FUNGAL ANALYSIS AND EFFECTS OF SODIUM METABISULPHITE PRESERVATION ON TOMATO PUREE

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CERTIFICATION

This is to certify the project work was carried out by **Sarah Anuoluwapo LAWAL (Miss)** with matriculation number **LSC1806760** in the Department of Microbiology, Faculty of Life Sciences, and University of Benin, Benin city under the supervision of

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APPROVAL

This project was carried out by **Sarah Anuoluwapo LAWAL (Miss)** under the supervision of **PROF C.E OSHOMA** in partial fulfillment of the award of a Bachelor of Science B. Sc degree in microbiology.

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DEDICATION

I humbly dedicate this project to the Almighty God for His love and mercy over my life. It is by His grace that I was able to bring this project to completion in good health.

ACKNOWLEDGEMENT

My sincerest gratitude goes to the Almighty God who has preserved me all through the period of my stay in the University of Benin. My gratitude also goes to my supervisor as I count it a privilege to have worked under the supervision of **PROF C.E OSHOMA**. My appreciation goes to the head of the department **PROF (MRS) F.I AKINNIBOSUN** and all departmental staff. Special thanks also goes to **Mr. Cedric Obasuyi** for his support in carrying out this project. I would like to express my profound gratitude to my parents, **Mr. Rasaq Lawal** and **Mrs. Mary Lawal** and to my siblings **Lawal Faith, Dada Martha, Ekemezie Dinah and Glory Lawal** for their support financially, morally and the love shown towards me. I'd also like to express and acknowledge my friends; **Becky Ogbar, Esther Otoibhi, Yemi Rufai, Victory Ighadaro, Johnson Darlington** and many others for their support and motivation in carrying out this project.

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ABSTRACT

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated and extensively consumed horticultural crops worldwide. Spoilage of tomato fruit by contaminating microorganism is a major agricultural and economic problem. The search for techniques to prevent or control spoilage is a welcome development. In this study, was aimed at determination of fungal analysis and effects of sodium metabisulphite and pasteurization as a preservative of tomato fruit puree. Fresh, uninfected tomatoes were purchased at Aduwawa tomatoes market, Ikpoba-Okha local government Area, Edo state, and prepared into puree. The samples were treated with the sodium metabisulphite at the concentrations of 0, 0.1, 0.2, 0.3, 0.4 and 0.5g/l and pasteurized at 65°C for 15 mins. The treated samples were stored for a period of 15 days and analyzed every 5 days interval. The analysis carried out were fungal count and identification, pH and titratable acidity. The result showed that total fungal count ranged from 5.00$\times 10^4$ sfu/ml to 1.00$\times 10^4$ sfu/ml. The identified fungal isolates were *Aspergillus niger, Fusarium* sp*, Rhizopus stolonifer, Geotrichum* sp*, Trichoderma* sp*, Candida* sp *and Saccharomyces Cerevisae.* The pH ranged from 4.10±0.07 to 5.80±0.57 while the titratable acidity ranged from 0.083% to 1.345%. From this study, the concentrations of 0.4g/L and 0.5g/L proved to be effective in preserving the tomato puree and inhibiting fungal growth.

CHAPTER ONE

1.0 INTRODUCTION

Botanically, tomato (***Solanum lycopersicum)*** is a fruit, a berry consisting of ovary, together with its seeds of a flowering plant. It belongs to the *Solanaceae* family widely grown throughout the warm temperate and tropical region of the world (Nyam *et al,* 2018). Tomatoes have formed a very important aspect of human diet in Nigeria and are also processed in the form of various products such as tomato puree, tomato juice, ketchup and paste. Tomatoes are rich in Vitamin C and provide potassium, Iron, Phosphorus and B vitamins which are a good source of dietary fiber. Tomato is a high water-containing perishable and is very much susceptible to microbial spoilage. Over the years, various methods have been developed for the preservation of tomatoes such as canning, sun-drying and use of chemical preservatives. Fresh tomatoes are appetizing but cannot be stored for a long period. Tomatoes contain a lot of water, making them more prone to deterioration due to the action of numerous microbes. (Chawafambira *et al,* 2021).

Tomato puree is a food product prepare from processing of tomatoes as their main constituent. Concentration of tomato juice was made for puree preparation containing minimum 8.37% total solids. Considering nutritional aspects tomato puree is healthier than other processed tomato products like paste, ketchup and sauce. Normally by cooking and processing the nutritional value of fruits and vegetables decreases but processing of tomato into puree increase the bioavailable lycopene concentration (Alam *et al,* 2018). Tomato puree has a high moisture content and is vulnerable to microbial spoilage and unfit for consumption. One way by which this can be prevented is through the use of chemical preservatives. These chemicals are substance of no nutritional significance. They are added to foods as antimicrobial agents to preserve them from deterioration and extend shelf life.

Food deterioration refers to a variety of changes in food that make it less appealing or even harmful to consumers (Tandel *et al,*2022). Changes in fragrance, taste, appearance, or texture may accompany these changes. Among the major challenge facing production and marketing of tomato is the rapid quality deterioration, reduced shelf-life. The quality deterioration of tomatoes leads into undesirable changes which are characterized by changes in color, texture, flavour, and nutritive value (Kader, 2005). Overall, the management of these quality challenges may result in reductions in availability, edibility, quality as well as wholesomeness, contributing to the incidence of postharvest food losses and subsequent financial losses. Some of the major factors contributing to these post-harvest losses are inadequate post-harvest handling, lack of appropriate processing technology, poor storage facilities and poor infrastructures. The microbial degradation of tomato lowers the market value and nutritional quality of the product. Contaminations with mycotoxins, which form aflatoxins in humans after inhalation or ingestion, render tomato fruits unfit for consumption, resulting in food poisoning (Bello *et al,* 2016).

Preservation of tomato puree to prevent spoilage can be achieved by the addition of a chemical preservative known as sodium metabisulphite. It is an inorganic compound with molecular formula NaS2O5 and sometimes referred to as disodium metabisulphite and is prepared by treating a solution of sodium hydroxide with Sulphur dioxide. It is a white or yellowish crystalline powder with a light odor of rotten egg (Golfshan *et al,* 2014). Sodium metabisulphite is extensively used as food preservatives (due to their antimicrobial activity) and have been accepted as safe by the Food and Drug Administration since 1959 (Noorafsham *et al,* 2014).

1.1 AIM AND OBJECTIVE

The aim of the study was to determine fungal quality of tomato fruit puree product pasteurized, treated with sodium metabisulphite and stored under anaerobic condition.

The specific objectives were to:

1. enumerate, isolate and characterize the fungi associated with tomato fruit puree pasteurized, treated with sodium metabisulphite and stored under anaerobic condition.
2. examine the effect of the chemical preservative sodium metabisulphite on the physiochemical characteristics of tomato puree.
3. determine the pH, titratable acidity of tomato fruit puree preserved and treated with sodium metabisulphite under anaerobic condition.

1.2 JUSTIFICATION OF STUDY

Preservation techniques play a vital role in extending the shelf life and maintaining the quality of perishable food products. Tomato puree is a widely consumed and commercially important product, often used as a base in various culinary applications. However, due to its high water content and susceptibility to spoilage, tomato puree requires effective preservation methods to ensure its long-term stability and safety for consumption. This study aims to investigate the use of sodium metabisulphite as a preservative for tomato puree and provide a comprehensive justification for its application and also aims to assess the mycological stability of tomato puree treated with different concentrations of sodium metabisulphite to determine the optimal level that effectively controls microbial growth, ensuring the safety of the product during storage.

Preservation methods should not only ensure product quality but also be economically feasible for large-scale production. Sodium metabisulphite is a cost-effective preservative widely available in the market. Conducting a cost-benefit analysis will provide valuable insights into the economic viability of using sodium metabisulphite for tomato puree preservation, allowing food producers to make informed decisions regarding its implementation.

This study's justification lies in its potential to enhance the mycological stability, nutritional preservation, flavor and color retention, and economic viability of tomato puree. The findings from this research will contribute to the development of efficient preservation strategies, ensuring the availability of safe and high-quality tomato puree to consumers while minimizing food waste and economic losses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF TOMATOES (*Solanum lycopersicum*)

Tomato is one of the most widely cultivated vegetable crops in Africa and in the world as a whole and it belongs to the nightshade family, *Solanaceae*. Tomato classification has been the subject of much discussion and the diversity of the genus has led to reassessment of earlier taxonomic treatments. For a long time tomatoes were known as *Lycopersicon esculentum*, but recent research has shown that they are part of the genus *Solanum* and are now again broadly referred to as *Solanum lycopersicum.* Tomato has its origin in the South American Andes and it was brought to Europe by the Spanish conquistadors in the sixteenth century and later introduced from Europe to southern and eastern Asia, Africa and the Middle East (Shankara *et al.,* 2005). It is botanically a fruit however it is considered a vegetable for culinary purposes.

However, the tomato has much lower sugar content than other edible fruits, and is therefore not as sweet. 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. Tomato can be cultivated on almost any type of the soil. They are grown both in summer and winter but those grown in winter are superior, because they contain more solids and contain usually from 7 to 8.5% of total solids of which about 1% is skin and seeds. Tomato is a popular and versatile food that comes in over a thousand different varieties that vary in shape, size, and colour such as cherry tomatoes, roma tomatoes (India hybrid), heirloom tomatoes, plum tomatoes, beefsteak tomatoes, grape tomatoes, and campari tomatoes among others. Ripe tomatoes are red or orange in colour and slightly soft to the touch. Tomatoes for fresh market are under ripened and packed in crates to avoid damage while those for canning should be fully ripe. Fully ripe tomatoes can stay for 3-4 days under ambient temperature or longer when stored in the refrigerator but it is best kept at room temperature (Olumakaiye ., 2014). Tomato stored cold will still be edible but tend to lose flavour. Tomato is a versatile product that can be eaten fresh or processed into wide range of products such as tomato paste, tomato puree, ketchup and tomato juice.

Tomatoes grows best in light, free draining, and fertile loam soil with pH of 5 – 7. However tomatoes can be grown in a variety of soils (Purseglove, 1988, Naika et al., 2005). Regarding fertilizer requirements, tomatoes require an abundance of the three major elements namely, nitrogen, phosphorus, and potassium. Adequate soil nitrogen application is important to enhance foliage growth which has a major bearing on crop maturity and protects the fruits from sunscald. Phosphorus influences fruit quality by stimulating vigorous root growth that enables more nutrients to enter the plant thereby promoting sturdy stem growth and healthy leaf formation. They also use large amount of potassium. This element is important in stimulating early plant growth and regulating normal carbohydrate and protein metabolism. It is relatively a short duration crop and gives a high yield, it is economically attractive. After fruit setting, fruit ripens over a period of 45 - 70 days, depending upon the cultivar, climate and growth conditions. The fruit continues growing until the stage of green ripeness. Three fruit developmental stages are noted. Ripening occurs as the fruit changes color from light green to off-white, pink, red, and finally dark red or orange. Depending on the distance and time to market, harvest may occur anytime between the pink to dark red stage, the later stages producing more flavorful fruit.

Tomato is a high water-containing perishable and is very much susceptible to microbial spoilage. Over the years, various methods have been developed for the preservation of tomatoes such as canning, sun-drying and the use of chemical preservatives (Nyam *et al*., 2018).

2.2 NUTRITIONAL COMPOSITION OF TOMATO

Tomatoes contain numerous phytochemicals, the most well-known of which is lycopene. Tomatoes are one of the important vegetables/fruits in our diet, since they are rich in health valued food components such as carotenoid (lycopene), ascorbic acid, (vitamin C), vitamin E, folate and dietary fiber. Carotenoids are present in many vegetables and fruit but lycopene is more restricted in its distribution, being concentrated in tomatoes, guava, rosehip, watermelon and pink grapefruit (Hedges., 2005). Water comprises 90% of the fresh weight of tomato and the size is influenced by the availability of water to the plant. The presence of large amount of water in the fruit makes it perishable. As the tomato fruit develops, starch decreases while carbohydrates such as sucrose and reducing sugars increase. Sugars are mostly found in ripe fruit and starches in unripe fruit. In a ripe tomato, solids form about 5-7% of the total fruit weight. About half of the solids comprise sugars and one eighth is acids. The main sugar in tomatoes is glucose and the pH of fruit is normally between 4.0 and 4.5.

2.2.1 Lycopene

Lycopene ($C_{40}H_{56}$) is a carotenoid pigment that is primarily responsible for the deep red color of ripe tomato fruits and tomato products. It is absorbed in the human body and is one of the most common circulating carotenoids (Muhammed *et al*., 2015).

2.2.2 Carbohydrates and fiber

Tomatoes are low in calories, with approximately 18 kcal per 100 grams. They are primarily composed of carbohydrates, providing 3.9 grams per serving. Crucially, tomatoes are an excellent source of dietary fiber, with 1.2 grams per 100 grams. Fiber plays a vital role in digestive health, promoting regular bowel movements, and reducing the risk of conditions such as constipation and diverticulitis.

2.2.3 Ascorbic acid

Tomatoes are known for their relatively high content of ascorbic acid, commonly known as vitamin C. Ascorbic acid is an essential water-soluble vitamin that acts as an antioxidant in the body, playing a vital role in various physiological processes.

The ascorbic acid content in tomatoes can vary depending on factors such as tomato variety, ripeness, and growing conditions. On average, tomatoes contain around 14 milligrams of vitamin C per 100 grams of the fruit. However, this value can fluctuate, with some varieties of tomatoes containing higher levels of ascorbic acid (Pekar *et al*., 2016).

2.3 POTENTIAL HEALTH BENEFITS OF TOMATO

Tomatoes are loaded with all kinds of health benefits for the body. They are in fact, a highly versatile health product and due to their equally versatile preparation options, there's really no reason to neglect the tomato as part of a healthy diet. One of the most well-known tomato eating benefit is its' Lycopene content. Lycopene is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and diseases. Free radicals in the body can be flushed out with high levels of Lycopene, and the tomato is so amply loaded with this vital anti-oxidant that it actually derives its rich redness from the nutrient. Lycopene is not a naturally produced element within the body and the human body requires sources of Lycopene in order to make use of this powerful anti-oxidant. While other fruits and vegetables do contain this necessary health ingredient, no other fruit or vegetable has the high concentration of Lycopene that the tomato takes pride in. Studies involving the tomato have cropped up all over the world of medical science. There are more health benefits derived from eating a tomato than the scientific community is able to print, at least yet. These studies have proven not only the benefits in preventing cancer, heart disease as well as high cholesterol are also in the tomato's sights. The health benefits of tomatoes are becoming more and more documented every day as we learn new uses of it. (Debjit *et al*., 2012).

2.3.1 Medicinal uses of tomatoes

- Tomatoes are good for the skin: Tomatoes contain a high level of lycopene, which is a substance that is used in some of the more pricy facial cleansers that are available for purchase over-the counter
- Tomatoes help prevent several types of cancer: A number of studies have been conducted that indicate that the high levels of lycopene in tomatoes works to reduce chances of developing prostate, colorectal and stomach cancer. Lycopene

is a natural antioxidant that works effectively to slow the growth of cancerous cells. Cooked tomatoes produce even more lycopene.

- Tomatoes help maintain strong bones: Tomatoes contain a considerable amount of calcium and Vitamin K. Both of these nutrients are essential in strengthening and performing minor repairs on the bones as well as the bone tissue.
- Tomatoes provide essential antioxidants: Tomatoes contain a great deal of Vitamin A and Vitamin C. This is primarily because these vitamins and beta-carotene work as antioxidants to neutralize harmful free radicals in the blood. Free radicals in the blood stream are dangerous because it may lead to cell damage. Remember, the redder the tomato is, the more beta-carotene it contains. In addition, it is important to keep in mind that cooking destroys the Vitamin C, so for these benefits, the tomatoes need to be eaten raw.
- Tomatoes are good for the heart: Because of the Vitamin B and potassium in tomatoes, they are effective in reducing cholesterol levels and lowering blood pressure. Therefore, by including tomatoes in regular balanced diet one can effectively prevent heart attacks, strokes as well as many other heart related problems that may threaten one’s life.

Tomatoes are also good for liver health. Tomatoes detoxify the body because of chlorine and sulfur present in it. Natural chlorine stimulates the liver and its function is filtering and detoxifying the body waste. Sulfur protects the liver from cirrhosis. According to herbalists, tomatoes and its product can reduce the cardiovascular diseases as lycopene present in it. Lycopene is a very powerful antioxidant which is present in red tomato and helps in prevent cancerous cell formation and other health disorders. Free radicals present in the body can be flushed out by lycopene present in tomato. The pulp of fruit is used as skin-wash for people with oily skin. The tomato fruits are used as an easy first aid treatment for burns, scalds and sunburn. The strong smell of this plant is useful to repel insects from nearby plant. The decoction of the root is useful in the treatment of toothache/ Tomatoes are used to cure scorpion sting and fresh fruit is used by Americans orally for kidney and liver problems (Pushpa., 2021).

2.4 FUNGAL DISEASES ASSOCIATED WITH TOMATO

Diseases are a major limiting factor for tomato production. Diseases are often considered as one of the major limiting factors in the cultivation of tomato. Tomato crops are highly affected by diseases, which causes dramatic losses in agriculture economy (Brahimi *et al*., 2017).

Fungi are the most common cause of infectious plant diseases and can be very destructive. Some of the most common fungal diseases that infect tomatoes include Damping-off, Early blight, Septoria leaf spot, Late blight, Fusarium wilt, Grey leaf spot, Powdery mildew, White mold, Alternaria stem canker, Corky root rot, Didymella stem rot, Fusarium crown and root rot, Fusarium foot rot, Southern blight, Buckeye rot. These diseases are contagious and can spread from plant to plant in a field, often very rapidly when environmental conditions are favorable.

2.4.1 Fusarium wilt

The disease is caused by *Fusarium oxysporum f. sp. lycopersici* that can cause significant yield losses of tomato production in greenhouse, high tunnel, and field. Wilt diseases are caused by pathogens that invade the vascular system (xylem tissue) and disrupt water flow through the plant (Kumar *et al.,* 2018). The initial symptoms of the disease are drooping and yellowing of leaves, often on one side of the plant, which may recover during the evening hours. As the disease progresses, wilting of the plant gets worse and become permanent. The disease also causes stunted growth of the plant.

2.4.2 Damping off

Damping-off is an important disease of tomato, which causes significant losses in nurseries on young susceptible transplants. Damping-off is caused by a number of fungi including, *Pythium* species (spp), *Rhizoctonia* spp, *Fusarium* spp and *Phytophthora* spp. Conditions for the development of this disease are high temperature, high humidity, high soil moisture, poor aeration, high levels of nitrogen fertilizer, and closely sown seed [1] Damping off of tomato occurs in two stages, i.e. the pre-emergence and the post-emergence phase. In the pre-emergence the phase the seedlings are killed just before they reach the soil surface. The young radical and the plumule are killed and there is complete rotting of the seedlings. The post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level. The infected tissues become soft and water soaked. The seedlings topple over or collapse (Kumar *et al.,* 2018).

2.4.3 Early blight

Early blight is a common leaf-spotting fungal disease of tomato. Extensive defoliation from early blight exposes fruit to sunscald and increases fruit rot. Early blight also attacks stems and fruit. Early blight of tomato is caused primarily by the fungus *Alternaria linariae.* It appears as large, irregular spots with yellow halos on leaves that eventually yellow. The disease first appears on lower older leaves and moves upward as the plant becomes mature. Older leaves are more susceptible than younger leaves. As disease progresses, heavy defoliation occurs, raising the respiration rate and lowering the photosynthetic rate. The infection on fruit causes dark, sunken, leathery and purple lesions on the stem-end. These lesions expand to a significant size and extend deep into the flesh of the fruit. Infected fruits mostly drop prematurely, and those reaching to maturity also become unmarketable (Adhikari et al., 2017).

2.4.4 Late Blight

Late blight is a water mold caused by the fungus *Phytophthora infestans.* Leaves have large, dark brown blotches with grayish edges that turn to large sections of dry brown foliage (Khaire *et al*., 2021). Symptoms of late blight may be found on any above-ground part of the tomato plant. Infected leaves typically have green to brown patches of dead tissue surrounded by a pale green or gray border. When the weather is very humid and wet, late blight infections can appear water-soaked or dark brown in color, and are often described as appearing greasy. White, fuzzy growth may be found on the undersides of leaves or on lower stems. Stem and petiole lesions are brown and are typically not well defined in shape. Discoloration may also occur on the flowers, causing them to drop. Symptomatic tomato fruits appear mottled, often with golden to dark brown, firm, sunken surfaces.

2.4.5 Septoria leaf spot

Septoria leaf spot, caused by the fungus *Septoria lycopersici*, is the most common foliar disease of tomatoes. It first appears as small, water-soaked spots that soon become circular spots about 1 ⁄8 inch in diameter. The lesions gradually develop grayish white centers with dark edges. The light-colored centers of these spots are the most distinctive symptom of Septoria leaf spot. When conditions are favorable, fungal fruiting bodies appear as tiny black specks in the centers of the spots. Spores are spread to new leaves by splashing rain. Heavily infected leaves turn yellow, wither, and eventually fall off. Lower leaves are infected first, and the disease progresses upward if rainy weather persists (Kumar *et al.,* 2018).

2.4.6 Southern blight

Southern blight is caused by *Sclerotium rolfsii*, a soil-borne fungus that is almost hard to eradicate, even though it remains at relatively low levels. Near the surface of the soil, the fungus infects the lower stem of the plant. It is called Southern blight because it does not live in frozen soil for long stretches and therefore only thrives in hot weather. High humidity and soil moisture and mild to hot temperatures (29-35 0C) favor Southern blight. A fast wilting of the entire plant is the initial symptom of southern blight. Near the soil line, a water-soaked lesion on the stem quickly spreads, turns brown, and girdles the stem. The fungus develops white fungal colonies around the infectious stem (mycelia or hyphae) and can be seen on the soil surrounding the crop (Khaire *et al.,* 2021).

2.5 TOMATO PUREE

Tomato puree is a food product prepare from processing of tomatoes as their main constituent. Tomato puree is a thick, smooth consistency product made by cooking and straining tomatoes to remove the seeds, skin, and any remaining solids. It is widely used in cooking and food preparation due to its concentrated flavor and versatile nature.

Tomato puree has a rich and intense tomato flavor, as it is made from concentrated tomatoes. Its texture is smooth and free from any chunks or solid pieces. The consistency can vary depending on the cooking time and straining method used, with some purees being thicker or more watery than other. Tomato puree is a versatile ingredient that adds depth and flavor to a wide range of dishes. It serves as a base for many sauces, soups, stews, and curries. It can be used as a foundation for pasta sauces, pizza sauce, chili, or as an ingredient in tomato-based dishes. Tomato puree can also be used to enhance the flavor of dips, dressings, and marinades. The origin of tomato puree can be traced back to the practice of preserving and utilizing tomatoes, which have a long history in Mesoamerica. Tomatoes were first cultivated by ancient civilizations in the region that is now Mexico and parts of Central and South America. They were used in various culinary preparations, and methods of processing tomatoes into concentrated forms likely developed as a means of preservation.

The nutritional composition of tomato puree is similar to that of fresh tomatoes, as it is essentially concentrated tomato flesh. It retains the vitamins, minerals, and antioxidants present in tomatoes, such as vitamin C, vitamin A, potassium, and lycopene. However, the cooking process may result in some loss of heat-sensitive nutrients like vitamin C. It is important to note that some brands of tomato puree may contain added salt or other ingredients, so it is advisable to check the label for specific nutritional information.

Tomato puree is a processed food product consisting only of pulped tomatoes as its major ingredient. It is usually prepared by vacuum concentration of tomato juice and must contain at least 8.37% of tomato solids. Tomato puree has been found to be nutritionally better than other forms of processed tomato such as tomato ketchups, tomato paste and tomato sauce. Unlike most fruits and vegetables where nutritional content decreases with cooking, processing tomato into puree increases the concentration of bioavailable lycopene. The rationale for concentration of tomato puree is to reduce the moisture content and increase the acidity to a level that will prolong shelf life during storage, to reduce colonization by microorganisms and to make tomato available all year round (Ekundayo *et al*., 2015).

Tomato puree is typically available in cans or jars and can be stored at room temperature before opening. Once opened, it should be refrigerated and consumed within a few days. Some tomato puree products may come in re-sealable packaging for easier storage.

2.6 OVERVIEW OF PRESERVATION TECHNOLOGY

The quality of food can be adversely affected by physical, chemical, biochemical and microbiological processes. Quality deterioration caused by microorganisms may include a wide range of types of spoilage that are undesirable commercially, because they limit shelf life or lead to quality complaints, but are safe from a public health point of view. More seriously, the presence or growth of infectious or toxicogenic microorganisms (foodborne pathogens) represent the worst forms of quality deterioration, because they threaten the health of the consumer. The quality of produce cannot be improved after harvest; nevertheless it remains possible to slow down the rate of undesirable changes and maintain the quality for a longer time. In general, it is defined as the degree of fitness for use or the condition indicated by the satisfaction level of consumers, it can also be defined using many factors, including appearance, yield, eating characteristics, and microbial characteristics, but ultimately the final use must provide a pleasurable experience for the consumer. When food has deteriorated to such an extent that it is considered unsuitable for consumption, it is said to have reached the end of its shelf life. In studying the shelf life of foods, it is important to measure the rate of change of a given quality attribute. In all cases, safety is the first attribute, followed by other quality. Therefore, while the aim of effective food preservation is to control all forms of quality deterioration, the overriding priority is always to minimize the potential for the occurrence and growth of food spoilage and food poisoning microorganisms.

2.7 FOOD PRESERVATION METHODS

Some preservation methods require the food to be sealed after treatment to prevent recontamination with microbes; others, such as drying, allow food to be stored without any special containment for long periods. Preservation processes include:

- Heating to kill or denature micro-organisms (e.g. boiling)
- Dehydration (drying)
- Osmotic inhibition (e.g. use of syrups)
- Low temperature inactivation (e.g. freezing)
- Salting
- Food irradiation
- Use of preservatives

Based on the mode of action, the major food preservation techniques can be categorized as (1) slowing down or inhibiting chemical deterioration and microbial growth, (2) directly inactivating bacteria, yeasts, molds, or enzymes, and (3) avoiding recontamination before and after processing.

2.8 OVERVIEW OF FOOD PRESERVATIVES

Food additives are substances added intentionally to foodstuff, to increase the durability of the product and enhance or modify its properties, including its appearance, flavor or structure, provided it does not detract from its nutritional value. Substances can be of natural or synthetic origin, usually without appreciable nutritional value, that are added to food in small amounts during the manufacture (industrial change or during packaging). Preservatives, also known as antimicrobial agents, are used to extend the shelf-life of foods by protecting them against deterioration caused by microorganisms. (Silva and Lidon, 2016). Preservatives are often added to food to prevent their spoilage, or to retain their nutritional value and/or flavor for a longer period. The basic approach is to eliminate microorganisms from the food and prevent their growth. This is achieved by methods such as a high concentration of salt, or reducing the water content. This inhibits spoilage of the food item by microbial growth. Preservatives can be divided into two types, depending on their origin. Class I preservatives refers to those preservatives which are naturally occurring, everyday substances. Examples include salt, honey and wood smoke. Class II preservatives refer to preservatives which are synthetically manufactured

Food preservatives traditionally has three goals: the preservation of nutritional characteristics, the preservation of appearance, and a prolongation of the time that the food can be stored. (Abdulmumeen *et al*., 2012).

2.9 USE OF CHEMICALS

The use of chemicals in foods is a well-known method of food preservation. Wide varieties of chemicals or additives are used in food preservations to control pH, as antimicrobials and antioxidants, and to provide food functionality as well as preservation action. Many legally permitted preservatives in foods are organic acids and esters, including sulfites, nitrites, acetic acid, citric acid, lactic acid, sorbic acid, benzoic acid, sodium metabisulphite, sodium benzoate, methyl paraben, ethyl paraben, propyl paraben, and sodium propionate.

2.9.1 Sodium metabisulphite

Sodium metabisulphite is a chemical compound with the formula $Na_2S_2O_5$. It is also known as sodium pyrosulphite and sodium disulphite. $Na_2S_2O_5$ is an ionic compound containing the sodium cation (Na^+) and the metabisulphite anion ($S_2O_5^{2-}$). In its standard state, sodium metabisulphite exists as a white or yellowish-white powder and is commonly used in the salt form due to its high solubility in water. Sodium metabisulphite is also known as E223 in the food industry. Sulphites are primarily used as antioxidants or antimicrobial agents to prevent or reduce the discoloration of light colored fruits and vegetables. Sulfur dioxide, the active antimicrobial form, is a colorless gas that was first used to treat wines in Rome and was used to treat cider in the 17thcentury. This compound has a faintly pungent odor similar to that of sulfur dioxide. It is important to note that sodium metabisulphite may induce allergic reactions in humans who are sensitive to sulfites.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 SAMPLE COLLECTION

The samples were obtained from the Aduwawa tomatoes market, Ikpoba-Okha local government Area, Edo state, Nigeria. The samples were then transferred to the microbiology laboratory for processing. Only ripe, whole and uninfected tomato fruits were selected for use.

3.2 MATERIALS USED

Sterile jars, blender, reagents, test tubes, bijou bottles, petri-dishes, media, microscope, glass slides, distilled water, sodium metabisulphite.

3.3 STERILIZATION OF GLASSWARE

All glassware including conical flask, bijou bottles, and slides were washed thoroughly with cleansing agent and rinsed in clean water. They were then sterilized by autoclaving for 5mins.

3.4 SAMPLE PROCESSING

The samples were sorted, washed twice in sterile water and then thrice in hot water after which they were transferred to a bowl containing freshly boiled water. This was done to soften the tomatoes and eliminate surface microorganisms. This process is known as blanching. After blanching for about an hour, the sample was transferred to a sterilized container. The blender was sterilized by soaking overnight in a solution of water, ethanol and acetone. It was then rinsed thoroughly in hot water the next day before blending. The samples were blended smoothly and the blender was re-washed with hot water. After blending, the puree was transferred to a sterile container. All procedures were carried out aseptically.

3.4.1 Preparation of preservative solution

Preparation of different concentrations of sodium metabisulphite was carried out. Concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5g/L were prepared. 10mls of the solution was dispensed into clean jars and sterilized at 121 degrees Celsius for 15mins. After sterilizing, the jars were allowed to cool and about 100mls of prepared tomato puree were transferred into the jars containing varying concentrations of the preservative following aseptic procedures. The bottles were sealed tightly and stored for 15 days with a 5 days analysis interval. The analysis carried out were microbiological analysis, pH, and titratable acidity.

3.5 SENSORY EVALUATION OF SAMPLE

Organoleptic observation involving notable changes in color, smell, consistency and visible cell growth were carried out at every 5 days interval for a period of 15 days.

3.6 MICROBIOLOGICAL ANALYSIS OF SAMPLE

The stored samples were analyzed every 5 days including day 0 for a period of 15 days. The microbiological analysis carried out included enumeration, characterization and identification.

3.6.1 Preparation of culture media

The media used was potato dextrose agar. This medium was used to culture fungi. The media was prepared from commercially available dehydrated powder, available from most suppliers of the culture media. In the preparation, 39g of potato dextrose agar powder was dissolved in 1Litre of distilled water in a conical flask and then covered tightly with aluminum foil. It was mixed thoroughly and sterilized by autoclaving at 121C for 15mins and then left to cool.

3.6.2 Enumeration

9mls of sterile peptone water was pipetted into bijou bottles and 1ml of the puree sample was pipetted into 45mls of sterile peptone water. From here, each sample was serially diluted up to 5 folds. Using pour plate technique, 0.1ml of the diluent was aseptically transferred to petri dishes after which cooled molten agar was poured. The plates were rocked gently and then incubated at 25°C. The fungal plates were incubated for 48-72 hours. After incubation, the plates were observed and visible, distinct colonies were counted.

3.6.2.1 Sub-culturing of microorganisms

Freshly prepared PDA was poured into already sterilized bijou bottles, then allowed to solidify. Using streaking method, the old plates containing the colonies were used to isolate the individual fungal cultures into slants and afterward stored under refrigeration.

3.7 CHARACTERIZATION AND IDENTIFICATION OF FUNGAL ISOLATES

3.7.1 Morphological characteristics and microscopy

Fungal colonies were examined for their morphological characteristics.

Microscopy was performed thereafter.

3.7.2 Microcopy

A drop of lactophenol cotton blue was put on a clean glass slide and the fungal isolate was smeared on the surface using a sterile wire loop. A glass cover slide was then placed over the smear and then viewed under the microscope.

3.8 DETERMINATION OF PH

PH was determined using the pH meter.

3.9 DETERMINATION OF TITRATABLE ACIDITY

100mls of 0.2M NaOH and 100mls of water was mixed together. 1g of each puree sample was diluted in 2mls of distilled water and mixed. Titration was carried out by titrating 2mls of the filtrate against NaOH using 2 drops of phenolphthalein as the indicator. End point was read when the solution turned pink. The formula for calculating titratable acidity is given below;

% titratable acidity = $\frac{\text{N x V x M}}{W}$

: Where N= Normality of NaOH (0.1)

V= Average titer value

M= Milli-equivalent of citric acid (0.06404)

W= Sample weight (1g)

CHAPTER FOUR

RESULTS

Table 4:1 shows the total fungal count in stored tomato puree treated with sodium metabisulphite over the course of 15 days. Potato dextrose agar was used. At the end of the storage duration (15 days), UP (control) recorded the highest fungal count. Samples P 0.4 and P 0.5 inhibited fungal growth thereby leading to no count.

Table 4:2 shows the pH recorded for all sample groups over the period of 15 days. On day 0, the highest pH was recorded by UP (control) (4.60±0.28) while the lowest was recorded by P 0.1 and P 0.00 (4.10±0.14). At the end of storage duration (15 days), UP (control) recorded the highest pH at (5.80±0.57) and P 0.5 recorded the lowest pH (4.1±0.07).

Table 4:3 presents the titratable acidity concentration realized from preserved tomato puree samples with sodium metabisulphite. On day 0, P 0.2 and P 0.4 recorded the highest concentration (0.941%) while P 0.3 recorded the lowest concentration at (0.083%). At the end of the storage, the highest concentration (0.877%) was recorded in P 0.00 and the lowest concentration (0.403%) was recorded in UP (control).

Table 4:4 shows the cultural, morphological and microscopic characteristics of the isolated fungi from the stored tomato puree samples treated with sodium metabisulphite.

Figure 1 shows the percentage frequency occurrence of the isolated fungi from stored tomato puree treated with sodium metabisulphite.

Table 4:5 shows the distribution of isolated fungi from stored tomato puree treated with sodium metabisulphite. *Saccharomyces cerevisae* had the highest occurrence.

Table 4:1: Total Fungal Count (x 10^3 sfu/ml) of Tomato Puree Treated with Sodium Metabisulphite Over the Course of 15 days.

SAMPLE	Storage duration (days)			
	0	5	10	15
UP (control)	7.00×10^4	1.30×10^5	2.70×10^5	OG
P 0.00	-	7.00×10^4	2.30×10^5	4.20×10^5
P 0.1	-	2.00×10^4	4.00×10^4	5.00×10^4
P 0.2	-	-	4.00×10^4	3.00×10^4
P 0.3	-	-	2.00×10^4	3.00×10^4
P 0.4	-	-	-	-
P 0.5	-	-	-	-

Where UP is unpasteurized without preservative

P 0.0 is pasteurized without preservative

P 0.1-0.5 is pasteurized with preservative

OG is overgrowth

(-) is No growth

Table 4:2: pH of Tomato Puree Samples Treated with Sodium metabisulphite over the course of 15 days

SAMPLE	Storage duration (days)			
	0	5	10	15
UP (control)	4.60±0.28	4.80±0.14	3.90±0.35	5.80±0.57
P 0.00	4.10±0.14	4.50±0.35	4.60±0.42	4.40±0.28
P 0.1	4.20±0.00	5.60±0.28	4.40±0.42	4.40±0.07
P 0.2	4.30±0.07	5.50±0.49	4.50±0.49	4.50±0.21
P 0.3	4.20±0.21	5.20±0.28	4.10±0.21	4.30±0.14
P 0.4	4.10±0.14	5.60±0.07	4.20±0.07	4.30±0.00
P 0.5	4.30±0.14	4.40±0.14	4.30±0.35	4.10±0.07

Where UP is unpasteurized without preservative

P 0.0 is pasteurized without preservative

P 0.1-0.5 is pasteurized with preservative

Table 4:3: Titratable Acidity of Tomato Puree Treated with Sodium Metabisulphite Over the Course of 15 days (%).

SAMPLE	Storage duration (days)			
	0	5	10	15
UP (control)	0.279	1.069	0.787	0.403
P 0.00	0.787	0.768	1.025	0.877
P 0.1	0.787	0.814	1.088	0.576
P 0.2	0.941	0.814	1.025	0.787
P 0.3	0.083	0.768	1.345	0.852
P 0.4	0.941	0.979	1.172	0.467
P 0.5	0.814	1.069	0.877	0.749

Where UP is unpasteurized without preservative

P 0.0 is pasteurized without preservative

P 0.1-0.5 is pasteurized with preservative

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Table 4:4: Cultural, Morphological and Microscopic Characteristics of Isolated Fungi Treated with Sodium Metabisulphite Over the Course of 15 days

ISOLATES	MORPHOLOGY	MICROSCOPY	POSSIBLE IDENTITY
1	Black center with white margin	Branched septate hyphae Globose conidia	*Aspergillus niger*
2	Fluffy white growth	Septate hyphae	*Fusarium* sp.
3	White cottony growth with black dots	Septate hyphae Large sac-like sporangia	*Rhizopus stolonifer*
4	White creamy cottony growth	Branching hyphae No conidia	*Geotrichum* sp.
5	White to green cottony growth	Branched septate Oval conidia Spherical conidium	*Trichoderma* sp.
6	Round, dry, creamy growth	Oval blastospores No hyphae	*Saccharomyces cerevisae*
7	Smooth white growth	Round blastoconidia in singles or clusters	*Candida* sp.

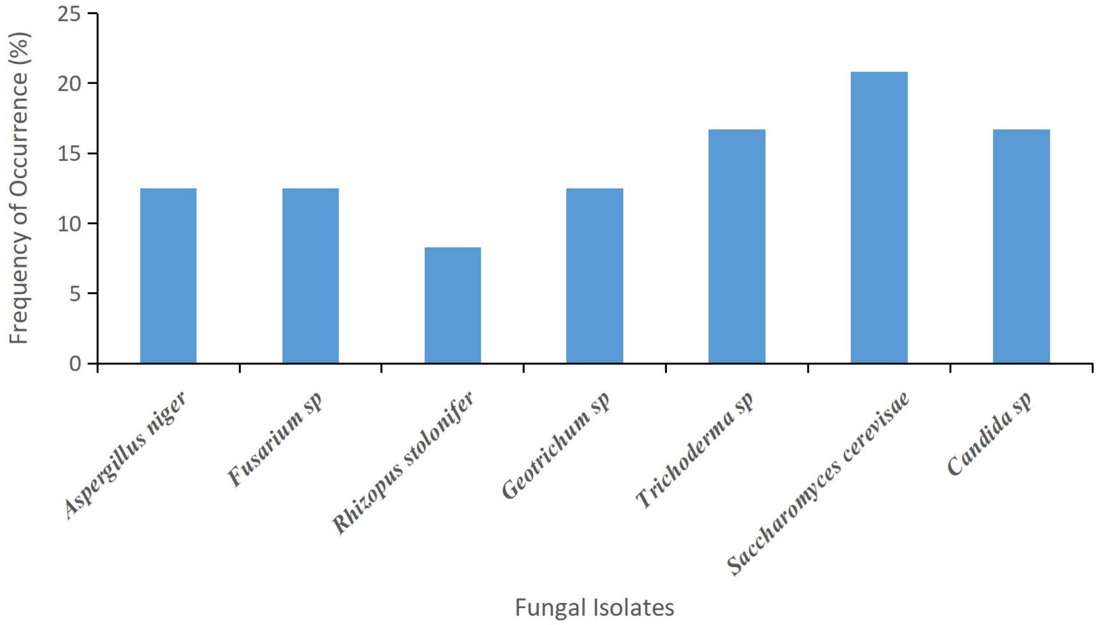

Figure 1: Percentage frequency of occurrence of Isolated Fungi from Tomato Puree Treated with Sodium Metabisulphite

Table 4:5: Frequency Distribution of Isolated Fungi from Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 15 days

SAMPLE	*A.niger*	*Fusarium* sp	*R.stolonifer*	*Geotrichum* sp	*Trichoderma* sp	*S.cerevisae*	*Candida* sp
UP (Control)	+	+	+	+	+	+	+
P 0.00	+	+	-	+	-	+	+
P 0.1	+	-	-	+	+	+	-
P 0.2	-	+	+	-	+	+	+
P 0.3	-	-	-	-	+	+	+
P 0.4	-	-	-	-	-	-	-
P 0.5	-	-	-	-	-	-	-

CHAPTER FIVE

DISCUSSION

Tomato (*Solanum Lycopersicum*) is one of the most important crops worldwide and constitutes part of our daily diets as cooked or salads. Tomato is an extremely valuable raw material for a wide range of processed foods. Large quantities of tomatoes are used to produce soup, juice, sauce, ketchup, puree, paste, powder and also in preparing traditional curries. They are a good source of vitamins particularly vitamins C. However, tomatoes contain a lot of water, making them prone to deterioration by the action of numerous microorganisms. This makes storage and shipping difficult. (Tandel., 2022; Abrar *et al*., 2016). Pre and post-harvest illnesses, in-appropriate handling and other circumstances can have an impact on the nutritional value and quality of newly produced tomato products. The problem of rapid spoilage in tomatoes coupled with inadequate storage capabilities has led to a series of research into prolonging its shelf life for safe consumption. Various methods have been employed over the years with the aim of prolonging the shelf life such as thermal treatments, Good Manufacturing Practices (GMP), canning and chemical treatments. This present study investigated the preservation of tomato puree treated with varying concentrations of sodium metabisulphite.

The occurrence of the fungi isolates indicated a diversity of microbial species in the puree sample. The fungi *Aspergillus niger, Fusarium* sp*, Rhizopus stolonifer, Candida* sp *and Saccharomyces cerevisae* were also isolated in the study by (Goka *et al.,* 2021) who worked on the evaluation of the microbiological and physic-chemical characteristics of local tomato puree produced on a small scale in Togo. The UP (Control) sample recorded the highest growth and spoiled rapidly. This was as a result of the absence of preservative and pasteurization. The most widely distributed isolate in this present study was *Saccharomyces* *cerevisae.* Another reason for the presence of high number of microorganisms in the tomato could be the nutritional content of the tomato puree. Tomato is rich in minerals which support the growth of microorganisms and this can lead to deterioration of the food. This agrees with (Ogodo *et al.,* 2020) who reported the presence of *Aspergillus niger, Fusarium* sp and Saccharomyces cerevisae as fungal agents responsible for spoilage. A report of (Asoso *et al*., 2022) indicated fungal isolates obtained from spoiled tomato as *Rhizopus stolonifer, Saccharomyces cerevisae, Geotrichum* sp, *Fusarium* sp and *Aspergillus niger.* They reported that *Aspergillus niger* had the highest occurrence in tomato fruits they studied and concluded that the fungus may be the major organism responsible for the spoilage of tomato fruits.

As the storage duration continued, the effect of increased microbial activity was reflected in the pH. The preservatives concentration conditions being employed and their reflection on the shelf life and sensory attributes of the tomato puree were analyzed. In samples without preservatives or pasteurization, significant changes in the color, smell and consistency were recorded on the 5^{th} sampling day. After the total preservation of 15 days, samples containing sodium metabisulphite with concentrations of 0.4g/L and 0.5g/L experienced delayed spoilage. Sodium metabisulphite is a potent preservative that can help extend the shelf life of tomato products by inhibiting the growth of yeasts and molds as well as other spoilage microorganisms. It helps in maintaining the red color and texture of the tomato ensuring that the preserved product remains visually appealing to consumers. Although, sodium metabisulphite is effective in preserving tomato puree, it is also known to cause many health problems to some individuals who are sulfite sensitive (FDA., 2016). It is important that food product be regularly assessed for the presence of microorganisms in order to track spoilage and maintain safety in food consumption. Harvesting, processing, transporting and sales operations must be carried out according to standard quality protocols.

CONCLUSION

Conclusively, applying metabisulphite and pasteurization resulted in the inhibition of fungi and prolongment of the shelf life in tomato puree at a concentration of 0.4g/L and 0.5g/L up until 15 days. From this study, the fungi responsible for the deterioration of the tomato puree at lower concentrations were *Aspergillus niger, Fusarium* sp*, Rhizopus stolonifer, Geotrichum* sp*, Trichoderma* sp*, Candida* sp and *Saccharomyces cerevisae.* Sodium metabisulphite applications to products could be suggested for reducing surface browning, extending the shelf life of tomato puree and minimizing the problem of post-harvest food losses.

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