BACTERIOLOGICAL QUALITY AND EFFECT OF ANAEROBIC METABISULPHITE PRESERVATION ON TOMATO PUREE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc HONS) IN MICROBIOLOGY.

DECEMBER , 2022

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CERTIFICATION

This is to certify that the project work was carried out by **Blessing Ehijiame ALEGBE (Miss)** with matriculation number **LSCI705451** the Department of Microbiology, University of Benin, Benin City under the supervison of

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APPROVAL

This project was carried out by **Blessing Ehijiame ALEGBE** (Miss) under the supervision of **DR. C.E OSHOMA** in partial fulfilment of the Award of a Bachelor of Science B.Sc degree in Microbiology.

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DEDICATION

I humbly dedicate this work to the Almighty God, who has given me the divine health, wisdom, strength and grace till this point of my life. All glory and honor belongs to Him and Him alone.

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ACKNOWLEDGEMENT

My greatest gratitude and thanks go to the Almighty God who has kept me all through this period and has also given me the grace throughout my stay in UNIVERSITY OF BENIN. My appreciation also goes to my ever ardent project supervisor. It was a great privilege and honour working under the supervision of **DR.C.E.OSHOMA**. He is an understanding and keen father who wants and pushes his students to be the best that they can be. My appreciation also goes to all lecturers in the Department of Microbiology. I wish to express my profound gratitude to my parents, **Mr. Lucky.E and Mrs. Ikodiya.G ALEGBE**, and my siblings Favour Alegbe, Harmony Alegbe and Stephen Alegbe for their support, financially, morally, spiritually and for their love and care throughout the period I carried out my project. Lastly, I will not forget to acknowledge my friends who were with me throughout my stay in school. They are: **Kennedy Ordia, Ruemus Ekwevugbe, Chiwike Chinyerem, Ebuka Achugo, Patience Nwaobi, Favour Alegbe,David Rebecca, Simeon Mensah, John Unuane, Sefia Ufuoma Tamie** and many others for their love, empathy, sincerity, motivation, great sense of humor and more importantly the crucial roles they all played which has inspired me deeply in carrying out this project successfully.

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ABSTRACT

Tomato is an economically important fruit in Nigeria. Tomato has a high amount of water which makes them more susceptible to spoilage by the action of various microorganisms. The aim of this study was to achieve the bacteriological quality of tomato fruit puree product preserved and treated with sodium metabisulphite under anaerobic condition. Tomato fruits were purchased at Okoko's village farm, Ovia North East Local Government Area, Edo state and blended into tomato puree. The tomato puree samples were treated with differing concentrations (0, 0.1, 0.2, 0.3, 0.4 and 0.5g/l) of sodium metabisulphite pasteurized at 65^{0}C for 15 minutes and then stored for 21 days under anaerobic conditions and analysed at every 7 days interval. The analysis carried out were Total Heterotrophic bacteria, Coliform , and Salmonella Shigella count, as well as lycopene content, ascorbic content and changes in pH. From the result, the highest heterotrophic bacteria count was 23 ± 2.8310^{4}cfu/ml, while the lowest was $1\pm1.41\text{x}10^{4}$cfu/ml from puree sample treated with 0 and 0.5g/l of metabisulphite respectively. The highest lycopene content was 22.01 ± 0.1510^{4}cfu/ml while the lowest was $7.71\pm0.15\text{x}10^{4}$cfu/ml from puree sample treated with 0 and 0.2g/l of metabisulphite respectively. The identified isolates were: *Klebisella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas putida, Proteus vulgaris, Proteus mirabilis* and *Citrobacter sp*. From this study, both pasteurization and preservation by sodium metabisulphite has shown potential as effective ways of prevention against spoilage in tomatoes.

CHAPTER ONE

INTRODUCTION

The tomato is the edible berry of the plant *Solanum lycopersicum,* commonly called tomato plant. The species is native to western South America, Mexico, and Central America. The Mexican Nahuatl word tomato gave rise to the Spanish word tomate, from which the English word tomato derived. Its domestication and use as a cultivated food may originate from the indigenous peoples of Mexico. The Aztecs used tomatoes in their cooking at the time of the Spanish conquest of the Aztec Empire, and after the Spanish encountered the tomato for the first time after their contact with the Aztecs, they brought the plant to Europe, in a widespread transfer of plants known as the Columbian exchange. From there, the tomato was introduced to other parts of the European-colonized world during the 16th century including Nigeria and Ghana (Sharma *et al*., 2012).Tomatoes are a highly nutritious plant food. The benefits of consuming a variety of fruit and vegetable are impressive, and tomatoes are no different. The higher the proportion of plant foods in the diet, the risk of developing heart disease, diabetes, and cancer is reduced. There are different types and sizes of tomato, and they can be prepared in differently. These include cherry tomatoes, stewed tomatoes, raw tomatoes, soups, juices, and purees. The health benefits may vary according to types. For example, cherry tomatoes are higher in beta-carotene than regular tomatoes. High consumption of fruit and vegetable is also related to healthy skin and hair, increased energy, and reduced weight. Increasing the consumption of fruits and vegetables significantly decreases the risk of obesity and overall mortality .In most developing countries, microbial infestation of tomatoes can occur during the harvesting period, post harvesting, handling, storage, transportation, and processing by customers .(Baiyewu *et al).* have also reported that another means of bacterial contamination is by exposing them on benches and baskets in the open markets for customers. The spread of bacteria especially in damaged tomatoes could be considered to be more harmful when such contaminated tomatoes are eaten in undercooked

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foods. In some countries, research have been conducted on bacteria associated with tomatoes and tomato products . A study carried out by Ajayi in the United State has revealed that *Clostridium* sp., *Staphylococcus* sp., and *Bacillus* sp. were predominant bacteria isolated from both canned and raw tomatoes. In India, a study carried out on tomato puree revealed the presence of *Klebsiella* sp., *Proteus mirabilis, Vibrio* sp., *and Pseudomonas* sp. .In Nigeria, Wogu and Ofuase isolated *Bacillus subtils*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis,* and *Staphylococcus aureus* from spoilt tomatoes in Benin City. A similar study also revealed high levels of *Staphylococcus* sp. (22.5%), *Bacillus* sp. (20%), and *Escherichia coli* (15%) in Lagos State, Nigeria. In Ghana, limited information is available on the types of pathogenic bacteria associated with tomatoes sold in markets in Accra, Ghana. This study aimed to isolate and identify pathogenic bacterial agents associated with two different grades of raw tomatoes (fresh and spoilt) sold in three central markets in Accra.

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1.1 AIM AND OBJECTIVES

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This study aimed to achieve the bacteriological quality of tomato fruit puree product preserved and treated with sodium metabisulphite under anaerobic condition.

The objectives of this study were to:

1. enumerate, isolate and characterize the bacteria associated with tomato fruit puree preserved and treated with sodium metabisulphite under anaerobic condition
2. determine ascorbic acid, lycopene contents of tomato fruit puree preserved and treated with sodium metabisulphite under anaerobic condition.

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CHAPTER TWO

LITERATURE REVIEW

2.1 BRIEF HISTORY OF TOMATO CULTIVATION

Tomato (Lycopersicon esculentum Mill.) is one of the most popular and widely grown plants in the world as well as in Africa (Osemwegie, *et al*., 2010). It is the second most important vegetable worldwide, in terms of the amount of vitamins and minerals it contributes to the diet (Enrique & Eduardo, 2006). It originated in South America, but was introduced into Europe and became known to botanists around the middle of the sixteenth century. Genetic evidence shows that the progenitors of tomatoes were herbaceous green plants with small green fruit and a center of diversity in the high lands of Peru (Smith 1994). One species, *Solanum lycopersicum,* was transported to Mexico where it was grown and consumed by Mesoamerican civilization. The Spanish explorer, Cortes may have been the first to transfer the small yellow tomato fruits into Europe after he had captured the Aztec city of Tenochtitlan, now Mexico City in 1521; although Christopher Columbus, a Genoese working for the Spanish monarchy may have taken them back as early as 1493. The earliest discussion of tomato in European literature appeared in an herbal written in 1541 by Pietro Andrea Mathiolian Halian, an Italian physician and botanist, who named it pomod'oro, or 'gold apple' (Smith 1994). The word ''tomato'' is derived from the Nahuatl word tomato, literally 'the swelling fruit' and the latin name of tomato is Lycopersicon esculentum Mill. Syn. Solanum lycopersicum (Cutler, 1998). Tomatoes were not grown in England until the 1590s. One of the earliest cultivators was John Gerard, a Herbal-Surgeon. Smith (1994) reiterates that Gerard's Herbal, published in 1597, and largely plagiarized from continental sources, is indeed one of the earliest discussions of tomato in England. Similarly, Tindall (1988) remarked that the Portuguese trader and freed slaves from West Indies introduced this plant into West Africa Countries.

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2. 2 FRUITS AND VEGETABLES

An interesting feature of tomato history is the classical debate. Today, we are asking if tomato a fruit or vegetable. By definition, a fruit is the edible plant structure of a mature ovary of a flowering plant, usually eaten raw, some are sweet like apple, but the ones that are not sweet such as tomatoes, cucumbers, pepper, etc are commonly called vegetables. Botanists claim that a fruit is any fleshy material that covers a seed or seeds in which the point of view of a horticulturist is that the tomato is a vegetable plant. Until the late 1800's the tomato was classified as a fruit to avoid taxation, but this was changed after a Supreme Court ruling that the tomato is a vegetable and should be taxed accordingly.When it is all said and done, the history of the tomato has classified as a poisonous beautiful plant, a tax avoiding fruit and a taxable vegetable. Nonetheless, the tomato is the most popular vegetable in American and enjoyed by millions all over the world.

2.3 TOMATO PRODUCTS

There are different types of tomato products and usually there is some confusion about the differences between them, thus here are some definitions that were reported by (Thakur *et al*).

2.3.1 Tomato Juice

Tomato juice is a juice from whole crushed tomatoes with no skin or seeds, which has been subjected to fine screening, and is intended for consumption without dilution or concentration. It is smooth and in a liquid form available in cans or bottles. In recipes where a subtle tomato flavour and smooth texture are required, tomato juice can be included in small quantities

2.3.1.1 Tomato juice production

Fully ripe well developed colour tomatoes are washed, trimmed, steamed, crushed in a crusher or cut into pieces with knives. The crushed pieces are heated in the steam jacketed kettle till they become quite soften. The heated tomatoes are passed through the pulping machine using a fine mesh sieve to separate juice from seeds and the skin. The sugar and salt

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at 1% is added and heated to 85-90ºC. The hot juice is then filled in bottles, sealed immediately and pasteurized in boiling water for about 30 minutes and cooled

2.3.2 Tomato Paste

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Product obtained after concentration of tomato pulp, removing skin and seeds and containing more than 24% natural tomato soluble solids(NTSS)

2.3.2.1Tomato paste production

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Tomato pulp or juice is concentrated to 14-15%soluble solids in open pans followed by concentration in vacuum pans and packing in pre-sterilized bottles while still hot. In large scale processing units, the tomato paste is manufactured by using vacuum evaporators and packed either in tin can or in bulk aseptic packages. The tomato paste is utilized for manufacture of different products like ketchup, soup, sauce etc Hot Break and Cold Break are the two types of tomato paste and they are used in making different end products. The fresh tomatoes after chopping must be heated immediately to a very high temperature (ranging from 85 to 100°C) in order to make Hot Break (HB) paste while Cold Break (CB) paste requires the fresh, chopped tomatoes to be heated at a lower temperature(ranging from 65 to 75°C). Hot Break paste is usually used for ketchup and different types of tomato sauce requiring a 28-30° Brix, while Cold Break paste is mainly used for triple concentrate paste at 36-38° Brix which are then packaged for domestic use

2.3.3 Tomato Puree

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Tomato paste with low concentration of NTSS (8-24%). In USA it is also called tomato pulp or concentrated juice, it corresponds to the commercial tomato paste found in the market

2.3.3.1 Tomato puree production

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The pulp is concentrated under vacuum to about9% to 12% total solids so as to get tomato puree The sufficiently scalded product is mashed to puree by passing through a sieve, which retains the skins, the seeds, as well as the fibrous substances. It is necessary to evaporate the

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liquid contained in pulp by heating the product until the desired consistency is reach. The product is filled in bottles, crown corked and processed in boiling water for 30 min. and cooled

2.3.4 Tomato Powder

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Product obtained after drying using sun, oven or dehydrator and pulverised into powder.

2.3.4.1 Tomato powder production

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The process of removing moisture from food to inhibit the growth of mold and bacteria is known as dehydration and this process extends the shelf life of tomato. This process forms the basis for tomato powder .The fresh ripe tomatoes are sorted according to size and shape, the sorted tomato are cleaned by soaking for 1-2 minutes in 1% sodium hypochlorite solution and then rinsed with clean portable tap water and allowed to drain. The tomato will then be sliced into slices of about 5-10 mm and the seeds removed. The sliced tomato will be blanched for one minute at 90°Cand allowed to drain. It will then be placed inside the drying chamber of the oven and the oven temperature set at 60°C for oven drying or dehydrator and under the sun for sun drying method. Afterwards it will then be cooled, milled and packaged appropriately

2.4 TOMATO PRODUCTION AND POSTHARVEST SPOILAGE IN NIGERIA

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Nigeria is second largest producer of tomato in Africa second only to Egypt and 13th in the world, and produces 6 million tonnes of tomato annually prior to 1990 (Erinle, 1989). Tomato is cultivated almost all over Nigeria (Olanrewaju and Swamp, 1980). The major producing areas in Nigeria lie between latitudes 7.5 °N and 13 °N, and within a temperature range of 25 – 34 °C (Villareal,*et al*., 1980); these areas include most States in Northern Nigeria namely- Bauchi, Benue, Borno, Kaduna, Kano, Plateau, Sokoto, and a few southern states like Kwara and Oyo (Denton and Swarup, 1983; Olanrewaju and Swamp, 1980). The cultivation of tomato in the South-South region especially Bayelsa and Delta States is faced

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with serious constraints because the states are situated in the heart of the tropical rainfall region with its low topography. Another constraint is the fact that the states witness a greater period of rainfall (April to November) which does not support the growth of this heat-loving crop. For this reason, cultivation by farmers is primarily for subsistence purpose rather than commercial.

The relatively low production of tomato in Nigeria as compared to Asia, Europe, North and South America is attributable to biotic, social and environmental constraints. Prominent among such constraints are pests and diseases which reduce yield and the quality of marketable fruits. In the tropics, particularly in Nigeria, many insect pests are associated directly with tomato damage and yield losses, while others are important as vectors of diseases (Messiaen, 1992; Tindall, 1977; Umeh and Oyedun, 1995). Global postharvest losses of tomato are as high as 30-40% (Agrios, 2005; Kader, 1992), but this is much higher in developing countries like Nigeria due to improper handling procedures and lack of methods to prevent decay (Prigojin, *et al.,* 2005). Fruits, by nature have low pH, higher moisture content and nutrient composition, and these inherent attributes renders them very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxin (Philips, 1984; Moss, 2002; Stinson *et al*., 1981). Fungi are the most important and prevalent pathogens, infecting a wide range of fruit and causing destructive and economically important losses of fruits during storage, transportation and marketing (Sommer, 1985). The extent of postharvest damage due to spoilage fungi is reportedly dependent on tomato variety. A relatively recent study carried out in Oyo State, South western Nigeria showed that whilst up to 44% of postharvest spoilage was attributed to microorganism in a given tomato variety, only 14-23% of spoilage was attributed to the same microorganism among other varieties (Adeoye *et al*., 2009)

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Pre-harvest production practices may seriously affect postharvest return. Losses due to soil borne fungi like Phytophthora capsici is well documented (Hausbeck and Lamour, 2004; Ewin and Riberio, 1996). *Phytophtora capsici* cause late blight and other infections, resulting to wilting in tomato crops (Hausbeck and Lamour, 2004). Other soil borne fungi that can cause severe losses in tomato are Alternaria solani which causes early blight, *Septoria lycopersici* is responsible for Septoria leaf spot in tomato, *Fusarium oxysporum* cause Fusarium wilt etc. Postharvest tomato losses during storage and transportation are estimated to be as high as 20% in Nigeria (Olayemi, et al, 2010). Poor handling, packaging, storage and transportation eventually result in decay and increase the growth of microorganisms, which become activated because of the changing physiological state of the produce (Wilson *et al*., 1991). In Northern Nigeria where tomato is majorly produced, freshly harvested tomato fruits are stored, and conveyed in traditional weaved wicker baskets (Kutama*, et al*., 2007). These baskets are often used until they become infected with primary fungal spores that might have previously infected the fruits. Kora and associates (2005) observed that pathogenic innocula occurring on wooden boxes and baskets can initiate disease upon contact with healthy fruits, which eventually result to losses. It has also been pointed out that the inocula responsible for storage diseases among tomato often originates from infected and infested farm tools (Snowdon, 1992). As mentioned earlier on, postharvest tomato losses are also incurred during transportation from farms and locations where they are produced to markets. There are two main modes of transportation available to domestic transporters and handlers of fresh produce in Nigeria, the rail and the road system. However, transporters complain of the non-availability and unusual delays in rail system. Consequently, most the handlers use the road system for their regular and long distance haulage (Idah, *et al*., 2007; Olayemi, *et al*.,2010). Different types of vehicles are used to convey harvested tomato from the Northern part of Nigeria where it is majorly produced to other parts of the country. The major types of

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vehicles used in transporting their produce have been succinctly described by various workers (Idah, et al, 2007; Olayemi, et al, 2010). Most transporters use the 911 lorry shown in Figure 2 because of its capacity and superior ventilation. A typical example of vehicles used in transporting tomato from Northern Nigeria to the Southern parts of the country is shown in figure 2. A typical vehicle could carry between 250 and 300 baskets or jute bags load of fresh produce (about 7,500 and 9,000 kg). The problem of transportation is often exasperated by non-availability of vehicles when most needed. To avoid loss of their produce, the traders result to the use of any kind of available vehicle, sometimes even passenger buses are not spared. Marketers and distributors of tomato who convey the harvested product from Northern Nigeria to other parts of the country sometimes are seen to be very desperate as they fasten tomato consignments onto of fuel tankers and other articulated vehicles (Idah, et al, 2007; Olayemi, et al, 2010). These rather weird and desperate actions expose tomatoes to accidental fall off during the course of transportation and these losses could be heavy, though not usually quantified.The containers (baskets) are usually arranged in 5 to 6 layers inside the vehicle with woody planks in-between these layers. Some transporters use leaves to separate the layers, which normally do not prevent compression of the produce from the weight (load) of those on top. This practice is thus one of the sources of mechanical damage to the produce. Additionally, proper ventilation is sometimes either non-existent or grossly inadequate. The result is usually produce rot arising from high level of physiological activities of the produce occasioned by lack of proper and/or adequate ventilation (Idah, et al, 2007; Kra and Bani, 1988; Olayemi, et al, 2010).An assessment carried out at a market in Ilorin, Nigeria revealed quite a lot of tomato fruits damage. Damaged fruits mainly consisted of bruised, rotten, compressed and water soaked fruits. In particular, an average of 13.89% of fresh tomato fruits was reportedly damaged during transportation (Idah, *et al*., 2007). This means, in every consignment of 7,500 kg (lorry load) of tomato fruits about 1041.67 kg (representing 13.89%)

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of the fresh tomato fruits would be bad. In terms of money, for an average price of N200.00 per kg, the losses due to this damage is about N200,000.00 per lorry load if such damaged fruits are completely discarded, which is really substantial (Idah, *et al*., 2007). It is worthy to note that Ilorin where this assessment was done is situated in Northern Nigeria where tomato is produced. The amount of spoilt tomato would increase substantially by the time they get to markets situated down south of the country.At the market level, losses of tomato can be significant particularly in developing countries like Nigeria. A recent survey of markets in Yenagoa, capital of Bayelsa State, Nigeria revealed poor market facilities and storage arrangements of tomato displayed for sale (Fig. 2). Tomato fruits were seen washed with dirty water. Decaying and spoilt produce were indiscriminately left around packing house or shops. These conditions no doubt expose the fruits to pathogenic organisms and other environmental problems, leading to rapid deterioration of produce. Adeoye and Associates (2009) reiterate that mechanical damage to tomato ranks highest in economic postharvest losses followed by pathological damage while physiological damage causes the least, but nonetheless substantial. They further elucidated that at the market level postharvest losses were greater at retail level compared to wholesale.

2.5 OVER-VIEW OF DISEASE SYMPTOMS DUE TO FUNGI AND BACTERIA IN TOMATO

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The two primary classes of microorganisms that cause decay in tomato are bacteria and fungi. Certain other types of plant pathogens such as viruses and nematodes may be responsible for post harvest diseases and losses but do not cause progressive deterioration of tomatoes.

2.5.1Bacterial diseases

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Bacteria are single-celled organisms that can rapidly multiply and spread, particularly in water. Even a thin coating of water, such as on a wet fruit, leaf bin surface or packing house machinery, can support the rapid movement and growth of the organisms. Bacterial diseases

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of tomato have been described by various workers (Mehrotra and Ashok, 1980; Singh, 1998), examples include:

2.5.1.1Bacterial Spot: This is caused by several bacterial species of the genus Xanthomonas. The most common species of this genus that causes this disease is X. euvesicatoria (Jones et al, 2004), previously known as X. axonopodis pv. Vesicatoria or X.. campestris pv. vesicatoria . The bacterium is a gram negative rod, motile and strictly aerobic. The associated disease symptoms have been clearly captured by various workers (Mehrotra and Ashok, 1980; Singh, 1998). The disease is characterized by circular to irregularly shaped spots with a slightly greasy feel on leaves and stems. The spots are usually initially but as the lesion enlarges, they often become surrounded by a yellow halo. Numerous spots coalesce to become bigger, leaving the leaves wither and turn brown.Fruits symptoms are more distinctive than leaf or stem symptoms. Spots on green fruit first appear as black, raised, pimple-like dots surrounded by water-soaked areas. As the spots enlarge, they become gray-brown and scabby with sunken, pitted centers.

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2.5.1.2Bacterial Canker: This is caused by the bacterium Clavibacter michiganensis sub sp. Michiganensis. Symptoms are characterized by complete loss of leaves among young transplant. Older plants are often seen to have their leaflets turn brown at the edges at the onset of disease and progresses towards the leaf midrib. Diseased fruits are characterized by dark-coloured necrotic lesions with white halos of 16 – 32mm. At the beginning, the lesions appear white and slightly raised, but turn brown as the disease progresses and finally become dark-colored (Singh, 1998; Mehrotra and Ashok 1980).

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2.5.2Fungal diseases

Fungi are mostly filamentous microorganisms commonly known as molds. In nature, they often appear thread- like, cottony, or as yeast-like scum. Many fungal species can cause fruit

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decay in tomatoes. Fungi are generally more difficult to eradicate than bacteria, because fungal cells are much larger and produce spores that are highly resistant to drying and other environment stresses. The major postharvest diseases caused by fungi are Sour rot, Rhizopus rot, Buckeye rot and Black mold rot. Descriptions of these diseases have been well articulated by various workers (Mehrotra and Ashok 1980; Bartz *et al*., 2004).

2.5.2.1Sour rot: This is caused by the yeast Geotrichum candidum. The disease is characterized by lesions whose growth resembles a thick, gelatinous mass similar in appearance to cottage cheese. The lesions are usually watery in the early stages of the disease and later become coated with pathogen growth and remain relatively firm. Lesions give off an odor similar to that produce by lactic acid bacteria hence, the name, sour-rot (Bartz *et al.,* 2004).

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2.5.2.2Rhizopus rot: This disease is caused by Rhizopus stolonifer. Disease symptoms first appear as water-soaked lesions which exudes a clear liquid with time. Resulting lesion surfaces are covered with thin, cotton-like structure. Infection is usually through natural openings (Bartz *et al*., 2004) or wounds created by mechanical damage (Mehrotra and Ashok 1980). Studies on postharvest quality of tomato marketed in Nigeria have also shown to suffer from Rhizopus rot caused by R. stolonifer (Chuku et al., 2008).

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2.5.2.3Buckeye rot: Another postharvest disease of tomato is Buckeye rot caused by Phytophthora parasitica. The pathogen attacks both ripe and unripe tomato fruits. Symptoms first appear as water-soaked circular spots. As the disease progresses, the center of the spots become darkened and overgrown with sparse white mycelia of the fungus. The disease derived its name from the manner the fungal mycelia spreads from diseased fruit to adjacent healthy fruit (Bartz *et al*., 2004).

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2.6. PREPARATION OF TOMATO FOR STORAGE, PROCESSING AND PRESERVATION

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The preparation of tomato for preservation should be as soon as harvest is carried out, this is because the chances of deterioration increase rapidly as time passes. Preparation can be done using the processes outlined below.

2.6.1 Cleaning and Washing

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The tomatoes have to be thoroughly cleaned to get rid of any dirt or insecticide residues after harvesting and this can be done by the use of sodium hypochlorite or chlorine. The process of cleaning the tomato usually entails washing it in a pail with clean water that is frequently refreshed .

2.6.2 Peeling

Peeling of tomato before preservation is necessary and this can be done without difficulty with a stainless steel knife. In order to prevent the discolouration of the plant tissues, it is really important that the knife be made of stainless steel. It is best to first dip them in boiling water for 1 1⁄2 to 3 minutes in order to soften the skin, then the softened peel can now be removed without too much effort.(Cheryl *et al*) wrote a review paper on the Conventional and Alternative Methods for Tomato Peeling. The review highlighted the conventional methods used in tomato peeling, their efficacy and the potential applications of infrared, ohmic heating and power ultrasonic as a novel technology for tomato peeling. He stated that Steam/hot water and lye peeling have been the most commercialised methods, but compared to steam peeling, lye peeling is more preferred and has gained widespread application among processors due to its association with higher product yields and better product quality .

2.6.3 Cutting

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Cutting is most times employed before some preservation methods are carried out because approximately uniform pieces are usually required. Tomatoes are usually cut into cubes, thin

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slices, rings or shreds for the heating, drying and packing stages. To prevent microbial contamination, the cutting utensils have to be sharp and clean. The quality of the products decreases from the moment they are cut, due to the release of enzymes and nutrients for micro- organisms. The damage done to the plant tissues also leads to a decrease in quality. For this reason, the interval between peeling/cutting and preserving has to be as short as possible .

2.6.4 Blanching

Blanching or pre-cooking is done by immersing tomatoes in water at a temperature of 90-95°C. The result is that the tomatoes become somewhat soft and the enzymes are inactivated. In order to prevent unwanted colour and odour changes and an excessive loss of vitamins blanching is done before a product is dried . A study was carried out to determine the effect of blanching methods on drying kinetics of bell pepper and it was observed that the blanched samples generally had higher drying rates than the untreated samples .

2.7 PRESERVATIVES

Food preservatives are a group of compounds 0001 of very different molecular structures; they are organic and inorganic substances having different functional groups and ion forming tendencies. There are no commonly applicable procedures for the analysis of preservatives as a class of food additive; the procedures are specific to the preservative being analyzed. The lowest concentrations of commonly used preservatives are of the order of a few milligrams per kilogram of food, and, with few exceptions, recommended or statutory methods of analysis are designed to give a good accuracy at levels of 10 to > 1000 mg of preservative per kilogram of food. The question of the lower limit of detection is rarely an issue, unless it is desired to use small sample sizes, e.g., < 1 g, or to determine whether or not a food or its ingredients had been treated with a preservative. For solid foods, small sample sizes often lead to nonrepresentative sampling and should be avoided. Not all the procedures described

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constitute official methods of analysis. Frequently, for routine analysis, a food manufacturer would use a rapid or cheap analytical technique standardized against an official method. The official status of given procedures varies from country to country. Organic and inorganic acid preservatives may be 0002 added in the form of the undissociated acid or a variety of salts. In food, the ionic composition is determined largely by concentration and pH, but it is generally impossible to predict this accurately for any given situation. In order to avoid complications with the specification of the amount of preservative in a food, this is usually referred to as the weight for-weight concentration of the undissociated acid, e.g., benzoic acid, sorbic acid, or sulfur dioxide. Nitrite and nitrate levels are expressed in terms of the weight of the sodium salt.

2.7.1 Types of Preservatives

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There are two types of preservatives: natural and artificial/chemical. Both aim to preserve the life of an item, though they can differ in many ways. However, both use the same preservation methods:

- **Antimicrobial:** Prevents the growth of microorganisms such as bacteria and fungi.
- **Antioxidants:** Slows down or entirely stops the oxidation process.
- **Enzymes:** Halts the expiration of cosmetics and like products.

Natural preservatives are primarily used in foods and beverages to help reduce rotting and preserve the item's color and flavor. However, they are also found in cosmetics and other hygiene products. Common examples of natural preservatives include:

- Aloe vera
- Citric acid
- Lemon juice

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- Rosemary extract
- Sodium
- Sorbic acid
- Sugar

Artificial/chemical preservatives are man-made substances that are added to numerous products to extend their shelf life. While they too are created to prevent foods from spoiling and help them retain their shape and color, they are oftentimes filled with chemicals. Common examples of chemical preservatives include:

- Antimicrobial agents
- Antioxidants
- Benzoates
- Chelating agent.
- Nitrates
- Propionates
- Sorbates
- Sulfites.

2.7.2 METHODS OF PRESERVING TOMATO

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2.7.2.1 Drying

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One of the ancient ways of preserving tomato is drying. The basic procedure involves removal of moisture from the fruit to a point where decay is not likely. This can be achieved by using an oven, a dehydrator or the warm heat of the sun. Once finished, the produce should be stored in a dry place in air tight containers (Opega *et al*) worked on the Effect of Drying Methods and Storage Conditions on Nutritional Value and Sensory Properties of Dehydrated Tomato Powder (Lycopersicon esculentum mill)the effects of two drying

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methods, (oven drying and sun drying) of tomatoes and storage conditions of the products were studied to assess their effects on chemical, nutritional quality and sensory properties. Sensory evaluation which included four parameters, i.e. revealed taste, flavour, consistency, colour beside overall acceptability was significantly superior in the oven dried tomato product over the sun dried.

2.7.2.2 Preservatives (Chemical)

They prevent the growth of microorganisms and help preserve the tomato. There are many types of chemical preservatives that are used in food processing some of which include, sodium benzoate, sodium metabisulphite, sulphurdioxide, Sodium chloride (common salt) and citric acid. For example The efficacy of sulphur dioxide is more against moulds or bacteria than yeasts and has the additional advantage of slowingdown browning or darkening of some products (Shampa *et al*) also carried out a research on the Effect of Chemical Preservatives and Storage Conditions on the Nutritional Quality of Tomato Pulp .The results revealed that higher concentration of sodium benzoate and storage at-10°C might be a better way for long term preservation of tomato pulp.

2.7.2.3 Boiling

An effective way of preserving food is by using heat. The reason is because majority of harmful pathogens are killed at temperatures close to the boiling point of water. Therefore, heating food isa form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens

2.7.2.4 Pasteurisation

This is also known as post-heat treatment. Tomato paste can be pasteurised in their bottles using hot water. It is imperative to note that the product and the water should maintain the

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same temperature at all times, otherwise the bottles will burst. The filled bottles with their lids closed are placed in a larger pan of water with water heated to boiling point. This could be done for at least 45minutes (Nenad *et al*) looked into the impact of different thermal processing of tomato to its antioxidant activity, vitamin E, dry matte rand sugar content with the aim to establish the nutritive profile and distinguish superior genotypes in order to obtain high-quality final product with more benefit to human health.it was observed that Thermal processing by drying at60°C and pasteurization of tomato changed the level of total sugar and dry matter content. Total antioxidant activity decreased by drying, compared to fresh fruit while the level of vitamin E decreased in juice pasteurized at high temperature (100°C).

2.7.2.5 Freezing

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Tomatoes may be frozen whole, sliced, chopped or puree. Tomatoes can be frozen raw or cooked. Firm, ripe tomato should be sorted for freezing and should not be blanched. The pathogens responsible for food decay are killed or do not grow very quickly at reduced temperatures during freezing .

2.7.2.6 Curing

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It has been well acknowledged for centuries that adding salt to foods as preservative is of great value. Salt acts as a dehydrating agent in foods by binding with water molecules. The effect sugar has as a preservative is similar to those of salt in preventing spoilage of food. The use of either compound or certain other natural materials is known as curing.

i. Sugar: If the concentration of sugar in the preserved material is increased about 66 %, the water content is decreased to such an extent that the multiplication of microorganisms is checked and the present ones die in due course

ii. Salt: Strong salt solution never allows the microorganism to grow in the preserved products. It acts both by osmosis and as a poison and it is more effective than sugar. Abrine solution of 10 to 15% is sufficient for permanent preservation of most of the

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products. The pleasant flavor each compound adds to the final product is one of the desirable side effects of using salt or sugar as preservative.

2.7.3 Uses of Preservatives

While artificial and natural preservatives serve a similar purpose which is to extend the shelf life of products, the way in which they're used varies depending on the product the preservative is added to. For example, preservatives added to makeup products have a different purpose than those that are added to food.

2.7.3.1 Flavor Enhancement

One of the primary uses of food additives and preservatives is to enhance the existing flavor of different foods or products. Additives such as artificial sweeteners and sodium benzoate can be used to boost the flavor of our favorite carbonated drinks or juices. Mineral salts can enhance the texture and flavor of certain dishes and foaming agents help ensure that every time you pop the top on your favorite soft drink, it has that rich foamy consistency you've come to love.

2.7.3.2 Food Preservation

Another important component of additives and preservatives is to prevent premature spoilage in consumer food products. There are a host of different compounds used to this end, such as antioxidants, which prevent food from oxidizing or going rancid. Different types of benzoates are used to prevent mold and bacteria growth, in particular, potassium and sodium benzoate.

For meat and poultry products, a variety of liquid additives or preservatives are employed to extend the shelf life of certain products, in addition to increasing consumer food safety. One of the most commonly used compounds in this regard is potassium lactate, which inhibits spoilage and pathogenic bacteria and also provides overall protection from microbial bacteria.

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2.7.3.3 Enhanced Consistency & Appearance

Another popular use of food additives and preservatives is to help our favorite food products maintain their appealing appearance. Compounds like anti-caking agents stop ingredients from being lumpy and flour treatment additives improve baking quality. There are also glazing and gelling agents that can both protect food, in addition to altering its texture and appearance. Other additives can even increase the volume of different food products without altering its composition. (Mirza *et al*;2017).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Sample Collection

The sample were obtained from Okoko's village farm, Ovia North East Local Government Area, Edo state,Nigeria. After harvesting, samples were transferred to the laboratory for processing. Only whole, ripe, uninjured tomato fruits were selected for use.

3.2 Sample Processing

The samples were washed twice with clean, sterile water, after which they were transferred to a bowl containing freshly boiled water. This was to soften up the fruit and eliminate susceptible surface microorganisms. After blanching for about 30mins, the sample were then transferred into a sterilized container awaiting blending. The blender was first soaked with a solution of water, ethanol, and acetone overnight and then freely boiled in hot water twice before being used. Subsequently, after 5 sample blends, the blender was rewashed with freshly boiled water. All these operations were carried out aseptically. After blending, the puree was transferred to clean sterilized holding container awaiting microbial analysis.

3.3 Preparation of Preservative Solution

Preparation of varying concentrations of sodium metabisulphite was carried out. Concentrations of 0, 0.1, 0.2 , 0.3 , 0.4 and 0.5 g/L were prepared. Then 20ml was transferred into 1000ml capacity storage bottles each in triplicates and then sterilized at

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121°C for 15 min. The bottles were brought out, allowed to cool and then 200ml of prepared tomatoes puree was transferred to bottles containing varying concentrations of metabisulphite under aseptic conditions . The bottles were sealed and stored for 21 days and analyzed every 7 days interval. The analysis carried out were Bacteria load (Coliform count,Heterotrophic count and Salmonella Shighella count), Ascorbic content, pH, Titratable Acidity, Lycopene content.

3.4 Bacteriological analysis of sample

The stored samples were analyzed every 7 days including Day 0 for a duration of 21 days. The microbiological analysis carried out included enumeration, characterization, and identification. These were carried out according to the method of Cheesebrough, 2006.

3.4.1 Enumeration

To 45mls of sterile peptone water in a conical flask, 5mls of sample was added aseptically. From this, 1ml was pipetted out into a test tube containing 9mls of sterile peptone water. From here, the sample was serially diluted up to 4 folds. Using pour plate technique, 1ml was aseptically transferred into petri dishes, after which, cooled molting agar medium was poured . The plates were rocked gently and then incubated at 27±2°C for 24 hours. After 24 hours, plates were observed and visible, distinct colonies were counted.

3.4.1.1 Preparation of Culture Media

All media were prepared according to manufacturer's instruction. The media used in this study were Nutrient Agar and Potato dextrose agar. Streptomycin was added to the potato dextrose agar only to inhibit bacterial growth, and nystatin to the nutrient, eosin methylene blue and salmonella shigella agar plates to inhibit fungal growth.

3.4.1.1.1 Nutrient Agar

This was used to culture non-fastidious organisms and for bacterial heterotrophic plate count. This medium was prepared from commercially available dehydrated powder, available from

most suppliers of culture media. In the preparation, 28 g of nutrient agar powder was dissolved in one (1) litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C and then dispensed aseptically into Petri dishes already containing inoculum (pour plate).

3.4.1.1.2 Salmonella Shighella Agar

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Each single, isolated colony of bacteria was picked up with the help of sterilized wire loop and was streaked on fresh nutrient.SSA agar mediums or media (use one) respectively. The agar plates were incubated at 37°C for 24 h. The isolated and purified bacterial strains were also stored under refrigeration after preparing slants.

3.4.1.1.3 MacConkey Agar

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Each single, isolated colony of bacteria was picked up with the help of sterilized wire loop and was streaked on fresh nutrient.SSA agar mediums or media (use one) respectively. The agar plates were incubated at 37°C for 24 h. The isolated and purified bacterial strains were also stored under refrigeration after preparing slants.

3.4.2 Characterization and Identification of Isolated Microorganisms Bacteria

3.4.2.1 Cultural Characteristics and Biochemical Tests

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Colonies were examined for their cultural characteristics with reference to size, edge pattern, margin, surface texture, opacity, elevation, pigmentation, consistency and colour (pigmentation).

Biochemical characterization tests were thereafter performed. Gram staining was performed to differentiate the Gram positive from the Gram negative bacteria.

3.4.2.2 Gram staining test

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The Gram staining technique was used for differentiation between Gram positive and Gram negative bacterial strains. A drop of sterile distilled water was placed on a neat and clean

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glass slide, and a single isolated colony of 24 h old culture was mixed in it. The smear was made by spreading the culture. This smear was air dried and fixed by rapidly passing the slide three times over flame. It was then flooded with crystal violet for 1 min and then washed off with distilled water. Then Gram's iodine solution was added to the smear and the glass slide was left for one min and rinsed with distilled water. This step was followed by the application of decolorizing agent (ethanol) for 30 s. Decolorizing agent was immediately washed off with distilled water and the smear was counter stained with safranine for 1 min. The slide was washed with distilled water, air dried and was observed under the microscope.

3.4.2.3 Spore staining test

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The smear was prepared from the pure culture, air dried and fixed by passing over flame. The smear was covered with malachite green (5%) solution for 3-4 min, by heating the slide with continuous steaming. Then, the slide was washed with distilled water and counter stained with safranine for 1 min. The slide was washed with distilled water, air dried and examined under the microscope at 100X oil immersion objective. The presence of green colored oval or spherical bodies indicated a positive result.

3.4.2.4 Catalase production test

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Hydrogen peroxide solution (1 ml) was discharged unto a clean glass slide and a sterile inoculating loop was used to pick a small part of the colony of the test organism which was subsequently immersed in the hydrogen peroxide solution. A positive result was indicated by the production of gas bubbles, while its absence was regarded as a negative result.

3.4.2.5 Citrate utilization test

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Simmon citrate broth (5 ml) was inoculated with the test organism. The broth was incubated at 37°C for 48 h. A positive reaction was indicated by a change in the colour of the medium from green to blue color. Negative tubes were observed daily for 4 days to detect any delayed reaction.

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3.4.2.6 Indole test

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This was carried out according to the protocol outlined by Cheesbrough (2006). The test organism was inoculated into Bijou bottle containing 3 ml of sterile peptone water, incubated at 35-37°C for 48 h. This was followed by the addition of 0.5 ml Kovac's reagent. Red colour on the surface layer within 10 min indicated positive test for indole.

3.4.2.7 Oxidase test

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Filter paper (Whatman) was soaked with 2 drops of freshly prepared oxidase reagent. Colony of the test organism was smeared on the filter paper using sterile wire loop. Positive oxidase was indicated by the production of a deep purple/blue colour within 10 s (Cheesbrough, 2006).

3.4.2.8 Urease production test

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This was carried out by inoculating the urea slopes with colonies of the organism and incubated at 37°C for 24 h. A colour change of the medium from yellow to pink/red was indicative of a positive result (Cheesbrough, 2006).

3.4.2.9 Voges proskauer test

This test was used to demonstrate bacteria that ferment carbohydrate with the production of acetyl methyl carbinol (CH_3-CO-CHOH.CH_3). This compound is oxidized during the test to diacetyl which reacts with a guanido group under alkaline condition to give a pink colour. The bacteria culture of the test organism was inoculated into 2 ml of sterile glucose phosphate peptone water and incubated at 37°C for 48 h. 1 ml of 40% KOH and 3 ml of 5% alcoholic alpha-naphthol (Barritts reagent) was then added. It was then shaken and observed for colour formation. A pink colour within 2-5 min indicates a positive result.

3.4.2.10 Sugar fermentation test

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Many bacteria species can be differentiated on the basis of the sugars they utilize and ferment. The fermentation medium was prepared by the addition of 0.1 g of peptone, 0.1 g of sodium

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chloride and 0.1 g of fermentable sugar (glucose, mannitol, lactose, sucrose and mannose) in 10 ml of distilled water. 4 ml of the medium was pipetted into Bijou bottles containing Durham tubes. 1 ml of phenol red indicator was also added to the tubes. The Bijou bottles containing the sugar solution were inoculated with the test bacterial isolates and incubated at 37°C for 24-48 h. After incubation, a change of color from red to yellow indicates acid production and the presence of gas in the inverted Durham tubes was indicative of gas production.

3.5 Determination of pH

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pH was determined using a pH meter (GZ Industrial Supplies)

3.6 Determination of Titratable Acidity

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Firstly, 2g of sample was extracted in 20ml of 4% oxalic acid for 30mins.After extraction for 30 mins, the solution was filtered. After 30 mins, titration was carried out by titrating 5mls of the filtrate against 1M NaOH using phenolphthalein as the indicator. Endpoint was read when the solution turns pink. This experiment was carried out to determine the average titre value.

3.7 Determination of Lycopene

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Lycopene content was measured by a rapid spectrophotometric method. Approximately 0.6g of sample will be weighted and added to a mixture consisting of 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% USP grade ethanol and 10 ml of hexane. The homogenate will be centrifuged at 320g for 15 min on ice. After shaking, 3 ml of distilled water will be added. The vials will then be agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper, hexane layer, will be measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample will be estimated using the absorbance at 503 nm and the sample weight. Results will be expressed as mg of lycopene per kg of fresh weight (Davis *et al.,* 2003; Fist *et al.,* 2002; Ayala-Zavala *et al.,* 2008).

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3.8 Ascorbic Acid Determination:

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Five (5) of standard solution of ascorbic acid will be pipette into 100ml conical flask. Ten (10)ml of oxalic solution will be added and the solution titration against the dye -2,6 dichlorophenol indophenols)(V1 ml) until a pink colour persist for 15sec. The dye consumed is equivalent the amount of ascorbic acid. Also, 0.5kg of the sample will be extracted in 4% oxalic acid and made up to 100ml, the filtrated. Ten (10)ml of oxalic acid will be added to the filtrate above and above solution titration against the dye solution. The volume of dye use will be recorded as (V2 ml) (Alakali *et al.*, 2015).

$$\text{Ascorbic acid (mg/100g)} = \frac{0.5\text{mg} \times V2 \times 100\text{ml}}{V1 \times 5\text{ml} \times W} \times 100$$

Where W = sample weight

3.9 Statistical Analysis

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All experiments were performed in triplicates. Descriptive statistics was used to present result as mean plus or minus standard deviation. Analysis was done using SPSS version 23.

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CHAPTER FOUR

RESULTS

Table 1 shows the pH recorded for all sample groups over the period of 21 days. On day 0, the highest pH was recorded by Ps65^{o}C (5.30 ±0.36) while the lowest was recorded by Pv0.3g/l (3.70 ±0.12). At the end of storage duration (28 days), Ps65^{o}C recorded the highest pH (5.80 ±0.33) and Pv0.4g/l the lowest with 4.00 ±0.11.

Table 2 presents the titratable acidity concentration realized from preserved tomato fruit puree sample with sodium metabisulphite. Ps65^{o}C.Pv0.1g/l and Ps65^{o}C recorded the lowest concentration on day 0 (0.032%) while Pv0.4g/l had the highest (0.53%). At the end of storage, the highest concentration, 0.062%, was recorded Pv0.5g/l and the lowest, 0.030%, was recorded by Ps65^{o}C.Pv0.1g/l.

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Table 1:pH of Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65^{o}C	5.30±0.36	5.40±0.91	4.50±0.91	5.80±0.33
Pv0.1g/l	3.80±0.33	3.70±0.12	3.70±0.33	4.40±0.66
Pv0.2g/l	3.80±0.12	3.70±0.66	3.60±0.11	4.30±0.91
Pv0.3g/l	3.70±0.12	3.60±0.91	3.60±0.12	4.10±0.12
Pv0.4g/l	3.70±0.66	3.50±0.12	3.60±0.36	4.00±0.11
Pv0.5g/l	3.70±0.33	3.50±0.66	3.70±0.36	4.00±0.36
Ps65^{o}C.Pv0.1g/l	3.90±0.33	4.10±0.33	3.60±0.66	4.40±0.91
Ps65^{o}C.Pv0.2g/l	3.90±0.11	4.10±0.66	4.50±0.11	4.40±0.12
Ps65^{o}C.Pv0.3g/l	4.00±0.36	4.20±0.11	4.40±0.66	4.40±0.66
Ps65^{o}C.Pv0.4g/l	4.00±0.66	4.20±0.12	4.30±0.36	4.30±0.33
Ps65^{o}C.Pv0.5g/l	4.00±0.91	4.20±0.66	4.10±0.91	4.10±0.11

Where, Ps is Pasteurization applied at 65°C for 15 mins

Pv is Preservative (metabisulphite) applied at varying concenctrations

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Table 2: Titratable Acidity of Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days (%)

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65^{o}C	0.032	0.032	0.034	0.032
Pv0.1g/l	0.045	0.046	0.046	0.044
Pv0.2g/l	0.049	0.050	0.051	0.047
Pv0.3g/l	0.051	0.052	0.052	0.049
Pv0.4g/l	0.053	0.055	0.052	0.050
Pv0.5g/l	0.064	0.066	0.065	0.062
Ps65^{o}C.Pv0.1g/l	0.032	0.031	0.029	0.030
Ps65^{o}C.Pv0.2g/l	0.038	0.036	0.034	0.034
Ps65^{o}C.Pv0.3g/l	0.040	0.038	0.037	0.036
Ps65^{o}C.Pv0.4g/l	0.041	0.040	0.038	0.038
Ps65^{o}C.Pv0.5g/l	0.042	0.040	0.041	0.041

Where, Ps is Pasteurization applied at 65°C for 15 mins

Pv is Preservative (metabisulphite) applied at varying concentrations

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Tables 3-5 shows the total heterotrophic, coliform and salmonella and shigella bacterial count realised from cultural analyses of stored tomato fruit puree treated with sodium metabisulphite over the course of 21 days. Nutrient agar, MacConkey agar and Salmonella shigella agar were used. The total heterotrophic bacterial count data shows $23.00 \pm 2.11 \times 10^4$ cfu/ml on day 21 as the highest count (Ps65^{o}C) and $1.00 \pm 0.11 \times 10^4$ cfu/ml on day 14 (Ps65^{o}C.Pv0.1g/l). The total coliform count data shows $22 \pm 2.31 \times 10^4$ cfu/ml as the highest on day 21 (1), while the lowest, $1 \pm 0.11 \times 10^4$ cfu/ml was recorded on day 21 (2). The total salmonella and shigella count data shows $21 \pm 2.31\ 10^4$ cfu/ml as the highest count on day 21 (Ps65^{o}C), while $2 \pm 0.11\ 10^4$ cfu/ml, recorded on day 0 (Ps65^{o}C, Pv0.5g/l) was the lowest count. No growth was recorded on samples 1-5 for the duration of assessment.

Table 6 shows the cultural, morphological and biochemical characteristics of the isolated bacteria from stored tomato puree samples over 21 days.

Table 7 shows the names of the identified bacteria isolated from stored tomato fruit puree treated with sodium metabisulphite. The isolated bacteria include *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas putida, Protus mirabilis, Proteus vulgaris* and *Citrobacter* sp.

Table 8 shows the distribution of isolated bacteria from stored tomato fruit puree treated with sodium metabislphite. *Klebsiella pneumoniae* had the highest occurrence.

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Table 3: Total Hetrotrophic bacteria (x 10^4 cfu/ml) Count of Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65^{o}C	-	10±1.41	18±1.41	23±2.83
Pv0.1g/l	19±1.00	16±1.41	12±4.24	14±1.41
Pv0.2g/l	17±2.00	15±1.41	10±2.82	12±1.41
Pv0.3g/l	15±2.65	13±2.82	9±1.41	11±1.41
Pv0.4g/l	14±1.00	13±1.41	7±1.41	11±2.83
Pv0.5g/l	12±3.00	10±1.41	7±2.82	10±1.41
Ps65^{o}C.Pv0.1g/l	-	-	1±1.41	3±1.41
Ps65^{o}C.Pv0.2g/l	-	-	1±1.41	2±1.41
Ps65^{o}C.Pv0.3g/l	-	-	-	1±1.41
Ps65^{o}C.Pv0.4g/l	-	-	-	1±1.41
Ps65^{o}C.Pv0.5g/l	-	-	-	1±1.41

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Table 4: Salmonella Shighella (x 10^4 cfu/ml) Count of Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65^oC	-	7±1.41	14±1.41	22±1.41
Pv0.1g/l	9±2.00	6±1.41	5±1.41	10±7.07
Pv0.2g/l	8±3.00	6±2.83	5±2.83	9±1.41
Pv0.3g/l	5±1.00	4±1.41	4±1.41	8±1.41
Pv0.4g/l	4±1.73	4±2.83	3±1.41	7±1.41
Pv0.5g/l	3±1.00	3±1.	3±2.8	7±4.24
Ps65^oC.Pv0.1g/l	-	-	-	3±1.41
Ps65^oC.Pv0.2g/l	-	-	-	1±1.41
Ps65^oC.Pv0.3g/l	-	-	-	1±1.41
Ps65^oC.Pv0.4g/l	-	-	-	-
Ps65^oC.Pv0.5g/l	-	-	-	-

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Table 5: Coliform (x 10^4 cfu/ml) Count of Tomzato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65^{o}C	2±1.00	7±4.24	18±2.83	2±1.41
Pv0.1g/l	10±5.00	8±1.41	10±7.07	18±5.66
Pv0.2g/l	7,10,10	5±1.41	11±1.41	15±2.83
Pv0.3g/l	9±1.73	5±2.83	9±1.41	113±1.41
Pv0.4g/l	3±1.00	3±1.41	5±4.24	7±1.41
Pv0.5g/l	2±1.00	2±1.41	3±1.41	7±4.24
Ps65^{o}C.Pv0.1g/l	-	-	-	-
Ps65^{o}C.Pv0.2g/l	-	-	-	-
Ps65^{o}C.Pv0.3g/l	-	-	-	-
Ps65^{o}C.Pv0.4g/l	-	-	-	-
Ps65^{o}C.Pv0.5g/l	-	-	-	-

Values= Mean± S.D x 10^{4ee}

Where, Ps is Pasteurization applied at 65°C for 15 mins

Pv is Preservative (metabisulphite) applied at varying concentrations

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Table 6: Morphological, Cultural and Biochemical Characteristics of Isolated Bacteria from Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Colour	pinkish red	Golden yellow	Greenish-blue	Pink	Pink	Black	Colorless	Greyish white	Colorless	Greyish white	Cream	Pink
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Smooth	Smooth	Smooth	Entire	Smooth
Opacity	Opaque	Opaque	Translucent-opaque	Opaque	Opaque	Opaque	Translucent	Opaque	Opaque	Translucent	Translucent	Opaque
Elevation	Convex	Convex	Low convex	Convex	Convex	Convex	Convex	Umbonate	Convex	Effuse	Convex	Convex
Wet/dry	Wet	Wet	Wet	Wet	Wet	Dry	Dry	Wet	Wet	Wet	Wet	Wet
Gram stain	-	+	-	-	-	-	-	-	-	-	-	-
Shape	Bacilli	Cocci	Bacilli	Bacilli	bacilli	bacilli	bacilli	Rod	Rod	Bacilli	bacilli	Rod
Arrangement	Singles	Grape-like clusters	Singly	Singles	Singly	Pairs	Singly	singly	singly	Clusters	clusters	Pairs
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	+	+	-	-	-	-	+	-	-	-
Indole	-	-	-	-	-	-	-	+	-	-	-	-
Urease	+	+	-	-	+	-	-	-	-	+	+	Variable
Citrate	+	+	+	+	+	-	-	-	+	+	+	+
Lactose	+	+	-	-	-	-	-	+	-	-	-	+
Sucrose	+	+	-	+	+	-	-	Variable	-	-	-	+
Maltose	+	+	-	+	+	+	+	-	-	-	-	+
Xylose	+	-		+	-	+	+			+	+	+
MR	-	+	-	-	-	+	+	+	-	+	+	+
Glucose	+	+	-	+	+	+	+	+	-	+	+	+
VP	+	+	-	+	+	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	-	-	+
Gas Production	+	-	+	+	Variable	-	-	+	+	+	+	+
Spore	-	-	-	-	-	-	-	-	-	-	-	-
Probable isolate												

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Table 7: Bacterial Isolates and their % frequency of occurrance from Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

ISOLATE	NAME	% frequency of Occurrence
1	*Klebsiella pneumoniae*	10.6
2	*Staphylococcus aureus*	10.6
3	*Pseudomonas aeruginosa*	10.6
4	*Enterobacter cloacae*	6.4
5	*Serratia marcescens*	6.4
6	*Salmonella typhi*	6.4
7	*Salmonella paratyphi*	8.5
8	*Escherichia coli*	8.5
9	*Pseudomonas putida*	6.4
10	*Proteus mirabilis*	8.5
11	*Proteus vulgaris*	8.5
12	*Citrobacter sp.*	8.5

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Table 8: Distribution of Isolated Bacteria from Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days

SAMPLE	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*	*P. aeruginosa*	*S. marcescens*	*S. typhi*	*S. paratyphi*	*E. coli*	*P. putida*	*P. mirabilis*	*P. vulgaris*	*Citrobacter sp.*
Ps65^{o}C	+	+	+	+	+	+	+	+	+=	+	+	-
Pv0.1g/l	+	+	+	+	-	+	-	+	+	+	+	+
Pv0.2g/l	+	+	+	-	-	-	+	-	-	-	-	-
Pv0.3g/l	+	+	+	+	-	-	+	-	+	+	+	+
Pv0.4g/l	-	-	+	-	-	-	+	+	-	+	-	+
Pv0.5g/l	+	+	-	-	+	-	-	+	-	-	+	+
Ps65^{o}C.Pv0.1g/l	-	-	-	-	+	-	-	-	-	-	-	-
Ps65^{o}C.Pv0.2g/l	-	-	-	-	-	-	-	-	-	-	-	-
Ps65^{o}C.Pv0.3g/l	+	-	-	-	-	+	-	-	-	-	-	-
Ps65^{o}C.Pv0.4g/l	-	-	-	-	-	-	-	-	-	-	-	-
Ps65^{o}C.Pv0.5g/l	-	-	-	-	-	-	-	-	-	-	-	-

Table 9 shows the concentration of lycopene recorded from stored tomato puree treated with sodium metabisulphite over the course of 21 days. The highest concentration after 21 days was recorded by Pv0.4g/l (15.05±0.03mg/kg). The lowest concentration was recorded by Ps65^{o}C (7.71±1.03mg/kg).

Table 10- shows the concentration of Ascorbic acid recorded from stored tomato puree treated with sodium metabisulphite over the course of 21 days. The highest concentration after 21 days was recorded by Pv0.4g/l (15.05±0.03mg/kg). The lowest concentration was recorded by Ps65^{o}C (7.71±1.03mg/kg)

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Table 9: Lyocpene Evaluation of Tomato Puree Samples Treated with Sodium Metabisulphite Over the Course of 21 days (mg/kg)

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65ºC	19.31±0.06	13.30±0.15	10.02±0.11	7.71±0.15
Pv0.1g/l	20.86±0.26	14.60±0.26	10.22±0.13	8.06±0.03
Pv0.2g/l	22.01±0.15	14.96±0.11	12.50±0.03	9.79±0.15
Pv0.3g/l	21.13±0.11	17.79±0.13	13.30±0.03	11.98±0.11
Pv0.4g/l	21.11±0.03	17.69±0.15	15.15±0.30	13.89±0.03
Pv0.5g/l	21.00±1.03	17.30±0.03	16.50±0.13	13.57±0.06
Ps65ºC.Pv0.1g/l	20.06±0.03	15.61±0.03	13.02±0.11	10.16±0.15
Ps65ºC.Pv0.2g/l	19.25±0.23	18.28±1.03	12.22±0.13	10.02±0.11
Ps65ºC.Pv0.3g/l	19.02±0.09	17.01±0.26	15.23±0.26	13.80±1.03
Ps65ºC.Pv0.4g/l	20.88±0.03	18.01±0.33	16.48±0.03	15.05±1.03
Ps65ºC.Pv0.5g/l	20.61±0.13	18.00±0.26	17.50±1.03	15.30±1.26

Where, Ps is Pasteurization applied at 65ºC for 15 mins

Pv is Preservative (metabisulphite) applied at varying concentrations

Values= Mean± S.D x 10^5

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Table 10: Ascorbic acid Evaluation of tomato puree treated with sodium metabisulphite over the course of 21 days mg/kg.

SAMPLE	Storage duration (days)			
	0	7	14	21
Ps65°C	0.70±0.071	0.58 ± 0.007	0.38 ±0.007	0.17 ± 0.007
Pv0.1g/l	0.74±0.071	0.46± 0.007	0.32± 0.007	0.21± 0.007
Pv0.2g/l	0.74±0.071	0.51± 0.007	0.35± 0.007	0.23± 0.007
Pv0.3g/l	0.72±0.071	0.53± 0.071	0.42± 0.007	0.22± 0.007
Pv0.4g/l	0.73±0.071	0.55± 0.007	0.41± 0.007	0.25± 0.007
Pv0.5g/l	0.74±0.071	0.63± 0.007	0.44± 0.007	0.26± 0.007
Ps65°C.Pv0.1g/l	0.70±0.071	0.57± 0.007	0.33± 0.007	0.31± 0.007
Ps65°C.Pv0.2g/l	0.71±0.071	0.52± 0.007	0.45± 0.007	0.33± 0.007
Ps65°C.Pv0.3g/l	0.71±0.071	0.63± 0.007	0.48± 0.007	0.35± 0.007
Ps65°C.Pv0.4g/l	0.70±0.071	0.60± 0.007	0.44± 0.007	0.37± 0.007
Ps65°C.Pv0.5g/l	0.71±0.071	0.67± 0.071	0.50± 0.007	0.39± 0.007

Where, Ps is Pasteurization applied at 65°C for 15 mins

Pv is Preservative (metabisulphite) applied at varying concentrations

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CHAPTER 5

DISCUSSION

Tomato is widely used all over the world and has application in multiple dishes, both salad-based and none salad based. The can be incorporated, cooked or uncooked in food. However, the fruit is susceptible to rapid spoilage by microorganism. The consequence of this is the processing, transportation and sale of tomatoes, has to be carried out within short periods of time, otherwise, exposure to injuries and contamination by microorganisms can potentially lead to losses in the tune of millions of dollars. In Nigeria, tomato plays a vital role in meeting domestic and nutritional food requirement, generation of income and creation of employment (Oluwasola, 2015; Sigei *et al.*2014).

This problem of rapid spoilage in tomatoes coupled with inadequate storage capabilities in Nigeria has lead to series of research into prolonging its shelf life for safe consumption. Some of these methods include pasting, canning, drying and pickling. The present study investigated preservation of pureed tomatoes treated with varying concentrations of sodium metabisulphite.

The pH ranged from 3.70±0.12 to 5.80±0.33. For control group 1, there was a decrease in pH from day 0 to day 14 and an increase on day 21. This trend was also shown by control group 2. However, samples 1 to 5 showed a trend of increase up until day 21. Sodium metabisulphite, being acidic in pH contributed to the low pH recorded on day 0 for control group Pv0.2g/l and samples Ps65^{o}C.Pv0.1g/l to Ps65^{o}C.Pv0.5g/l.As the storage duration continues, the effect of increased microbial activity in the samples was reflected in the pH change. Samples with higher bacteria count recorded higher pH, while samples with lower bacteria count recorded low pH after day 0. This result agrees with the study by Sanchez-Clement *et al*. (2018) that found increasing pH levels with increased contamination rates.

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As indicated in Table 2, the highest Titratable Acidity recorded was 0.062% (Pv0.4g/l on day 21), while the lowest 0.032% (Ps65ºC and sample Ps65ºCPv0.1g/l on day 0). Titratable acidity has an inverse relationship with pH in organic mediums. As pH increases, concentration of titratable acidity decreases. This was indicated in the result of the present study and in the result of the work by Luck *et al.* (2013).

The total heterotrophic bacteria count (Table 3) showed Ps with the highest count ($23\pm2.11\times10^{4}$ cfu/ml) while the lowest was recorded by sample 3 ($1.00\pm0.33\times10^{4}$ cfu/ml). Samples treated with pasteurization temperature (65ºC for 15 min) and sodium metabisulphite showed the least count. This indicates the potential of a combined approach to arrest spoilage in tomato. Mhanga (2015) also recorded low contamination rate in their study investigating comparison study on the effectiveness of sodium metabisulphite, acetic acid and lemon juice in tomato preservation. The total coliform count data(Table 5) shows $22\pm2.31\times10^{4}$cfu/ml as the highest (Ps65ºC) and $1\pm0.11\times10^{4}$cfu/ml as the lowest (Ps65ºC.Pv0.2g/l). No count was recorded from days 0 to 14 for Ps65ºC.Pv0.3g/l (1-5). The total *Salmonella Shigella* count (Table 4) after 21 days ranged from 21 ± 2.31 to $7\pm0.36\times10^{4}$ cfu/ml. Ps65ºC.Pv0.3g/l recorded low counts from days 0-21. From these results, there is indication that a combined approach of heat plus preservative has potential of completely inhibiting the growth of *Salmonella* and *Shigella species* in tomato. This agrees with the work done by Efiuvwevwere *et al.* (2020) and Abalaka and Deibel (1980) who also reported decreasing counts of *Salmonella* and *Shigella* on sample treated with sodium metabisulphite., The isolated bacteria in Table 7 from stored tomato puree treated with sodium metabisulphite includes: *Klebisella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas putida, Proteus vulgaris, Proteus mirabilis, Citrobacter sp.* These bacteria has been implicated in spoilage of food products. It is important that food

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products be regularly assessed for bacterial presence, 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 (Obeng *et al.,* 2018).

The most widely distributed isolate in the present study was *Klebsiella pneumoniae*. The least distributed was *Salmonella typhi.* This result contrasts with the study done by (Mohammed and Kuhiyep, 2020; Mapiki *et al*., 2017) that recorded *Salmonella species* widely distributed in the investigated sample groups.

Lycopene is a non-provitamin A carotenoid that exhibits several health benefits. Epidemiological data support a correlation between lycopene intake and the attenuation of several chronic diseases, including certain types of cancers and cardiovascular diseases. It is currently unknown whether the beneficial effects are from the native structure of lycopene or its metabolic derivatives: lycopenals, lycopenols, and lycopenoic acids. .At the end of storage(21 days), the highest concentration of lycopene was recorded by sample 5(15.30±0.06 mg/kg)while the lowest was recorded by Ps(7.71±1.03mg/kg). Lycopene decreases in concentration as spoilage increases. From the present study, the sample that recorded the least contamination recorded higher lycopene concentractions. This may probably be due to the activities of the bacteria in these samples. This result is in agreement with the work done by (Vinha *et al.,* 2013).

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CONCLUSION

Combining pasteurization and treatment with metabisulphite resulted in inhibition of bacteria in tomato puree at a concentration of 0.4g/l (Ps65^{o}C.Pv0.4g/l) up until 21 days., The level of lycopene was also retained after 21 days storage. More research is necessary to exploit this approach in obtaining a product that is even more desirable with lower contamination value. Tomato preservation is of utmost economic importance as wastage and loss will be minimized on wild spread adoption.

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APPENDIX A

MATERIALS: AGARS, REAGENTS AND INSTRUMENTS

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The agars used include: Nutrient agar, MacConkey agar, Triple Sugar Iron agar , Simmons citrate agar , Peptone Water, The reagents include, Sterile water, Gram staining reagents (such as the Crystal violet, Lugol Iodine, Alcohol and Safranin), Hydrogen peroxide, Oxidase reagent (tetramethyl-p-phenylenediaminedihydrochloride), Kovac's reagent, Plasma, Immersion oil, Copper sulphate, 0.5ml MacFarland reagent, Antibiotic sensitivity disk.

The instruments used in this study include: Universal container, Forceps, Petri dishes, Autoclave, Pressure cooker, Bunsen burner, Inoculating loop, Binocular electrical microscope, Paper tape, Marker, Foil paper, Conical flasks, Beakers, Durham tubes, Test tubes, Microscopic slides, Cotton wool, Electrical weighing balance, Refrigerator, Slant bottles, Incubator, Pasteur pipettes, Test tube rack, Bijou bottles, Measuring cylinder, Whatman filter paper

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APPENDIX B

MEDIA USED, THEIR COMPOSITION AND METHODS OF PREPARATION AND USES

NUTRIENT AGAR

It was prepared by measuring 28.0 gram of the dehydrated TM media of nutrient agar and dissolved in 1000ml distilled water and heated gently in order to allow the medium dissolve completely. This medium was then sterilized by autoclaving using an autoclave at 121°C for 15 min and then cooled to 45-50°C and dispensed aseptically into sterile petri dishes.

Prior to use, the plates were dried at 30-37°C in an incubator, with lids partly ajar, for not more than 30 min or until excess surface moisture has evaporated in order for the media not be easily contaminated which may result to swarming bacterial growth, which could give inaccurate results.

Composition

Ingredients	**Composition (g/ltr)**
Agar	15.000
Peptone	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
pH	7.2 ± 0. 2 at 25°C

Uses of Nutrient agar

It is a general purpose medium. It is used for the isolation and purification of cultures. It can also be used as a means for producing the bacterial lawns needed for antibiotics sensitivity tests.

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MACCONKEY AGAR

It was prepared by measuring 47.0 gram of the dehydrated TM media of MacConkey agar and dissolved in 1000ml distilled water and heated gently to ensure that the medium was properly dissolved completely and was sterilized by autoclaving at 121°C for 15 min and then cooled to 45-50°C and then dispensed aseptically into sterile petri dishes.

Prior to use, the plates were dried at 30-37°C in an incubator, with lids partly ajar, for not more than 30 mins or until excess surface moisture has evaporated in order for the media not be easily contaminated which may result to swarming bacterial growth, which could give inaccurate results.

Ingredients	**Composition** **Composition (g/ltr)**
Peptic digest animal tissue	20.000
Agar	12.000
Lactose	10.000
Bile Salts	5.000
Neutral red	0.075
pH	7.4 ± 0. 2 at 25°C

Uses of MacConkey agar

It is a selective medium. It is used for the isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacteria. Lactose fermenting strains grow red or pink while non- fermenting strains are colourless and transparent and typically do not alter appearance of the medium.

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SIMMONS CITRATE AGAR

It was prepared by measuring 24.28 gram of the dehydrated TM media of Simmons citrate agar and dissolved in 1000ml distilled water and heated gently to ensure that the medium was properly dissolved completely and was sterilized by autoclaving at 121°C for 15 mins and then cooled to 50°C and then dispensed aseptically into bijou bottles and slanted.

Composition

Ingredients	**Composition (g/ltr)**
Agar	15.000
Sodium chloride	5.000
Sodium citrate	2.000
Dipotassium phosphate	1.000
Ammonium dihydrogenphospahate	1.000
Magnesium sulphate	0.200
Bromothymol blue	0.080
pH	7.4 ± 0. 2 at 25°C

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Uses of Citrate agar

It is used for differentiating Enterobacteriaceae

on the basis of their ability to utilize citrate.

PEPTONE WATER

It was prepared by measuring 15.0 gram of the dehydrated TM media of peptone water and was dissolved in 1000ml distilled water and heated gently to ensure that the medium was properly dissolved completely and was sterilized by autoclaving at 121°C for 15 mins and then dispensed aseptically into Durham tubes.

Composition

Ingredients	**Composition (g/ltr)**
Pancreatic digest of animal tissue	10.000
Sodium chloride	5.000
pH	7.2 ± 0. 2 at 25°C

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