almost a 30% reduction in the incidence of cardiovascular disease and coronary heart disease.

Vitamin Content

Tremendous amounts of natural vitamins including A, K, B1, B3, B5, B6, B7, excellent vitamin C, folate, and essential minerals.

Antioxidant Properties

Rich in beta carotenoids, lutein and zeaxanthin that help improve vision and protect eyes from degeneration.

Shelf Life of Foods

Shelf life is the length of time a commodity may be stored without becoming unfit for use, consumption, or sale (U.S. Food and Drug Administration, 2022). This distinction can mean it's no longer suitable for a pantry (unfit for use) or merely for a supermarket shelf (unfit for sale but still usable). It applies to diverse perishable items, including cosmetics, foods, beverages, medical devices, medicines, explosives, drugs, chemicals, car tires, and batteries. Some regions require an advisory 'best before', mandatory 'use by', or freshness date on packaged perishable foods.

According to the USDA, "canned foods are safe indefinitely as long as they are not exposed to freezing temperatures, or temperatures above 90 oF (32.20C)." If cans appear intact, they are safe to use; discard dented, rusted, or swollen cans. High-acid canned foods (tomatoes, fruits) retain best quality for 12 to 18 months, while low-acid canned foods (meats, vegetables) last 2 to 5 years (Meyer, 2007). Ideally, temperatures should remain below 800F (270C) (Meyer, 2007).

Degradation Factors

- Exposure to light, heat, and moisture
- Transmission of gases
- Mechanical stresses
- Contamination by microorganisms

Consumer Significance

- Enables safe, informed food use
- Requires intact, undamaged products
- Follow manufacturers' storage instructions
- Utilize shelf life data to reduce food waste

Shelf life depends on a product's degradation mechanism, often mathematically modeled around parameters like chemical compound concentration, microbiological index, or moisture content (Meyer, 2007). Health issues also influence shelf life for some foods; ubiquitous bacterial contaminants can render foods unsafe if stored too long, leading to food poisoning. However, shelf life alone isn't a precise indicator of safe storage duration. For instance, pasteurized milk can stay fresh for five days, but 'use-by' dates become irrelevant if harmful bacteria are already present (Meyer, 2007).

MATERIALS AND METHODS

Collection of Samples

The samples of tomato fruits were purchased from Yaba Market in Ondo City, Ondo State. They were taken to the laboratory for this research work.

Preparation of Media Used

Nutrient Agar

Twenty-eight grams of nutrient agar powder was measured with a weighing balance. It was poured into a two-litre conical flask and dissolved in 1 litre of distilled water. It was then autoclaved at 121°C for 15 minutes. After autoclaving, the medium was then placed in a water bath at 45°C and allowed to cool down before dispensing into Petri dishes, which were then allowed to solidify before introducing microorganisms.

Potato Dextrose Agar

Thirty-nine grams of potato dextrose agar powder was measured with a weighing balance, poured into a two-litre conical flask and dissolved in 1 litre of distilled water, then autoclaved at 121°C for 15 minutes. It was then placed in a water bath to cool at 45°C before dispensing into Petri dishes, which were then allowed to solidify before introducing microorganisms.

Sample Collection

Tomato fruits purchased from Yaba Market in Ondo City, Ondo State for laboratory research work.

Sterilisation Process

Disinfection using 70% ethanol solution to sterilise inoculation chamber and work bench areas.

Media Preparation

Nutrient agar and potato dextrose agar prepared and autoclaved at 121°C for 15 minutes.

Disinfection of Inoculation Chamber and Work Bench

This was done using a 70% ethanol solution with sterile cotton wool to sterilise the inoculation chamber and the work area in the laboratory. This process is carried out before and after working to disinfect any microorganisms present in the environment.

Culturing of Tomato Samples

The samples of the tomato that were obtained from Ondo market were washed very well to reduce the microbial load, after which sterile forceps were used to pull apart the tomatoes. Then, a part of the tomato was taken and transferred into 10 mL of sterile normal saline in various beakers according to the number of samples collected. The tomatoes were allowed to stand in the normal saline for 10 minutes, before being removed with the forceps from the beakers.

Serial Dilution

Four test tubes containing 9 mL distilled water were placed on a rack on a bench using a test tube rack. 1 mL of the sample solution was pipetted aseptically into the first test tube and mixed. This was then transferred to the second test tube and mixed, and the process repeated up to the last test tube (10^{-4}). Subsequently, 1 mL from the last test tube was discarded.

Culturing

Some sterile Petri dishes were obtained from the laboratory, and the medium to be used was prepared. 15 mL of the medium was measured into the Petri dishes, autoclaved at 121°C for 15 minutes. It was then allowed to cool to 45°C, so as not to kill the organisms. 1 mL of each dilution was aseptically transferred to sterile Petri dishes. The agar was then poured and gently rotated. It was then allowed to cool and gelled. The plates were transferred to the incubator and incubated at 37°C for 48 hours.

Sub-culturing of Isolates

For pure culture preparation, distinct colonies were aseptically transferred from the culture media into newly prepared sterile nutrient agar and potato dextrose agar plates. Streaking of the individual organisms was carried out using a sterile wire loop. After pure cultures had been established, they were transferred into bijou bottles, and the cultures were preserved in a refrigerator and maintained at 4°C until they were worked upon for identification.

Monitoring of Tomato Fruits Spoilage

Eight tomato fruits were picked from the sample of tomatoes purchased at Yaba Market, Ondo and monitored for 30 days, visually. Two setups were monitored, four fruits each in a plate and covered with a sieve to avoid rat contact. Results were taken for the 10 days.

Preparation of Tomato Paste and Addition of Preservatives

The tomato fruits were washed in three changes of water to reduce the microbial load, after which they were used for the production of tomato paste following the flowchart represented below. After washing, blanching was carried out, the removal of the epicarp was done, and then the tomatoes were milled using a blender. Cooking and thickening of the paste were done for 2 hours; it was then allowed to cool down for about 30 minutes, sterilised, and poured into bottles. They were preserved using three different preservatives to extend the shelf life. Recent studies have demonstrated the effectiveness of common spices and natural compounds in extending the shelf life of tomato paste, with specific combinations showing superior antimicrobial properties (Aati et al., 2024). The preservatives used were: vinegar, lemon, vegetable oil, and distilled water controls.

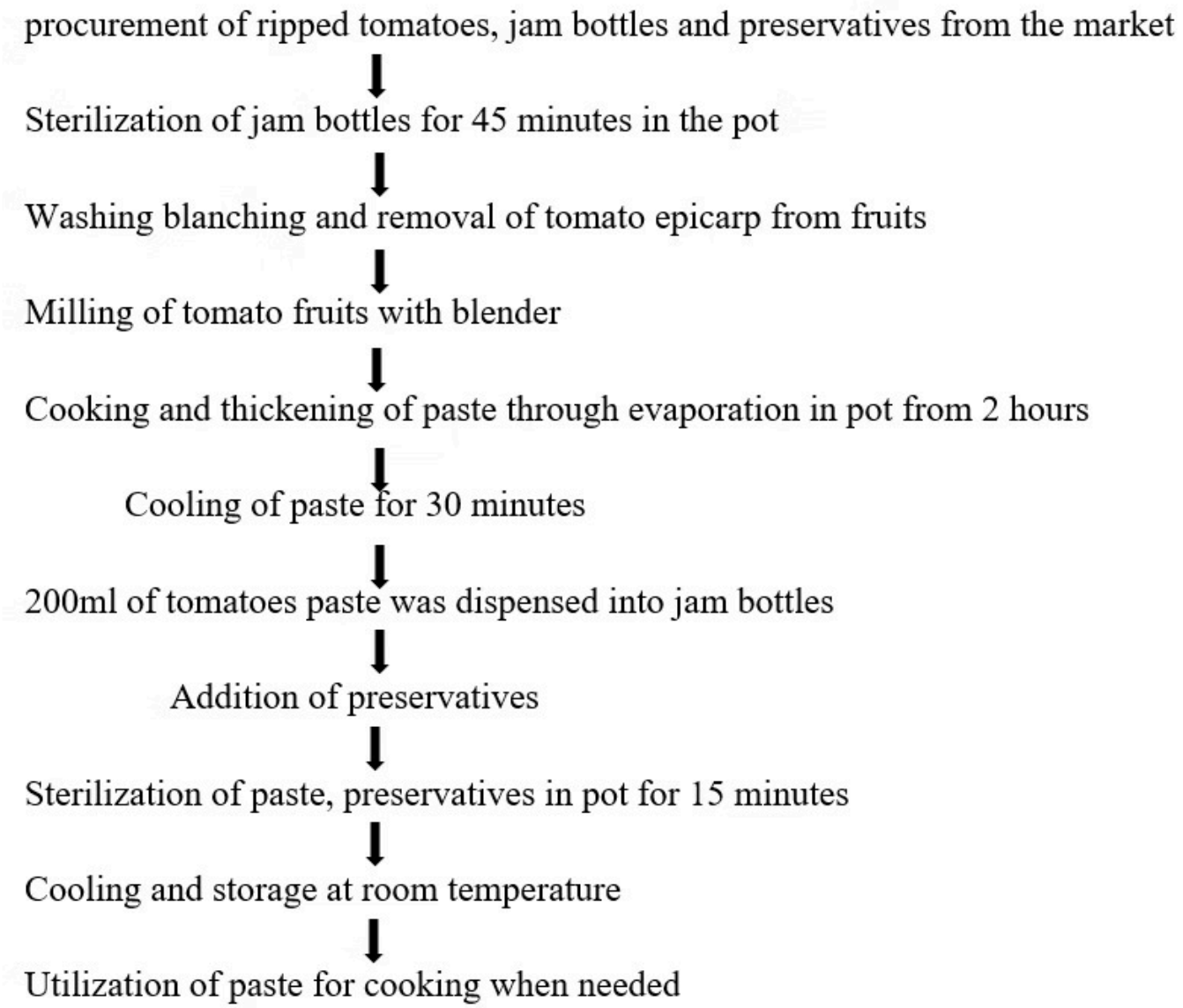

Figure 1: Procedure for tomato paste preparation on a flowchart

Proximate Analysis of the Preserved Tomato Pastes

The preserved tomato pastes and the preservatives that were used in preserving them were taken to Jaagee Laboratory, Ibadan, Oyo State, Nigeria for proximate analysis. The parameters analysed include moisture content, crude proteins, crude fats, crude fibre, total ash, and CHO.

Procedural technique for % moisture determination in samples

Materials: conventional oven, porcelain crucibles, analytical balance, spatula, desiccators, crucible tongs.

Procedure:

1. Dry empty crucibles were placed in the oven at 1050C for 30 minutes to get rid of any moisture present on the crucibles.
2. Transfer the porcelain crucibles into a desiccator and allow to cool at room temperature for about 20 minutes.
3. Weigh the empty porcelain crucibles and record this as W1.
4. Grind your samples into powder using a Cyclotec sample mill or pestle & mortar to increase the surface area.
5. Using an analytical balance, weigh 1.0000g of sample into the porcelain crucibles (record the combined weight as W1 and dry it in the oven at 1050C till constant weight or preferably for 2 hours.
6. Using crucible tongs, transfer the porcelain crucibles containing the samples from the oven into desiccators and allow to cool at room temperature for about 30 minutes.
7. Allow the porcelain crucibles containing the samples to cool for about 10 minutes in the ove
8. Take the final weight of the porcelain crucibles and content and record this as W2.

Calculation

$$\% \text{ moisture content} = \frac{(Wo + W1) - (Wo - W2)}{W1} \times 100$$

Crude Protein Determination

Equipment:

1. Kjeltec auto distillation unit, model: Kjeltec 8200
2. Tecator digestor, model: 8
3. Hanna magnetic stirrer, model: HI 300

Materials:

Digestion block, digestion tubes, Kjeldahl distillation unit, automatic titrator.

Reagents:

H_2SO_4, copper catalyst, 40% NaOH solution, receiver solution, distilled water, 0.1N HCl.

Preparation of receiver solution:

- Dissolve 0.1g methyl red indicator in 100ml 95% methanol.
- Dissolve 0.1g bromocresol green indicator in 100ml methanol or ethanol.
- Prepare 4% boric acid solution: dissolve 40g powder in ~5-6L very hot distilled water.
- Cool to room temperature, add 100ml bromocresol green solution and 70ml methyl red solution. Dilute to 10L with deionized water; mix carefully.

Procedure:

1. Weigh 1g (or 2.00ml if liquid) of well-prepared sample to 0.1mg accuracy into a 250ml digestion tube.
2. Add 2 Kjeltabs Cu 3.5 (or 7g K_2SO_4 and 0.8g $CuSO_4 \times 5H_2O$).
3. Carefully add 12ml concentrated H_2SO_4; gently shake to wet sample.
4. Attach exhaust system to digestion tubes in rack; set water aspirator to full effect.
5. Digest samples for 1 hour at 430oC.
6. Remove tube rack, place in stand; cool for 10-20 minutes.
7. Insert tubes into distillation unit and close safety door.
8. Carefully add 80ml deionized water to tubes.
9. Add 25-30ml receiver solution to conical flask; place into distillation unit. Position platform so distillate outlet is submerged.
10. Dispense 50ml of 40% NaOH into tube. (Note: Steps 8, 9, and 10 are automatic.)
11. Distill for about 4 minutes.

12 Titrate distillate with standardized HCl (0.1N or 0.2N) until blue-grey endpoint. Note volume of acid consumed.

NOTE: Run a blank through every batch.

Calculation

$$\% \text{ protein} = \frac{(T - B) \times N \times 14{,}007 \times 100}{W\ 1\ (mg)} \times F$$

$$gN/L = \frac{(T - B) \times N \times 14{,}007}{\text{Volume sample (ml)}}$$

Wi = Sample weight (mg)

T = Titration volume of sample (ml)

B = Titration volume of blank (ml)

N = Normality of acid to 4 decimal places

F = Conversion factor for nitrogen to protein = 6.25 for food & feeds

gN/L = Gram Nitrogen per Litre

Determination of crude fibre in food and feeds using Fibretec 2010 or M6

Equipment names:

1. Fibretec Hot/Hydrolysis Unit, Model: 1020
2. Fibretec Cold Extraction Unit, Model: 1021

Materials:

Analytical balance, fritted crucibles, air-ventilated oven capable of operating at 105± 20C and above, desiccator, grinding equipment, Fibretec Hot Extraction Unit, Fibretec Cold Extraction Unit, hot plate, wash bottle, muffle or incineration furnace 525 ± 150C

Reagents:

Acetone (technical grade), 1.25% H_2SO_4 solution, 1.25% NaOH solution.

Procedure

1. Pre-dry fritted crucibles at 130 ± 2°C for 30 minutes.

2. Place pre-dried crucibles on a balance and tare. To simplify filtration, weigh 1.000g of Celite 545. Weigh 1.0000g of well-prepared sample into the crucible containing the Celite.

Fibretec Hot Extraction Procedure

3. Switch on the Fibretec hot extraction unit, prepare 1.25% H_2SO_4, and heat on a hot plate.

4. Insert the crucibles using the holder and lock into position in front of the radiator in the Fibretec hot extraction unit, ensuring that the safety latch engages.

5. Place the deflector in front of the crucibles.

6. Close all valves.

7. Open the cold water tap (1-2 L/min) for the reflux system.

8. Add 150 mL of preheated 1.25% H_2SO_4 into each column (reagent 1).

9. Add 2-4 drops of n-Octanol to prevent foaming and turn the 'HEATER' control fully clockwise. When the reagent starts to boil, adjust to moderate boiling using the 'HEATER' control.

10. Measure boiling time from the moment the solution has reached its boiling point (30 min).

11. At the elapse of 30 min (end of extraction), turn off the heater.

12. Place valves in the 'VACUUM' position and open the cold water tap to full flow rate for the water suction pump, then start the filtration.

13. Use reversed pressure to wash the sample. Wash three times with hot deionised water. Use 30 mL portions of water and suck as dry as possible between washings.

14. Add 150 mL of preheated 1.25% NaOH solution into each column (reagent 2).

15. Repeat the operation from step 9-13 above.

16. Release the crucibles with the safety lock.

17. Using the crucible holder, transfer the crucibles to the Fibretec cold extraction unit.

Fibretec Cold Extraction Procedure

18. Position the crucibles in the fibretec cold extraction unit and close valves.

19. Add 25ml acetone to each crucible. Extract and filter solvent out by placing the valve in 'VACUUM' position. Repeat three times.

20. Remove crucibles and transfer them to a crucible stand. Leave at room temperature until the acetone has evaporated. Otherwise, there is a risk of burning the fibre during the drying process.

21. Dry crucibles for at least 2 hours at 130±2°C.

22. Cool the crucibles to room temperature in a desiccator and weigh accurately to 0.1mg.

23. Ash the samples in the crucibles for at least 3 hours at 525±15°C. Heat and cool crucibles with caution.

24. Cool crucibles slowly to room temperature in a desiccator and weigh accurately to 0.1mg.

Calculation

$$\% \text{ CRUDE FIBER} = \frac{W2 - (Wt + C)}{Wt} \times 100$$

Wt = Sample weight (g)

W2 = crucibles + residue weight after drying (g)

W3 = crucibles + residue weight after washing (g)

C = Blank

Solvent extraction using the Soxtec extraction system (ether extract/fat analysis)

Equipment name

Soxtec, model: 2050 Material: Analytical balance, aluminium extraction cups, thimbles, thimble adapters, air ventilation oven.

Reagent:

Petroleum ether 60 - 80°C

Procedure

1. Place thimbles fitted with the adaptors on a balance and tare.

2. Weigh 1.000g of well-prepared sample into the thimble and tare. Move them to the thimble stand using the thimble handler.

3. Switch on the Soxtee solvent extraction unit by pressing the MAIN button; the switch lamp should light up.

4. Set the temperature according to the solvent used to achieve a reflux of solvent that is 3-5 drops/second.

5. Select the proper program and check time settings for boiling, rinsing, evaporation, and pre-drying on the CONTROL unit.

6. Open the cold water tap for the reflux condensers with cooling water at approximately 15°C. The flow should be adjusted to 2 L/min to prevent solvent evaporation from the condensers.

7. Put a thin layer of defatted cotton on top of the sample.

8. Use the thimble handler and move the thimbles to the thimble supports. Insert the thimbles into the extraction unit. Attach them to the magnets.

9. Remove the thimble supports.

10. Use the cup holder to insert the aluminium extraction cups (pre-dried at 103 ± 2°C) loaded with 40-60°C petroleum ether.

11. Press the RUN/STOP key. The Soxtee auto fat extraction system will now perform the extraction automatically.

12. Remove the cups. Dry the cups at 103 ± 2°C for 30 minutes or until constant weight.

13. Weigh the cups.

Calculation

$$\% \text{ FAT} = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = Weight of sample (g)

W_2 = Empty extraction cup weight (g)

W_3 = Extraction cup + residue weight (g)

Procedural technique for % ash determination in samples

Materials:

Muffle furnace, conventional oven, crucibles, analytical balance, spatula, desiccator and crucible tongs.

Equipment name:

Carbolite, model:

Procedure: Dry Ashing

1. Dry empty crucibles in the oven at 130 ± 15°C for 30 minutes to remove moisture from the crucibles.
2. Transfer the crucibles into a desiccator and allow to cool at room temperature for approximately 20 minutes.
3. Weigh the empty crucibles and record the mass as W_0.
4. Blend the samples into a powder using a Cyclotec sample mill or a pestle and mortar to increase the surface area.
5. Using an analytical balance, weigh 1.0000g of sample into the crucibles (record as W_1) and ash it in the furnace at 500 ± 15°C for 5-6 hours.
6. Allow the crucibles containing the samples to cool for approximately 30 minutes in the furnace.
7. Using crucible tongs, transfer the crucibles into the desiccator and allow to cool at room temperature for approximately 45 minutes.
8. Weigh the crucibles with their contents and record the final mass as W_2.

Calculation

$$\% \text{ ASH content} = \frac{(W2-W3)}{W1} \times 100$$

MICROBIOLOGICAL IDENTIFICATION

Gram Staining Technique

A smear of the inoculum was prepared on a glass slide and was passed through flame slightly to heat-fix it. The smear was then stained with crystal violet (the primary stain that imparts its colour on the cells). After 60 seconds, the crystal violet was washed off with distilled water, and the smear was flooded with iodine. After 30 seconds, the iodine was washed off under slow running tap and then washed with ethanol (decolourising agent which removes the purple colour from the cells of some species). The ethanol was then rinsed off under slow running tap, and the smear was counter-stained with safranin (secondary stain: a basic red dye). After 30 seconds, the safranin was washed off under slow running tap, blotted dry and examined under the microscope. The Gram-positive bacteria retained the purple dye, while those that lost the purple colour (turning pink) were classified as Gram-negative.

Biochemical Tests Carried Out on Pure Isolates

Catalase Test

Used to differentiate bacteria that produce catalase enzyme. A loopful of bacterial isolate is transferred to a petri dish with 3% hydrogen peroxide. Evolution of oxygen bubbles indicates a positive result.

Sugar Fermentation Test

Fermentation media contain mannitol, a pH indicator (phenol red), and Durham tubes. A yellow colour change indicates acid formation, and gas production is noted in inverted tubes.

Starch Hydrolysis Test

1% soluble starch medium is inoculated with isolates. After incubation, plates are flooded with iodine, and a zone of clearance around the organism is observed.

Catalase Test

This test was used to differentiate bacteria that produce the enzyme catalase. The enzyme acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. In this test, a loopful of the bacterial isolate was aseptically transferred to a clean, sterile, non-vented petri dish for about two to three days, after which 3% hydrogen peroxide was introduced into the petri dish, closed, and tilted so the hydrogen peroxide would make contact with the bacterial growth. The evolution of oxygen or effervescence, observed as the production of gas bubbles, indicated a positive result.

Sugar Fermentation Test

The fermentation medium included a fermentable sugar (mannitol) for energy production, non-fermentable nitrogen sources and other nutritional requirements (as a basal medium), a pH indicator (phenol red), and vertically inverted Durham tubes that collect gas. A 24-hour-old culture of the organism was also used. The basal medium (peptone water) was prepared, and 1.0g of the desired sugar was added, followed by a few drops of phenol red. Five millilitres were dispensed into labelled test tubes containing inverted Durham tubes, corked with cotton wool, and autoclaved at 121°C for 15 minutes. After cooling, the sugar media were then inoculated with a loopful of 24-hour-old culture. The inoculated tubes were then incubated at 37°C for 72 hours, with daily examinations. Formation of acid, indicated by a yellow colour change of phenol red, is indicative of fermentation. Gas production was noted by checking for the formation of gas in the inverted Durham tubes.

Starch Hydrolysis Test

Soluble starch was separately prepared and added to nutrient agar to create a 1% soluble starch medium. The medium was sterilised at 121°C for 15 minutes. It was poured into petri dishes and allowed to cool. The isolates were aseptically inoculated onto the starch agar plates and incubated at 37°C for 45 hours. After incubation, the plates were flooded with iodine, and a zone of clearance or hollow around the test organism was observed.

Indole Test

A cotton wool swab was used to clean the work surface area. After preparing the tryptone soy broth, an inoculum from the pure culture was picked and allowed to go straight down to the bottom of the tube. The tube was then tightly closed and incubated for 24 hours at 37°C. After incubation, 1ml of chloroform and 1ml of Kovac's reagent were added to each test tube containing the inoculum, and then they were mixed together. A colour change from the creamy, yellowish peptone water to a somewhat pink colour indicates a positive indole test.

The Methyl Red and Voges-Proskauer Test

A methyl red and Voges-Proskauer test was used to differentiate between the *E. coli*. Many coliform organisms ferment dextrose rapidly, causing a fall in the pH of the medium. The acidic reaction of the medium is checked using a methyl red indicator. Development of a red coloration is indicative of a positive result for the methyl red test, while a yellow coloration indicates a negative result. The presence of a deep rose colour following the addition of acetylmethylcarbinol represents a positive result. The absence of a rose colour indicates a negative result.

Hydrogen Sulphide Test

Seventeen grams of medium were dispensed into 1 litre of distilled water and heated to dissolve. Ten to twenty millilitres were dispensed into test tubes, autoclaved, and left to solidify in a slanted position. A 24-hour old nutrient broth culture of the organism was stabbed into the butt and streaked along the slope, then incubated at 36°C for 24-48 hours. It was subsequently observed for acid production (pH change), CO_2 production, and blackening due to H_2S production. The results of reactions were recorded; a black change indicated a positive result, while a yellow change indicated a negative result.

Gelatin Hydrolysis

The test was conducted to highlight the proteolysis capabilities of microorganisms or their ability to produce gelatinase.

Procedure

One point seven grams of nutrient agar broth were prepared in 100 ml of distilled water, and 3g of gelatin was added. The mixture was sterilised by autoclaving at 121°C for 15 minutes. The medium was allowed to cool before introducing the organisms. A heavy inoculum of the organism was inoculated and incubated at 37°C for 1 week; the tubes were removed daily and placed in the fridge for 30 minutes to check for liquefaction.

RESULTS

The cultural and biochemical characteristics of bacteria isolated from tomato fruits are presented in Table 1, which details the cultural and biochemical characteristics of bacterial isolates from nutrient agar plates. The results for the first isolate showed cultural characteristics that were creamy, flat, smooth, and round. Gram staining indicated a purple colouration after adding immersion oil, signifying a positive result. Microscopic observation revealed a rod shape and single arrangement. The catalase test was positive, suggesting the probable organism. The second isolate displayed cultural characteristics of red, flat, smooth, and entire colonies. Gram staining was positive, microscopic observation showed a rod shape and single arrangement, and the catalase test was positive. The probable organism for the second isolate is *Klebsiella aeruginosa*. Table 2 presents the morphological and cultural characteristics of fungi isolated from tomato paste. The morphological characteristics of the first isolate showed branched and septate hyphae with greenish, filamentous conidiophores as cultural characteristics. The cultural characteristics were greenish, filamentous, with conidiophores, and rapid growth of black velvety spores. The second isolate's morphological observation revealed septate hyphae with canoe-shaped macroconidia. The third isolate, under microscopic observation, showed a non-septate view with enlarged conidiophores at the tip. The suspected organisms for these samples are *Aspergillus niger*, *Fusarium sp.*, and *Rhizopus stolonifer*.

Results of Tomato Deterioration

The results of tomato fruit deterioration, monitored for a period of 30 days, are presented in Table 3, detailing observations on the spoilage of tomato fruits. Spoilage became evident on the fourth day and progressed to full deterioration by day 28.

During the first week of observation, the tomatoes showed wrinkling and exuded water. By the second week, maggots were observed emerging from the tomatoes, and their presence was intense. In the third week, there was an excess of maggots, which subsequently developed into pupae. The maggots later dried, and the tomatoes, having lost water through evaporation, turned brownish with a reduced biomass.

Proximate Analysis of Tomato Pastes

Table 4 shows the results of the proximate analysis conducted on the tomato pastes and preservatives at the beginning and end of the research. The nutritional parameters demonstrated a reduction by day 28, indicating that day 1 values were significantly higher than day 28 values. The tomato paste preserved with vinegar and lemon exhibited microbial growth and degradation, along with some bubbling. In contrast, the tomato paste preserved with vegetable oil showed no microbial growth.

Table 1: Cultural and biochemical characteristics of samples isolated from tomato fruits

Isolates	Cultural characteristics	Gram reaction	Microscopy	Catalase	Glucose Utilization	H_2S Production	Methly red	Voges Prokaver	Indole test	Gelatin Hydrolysis	Starch Hydrolysis	Probable organisms
	Creamy, flat, Smooth, round	+	Rod, single	+	-	+	+	-	+	+	+	*Klebisiella aeromonas*
	Red flat, Smooth, entire	+	Rod, single	+	-	+	-	+	-	+	-	*Klebisiella aeruginosa*

Table 2 presents the morphological and cultural characteristics of fungi isolated from tomato paste. The morphological characteristics of the first isolate showed branched and septate hyphae conidiophores with greenish, filamentous cultural characteristics. The cultural characteristics included rapid growth of black velvety spores. The second isolate exhibited septate hyphae with canoe-shaped macroconidia during morphological observation. The third isolate, under microscopic observation, displayed a non-septate view with enlarged conidiophores at the tip. The suspected organisms for these samples are *Aspergillus niger*, *Fusarium sp.*, and *Rhizopus stolonifer*.

Table 2: Morphological and cultural characteristics of fungi isolated from tomato paste

Isolates	Morphologial characteristics	Cultural characteristic	Probable organisms
1	Branched and septate hyphae conidiophore with secondary branches, Enlarged conidiophore at the tips, producing round vesicle-like chains.	Greenish filamentous conidiophore, with rapid growing of black velvety spores	*Aspergillus niger*
2.	Conidiophores produced conidia in clusters or single forms. Septate hyphae with canoe-shaped macro conidia	At first, the conidia were cottony and white and thereafter change to brown	*Fusarium sp.*
3.	Hypae were non-septate and there were enlarged conidiophores at the tip producing round vesicle-like chains	Profuse proliferation of filamentous conidiophores	*Rhizopus stolonifer*

The results of tomato fruit deterioration, monitored for a period of 30 days, are shown in Table 3, detailing observations on the spoilage of tomato fruits. Spoilage became evident on the fourth day and progressed to full deterioration by day 28. During the first week of observation, the tomatoes were wrinkled with exudate water. By the second week, maggots were observed emerging from the tomatoes, and their presence was very intense. By the third week, there was an excess of maggots, which later turned into pupae. The maggots subsequently dried, and the tomatoes, with water evaporating, turned brownish with a reduced biomass.

Table 3: Observation of the spoilage stages of tomato fruits

Days	Visual Observation
1	The tomato fruits in both plates A and B looked fresh.
2.	The tomato fruits were fresh looking.
3.	There were sign of spoilage on the tomato fruits in both plates A and B.
4.	On the fourth day. 2 samples of the tomato fruits in plate A and B become soft.
5.	The softness in the tomato fruits in both plates (A and B) became intense. There was deformity in their shapes
6.	In plate A all the four samples were wrinkled and soft producing exudate water. While in plate B only two of the tomato fruits are wrinkled and soft.
7.	I had the same observation as day 6.
8.	Plate A and plate B were getting spoilt gradually, with intense water
9.	All the tomato fruits in the two plates were soft and rotten.
10.	Same observation as day 9.
11.	Two of the tomato fruits in plate A were producing maggots while the other 2 were wrinkled soft and with water. Plate B still remained as seen on the previous day.
12.	Same observation as day 11.
13.	Samples of the tomato fruits in plate A were producing maggots and very intense while only two of the samples in plate B were producing maggots. Other two remained the same.
14.	Same observation as day 13.
15.	Same observation as day 13.
16.	More maggots were produced and the size fo maggot had increased.
17.	Gradually the samples produced more water and bigger maggots on the plates.
18.	Same observations day 17.
19.	The maggots turned into pupae
20.	Same observation as day 19.
21.	The maggots were finally dried.
22.	The water were evaporating and tomatoes turning brownish with reduced biomass.
23-24	Further reduction in biomass.
25-27	The maggots and water were dried.
28-30	The tomato fruits deteriorated completely.

Table 4: Proximate analysis parameters of tomato paste at day 1 and day 28 of treatment application

Treatments	Moisture content%		Crude protein %		Crude fat%		Crude fiber%		Total ash%		CHO%	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Lemon + Tomato	89.00	9.50	2.34	1.99	0.00	0.02	0.93	1.73	0.79	0.67	6.94	5.09
Lemon Only	96.00		0.39		0.12		1.04		0.12		2.33	
Vinegar+ Tomato	89.10	91.50	1.81	1.95	0.96	0.49	0.00	1.23	0.65	0.73	7.58	4.10
Vinegar Only	99.08		0.00		0.00		0.00		0.02		0.00	
Veg oil + Tomato	88.40	85.50	1.93	2.31	2.60	3.71	1.99	1.91	0.80	0.86	4.28	5.71
Veg. oil	0.9		0.22		93.70		0.02		0.00		5.16	
Distilled Water	100	89.00	0.00	1.88	0.00	0.07	0.00	1.24	2.25	0.86	0.00	6.95

2.31%

Best Protein Retention

Vegetable oil preserved paste maintained highest crude protein at day 28.

5.71%

Carbohydrate Content

Vegetable oil treatment showed increased carbohydrate content over time.

28

Study Duration

Complete monitoring period for preservation effectiveness assessment.

Table 4 shows the results of the proximate analysis that was done on the tomato pastes and preservatives at the beginning and at the end of this research work. The nutritional parameter analysis showed that there was a reduction in the nutrient parameters on day 28. Day 1 values are higher than day 28 values. The tomato paste preserved with vinegar and lemon showed signs of microbial degradation and some bubbles. The tomato paste preserved with vegetable oil showed no growth.

DISCUSSION

In this study, spoilage of the tomato fruits started from the first day of purchase. After the tomato fruits were purchased in the market, they were placed inside a plate in the laboratory and kept at room temperature. On the third day, the tomato fruits were exuding water, which became evident on the fourth day of the set-up. Later, they began producing maggots and subsequently dried out, including the maggots, with the fruit finally deteriorating on day twenty-eight. The tomato pastes were preserved with vegetable oil, vinegar, lemon juice and water, which served as the control. Of all the preservatives applied to extend the shelf life of the tomato paste, vegetable oil was observed to be the best. Similar findings have been reported in recent systematic reviews examining biochemical treatments for tomato preservation, where natural preservatives demonstrated varying degrees of effectiveness depending on concentration and application methods (Bwade et al., 2025).

In this study, *Klebsiella aerogenes*, *Aspergillus niger*, *Fusarium sp.* and *Rhizopus stolonifer* bacteria were isolated and identified. During the 30-day monitoring of the tomato set-up, it was observed that the degradation process attracted houseflies and drosophila, which further contaminated the tomato fruits and accelerated the spoilage process. Tomatoes are considered one of the most economically important crops globally. Economically speaking, tomatoes are of tremendous value because they yield more (Hammerschmidt & Franklin, 2005). Thus, vegetable oil could serve as a preservative for tomato paste and an alternative to synthetic chemicals used in tomato paste preservation. This work was designed to ensure tomato paste is always available despite its highly perishable characteristics.

CONCLUSION

In the course of this project work, three different biological materials were used to preserve tomato paste, with vegetable oil providing the best preservation, where its appearance and nutritional value were not adversely reduced. The purpose of shelf life information is to help consumers make safe and informed use of foods. Food shelf life should be considered valid only if the product is bought intact and not damaged. Consumers should always follow manufacturers' storage instructions, particularly regarding the temperature and the use of the product after opening. It is also recommended that consumers consider the shelf life information when buying foods to avoid unnecessary food waste. For example, unlike foods with 'use by' dates, many foods with 'best before' dates will be safe to eat even if the date has passed. It is hereby recommended that consumers should consider the shelf life information when buying foods to avoid food poisoning. Tomatoes are highly perishable, and if not well preserved, they can spoil easily. Tomato fruits should be used within 3 days of purchase to avoid spoilage.

LIMITATIONS

This study provides preliminary insights into the biopreservation of tomato paste using vegetable oil. However, it is essential to acknowledge certain limitations that inform the scope and generalisability of these findings:

- Small Sample Size: The experimental design utilised a limited number of samples, which may not fully represent the variability inherent in tomato paste production and storage. Larger-scale studies are needed to confirm these initial observations.
- Limited Concentration Testing: Only a single concentration of vegetable oil was evaluated. Further research is required to determine the optimal concentration range for maximum preservation effectiveness across various conditions.
- Lack of Sensory Evaluation: The study did not include sensory evaluation data, such as taste, texture, and aroma, which are crucial for assessing consumer acceptance of the preserved tomato paste.
- Duration of Storage Study: The storage period was limited to 28 days. Longer-term studies are necessary to fully understand the efficacy of vegetable oil as a preservative over extended shelf lives.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed for the effective preservation of tomato paste and broader food safety practices:

- Optimal Vegetable Oil Concentration: Implement further studies to determine the precise optimal concentration ranges of vegetable oil for different storage conditions (e.g., ambient vs. refrigerated) to maximise shelf life extension without compromising product quality.
- Storage Temperatures and Packaging: Recommend specific storage temperatures (e.g., cool, dry place at X°C) and suitable packaging requirements (e.g., airtight, opaque containers) that complement vegetable oil preservation to prevent spoilage and maintain nutritional integrity.
- Quality Control Parameters: Establish clear quality control parameters, such as regular microbial counts, pH monitoring, and visual inspections, to consistently monitor the effectiveness of vegetable oil preservation throughout the product's shelf life.
- Guidelines for Commercial Adoption: Develop practical guidelines for food manufacturers on integrating vegetable oil as a natural preservative, including protocols for application, blending, and quality assurance, to facilitate commercial adoption as a safer alternative to synthetic chemicals.
- Timely Processing: Emphasise the importance of processing tomato fruits into paste within 3 days of purchase to ensure initial quality and minimise early spoilage, especially if not immediately preserved.

FUTURE RESEARCH DIRECTIONS

To build upon this research and address the identified limitations, the following areas warrant further investigation:

- **Investigation of Different Vegetable Oil Types and Concentrations:** Explore the preservative capabilities of various types of vegetable oils (e.g., olive oil, sunflower oil, coconut oil) and a wider range of concentrations to identify the most effective options.
- **Combination Studies with Other Biopreservation Methods:** Research the synergistic effects of combining vegetable oil with other natural preservation techniques, such as natural antimicrobials or fermentation, to enhance shelf life and safety.
- **Sensory Evaluation and Consumer Acceptance Studies:** Conduct comprehensive sensory evaluations to assess the impact of vegetable oil preservation on the organoleptic properties of tomato paste and gauge consumer acceptance.
- **Scale-Up Studies for Industrial Application:** Perform pilot-scale and industrial-scale trials to validate the effectiveness and feasibility of vegetable oil preservation in larger production environments.
- **Economic Feasibility Analysis for Commercial Implementation:** Evaluate the cost-effectiveness of using vegetable oil as a preservative compared to synthetic alternatives, considering raw material costs, processing expenses, and potential market advantages.

01

Use Vegetable Oil Preservation

For effective biopreservation and extension of shelf life of tomato paste, the vegetable oil technique of preservation should be used instead of chemical preservatives.

02

Quick Processing

Tomato fruits should be used within 3 days of purchase to avoid spoilage and maintain nutritional quality.

03

Follow Storage Instructions

Consumers should follow manufacturers' storage instructions regarding temperature and use after opening to maximise shelf life.

04

Further Research Needed

Conduct additional research on vegetable oil concentration and other biological preservation methods for tomato paste production.

Recently, people are shying away from chemical preservatives because of health hazards such as cancers. So biological methods of preservation are better, since no health and environmental hazards are associated with them.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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