**DETERMINATION OF MICROBIAL LOAD AND SURVEY OF DIFFERENT PRESERVATION METHODS FOR DATES (***Phoenix dactylifera***) FRUIT IN MINNA MARKETS**

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**ABSTRACT**

Fruits and vegetables have been a vital part of human diets, and there has been an increase in food contamination as a result of postharvest processing and preservation handling. This study was carried out to determine the microbial loads and survey on the different preservation methods of Dates fruit. Six date fruits were gotten from two different markets (Kpakungu and Mobil) with random selection of date fruits sellers using different or similar preservation methods. Samples were taken to the laboratory and subjected to test for the determination of bacterial and fungal loads, the major bacteria isolated were (*Bacillus aureus*, *Bacillus subtilis*, *Psuedomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*). To help in safeguarding the well-being of end users and consumers of fruits and vegetables, proper postharvest processing and preservation method should be in check by farmers/producers as well as buyers, to reduce the alarming rate of contamination of mycotoxins and presence of health challenging microbes on fruits being consumed directly or indirectly. Fruits vegetables sellers and consumers should ensure that the produce is more hygienic and fit for consumption, and improve on the handling process at their different ends. An example of using light salt solution to wash fruits before consumption.

Keywords: Bacterial, Contamination, Date, Fungi, Fruits, Markets, Microbes

**INTRODUCTION**

Date palm (*Phoenix dactylifera*) is considered to be a highly important fruit crop in several African and Middle Eastern countries due to its nutritional value and health-promoting properties (Afshin *et al.*, 2019, WHO, 2019). The fruit serves as a source of several minerals, vitamins, carbohydrates, and fiber, and is consumed on a regular basis. Annual production in the Middle East was estimated at 5.1 million tons, and world production has been estimated at 7.2 million tons produced on an area of 11.2 million hectares, of which 11% are destined for export (Ecocrop, 2011; El-Deek *et al.,* 2010). Microbes are found all over the globe with some few exceptions Earth (Swanson *et al*., 2022), including sterilized surfaces. Hence, the activities of humans cannot be completely separated from microbes. Thus, many pathogenic microbes have found their way into fresh fruits and vegetables which are a great source of a healthy diet for humans. Although some of these bacteria have been shown over time to cause harm, some bacteria are necessary for our daily lives (Hallen-Adams and Suhr, (2017) and help in digestion, decomposition, and the production of food such as cheese, bread, and yoghurt, such as some strains of *Lactobacillus, Bifidobacterium*, *Erwinia,* and *Streptococcus*. *Lactobacillus bulgaricus* is well known throughout the world for the production of yoghurt (Chen, 2019). Some industries also utilize *Streptococcus thermophiles*  in producing yoghurt. The growing demand for fresh fruits and vegetables has necessitated larger production. The larger production of vegetables within the shortest possible time to meet the growing demand has placed them at a higher risk of contamination with the pathogenic microbes, making the safety of consumers uncertain. Consumption of fresh fruits and vegetables is increasing as consumers strive to eat healthier diets. Production, handling, and packing processes may predispose certain produce to contamination with food borne pathogens. Thus suitable preservation method is one of the most important input contributing to fruit production because it increase the sustainability and longevity of produce.

Although the health benefits of fresh date produce are great, the proportion of food borne disease outbreaks linked to contaminated produce has increased over the past few decades (Ailes *et al.,* 2008). Challenges in the postharvest treatments of dates include identifying appropriate packaging technologies and the management of food safety issues, the latter of which includes contamination with mycotoxic fungi as well as contamination with human food borne pathogens. Kurtzman (1987) reported that mold growth on foods that are consumed directly can result in direct exposure to mycotoxins which is very harmful to humans.

Dates go through a series of treatments after harvest to prepare them for marketing or long-term storage. Microbiological contamination of fresh fruits and vegetables continues to be a serious food safety issue. This research was therefore setup to know the types of microbial loads that are present and to determine the suitable storage method for the date fruit and to compare the difference in microbial loads on date fruits using different pos-harvest storage.

**METHODOLOGY**

**Study Location:** The study was conducted at the Food Processing/Animal Production Laboratory, of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State.

**Materials Used:** The materials used were:- Date fruit (seeds), Culture medias (Sabouroud Dextrose Agar (SDA) Nutrient Agar), Conical flasks (500ml), Test tubes, Source of flame, Syringe and needles (10ml), Autoclave , Petri dishes, Distilled water (grams), Pipette, Oven, Incubator, Fungi Hood, salt, water.

**Source of Fruits:** The samples were collected from different market locations in Minna metropolis. Date fruits were selected from different local sellers in the markets after seeking for information on their preservation method for the date fruit purchased. The locations with storage methods selected are listed below

1. Polythene covering storage method at Kpakungu market

2. Powder application storage method 1 at Kpakungun market

3. Powder application storage method 2 at Kpakungun market

4. Polythene and sack plus clothings storage method at Mobil market

5. Sack clothing bags 1 storage method at Mobil market

6. Sack clothing bags 2 storage method at Mobil market

**Media Preparation:** Serial dilution was used for the enumeration of bacterial and fungal loads which is the step wise dilution of a substance in solution and could be used for getting more manageable results. Two different media were used, these are; Nutrient Agar (NA) and Saboraud Dextrose Agar (SDA). 28g of Nutrient agar was dissolved in 1000ml of distilled water and 65g of Saboraud dextrose agar was also dissolved in 1000ml of distilled water. After dispensing into distil water, they were brought to heat to dissolve agar-agar completely. All the prepared media were autoclaved at 121°C for 15 minutes and they were brought out to cool to 40-45°C before the inoculation of the bacteria and fungi.

**Preparation of Diluent:** Serial dilution was carried out on the samples by taking 1ml of the sample into the first test-tube using a micropipette and mixed. Sample aliquot was taken from the first test-tube again this was repeated until the last tube was achieved. 1ml of the diluted sample was taken and dispensed into the sterile Petri-dish and about 20ml of the molten agar was poured into the Petri-dish and rocked gently for homogeneity. The culture plate was allowed to solidify and then transferred into the incubator. The culture plate containing the Nutrient agar was cultured at 370C for 24hrs while that containing the Macconkey agar was cultured at 370 C between 24 hrs. The resulting growth of the cultures were counted to the colony forming unit per mil (CFU/ml). 9mls of distilled water was dispensed into test tube i.e six test tubes per samples and cork with foil paper and were autoclaved for 15minutes at 1210C and the test tubes were cooled at room temperature.

**Inoculation:** Two test tubes to be used for serial dilution was arranged in the test tubes rack per sample and 1g of the Dates fruit sample was introduce into the first test tube and was labelled as 10¹ and 1ml was taken from 10¹ and was introduced into the second test tube and labelled 102 and shaken. 1ml out of 10² was taken into the Petri dish in an aseptically order for bacterial inoculation and another 1ml into the second petri dish for fungi inoculation. The procedure was repeated to the remaining samples and molten Nutrient Agar (NA) was introduced into the Ptri dishes for bacteria and Saboraud Dextrose Agar (SDA) into the Petri dishes for fungi and were allowed to jell.

**Counting of Viable Colony:** The counting of the developed colony was done by counting the cell using colony click-counter machine and a pen. The number of colony obtained was multiplied by dilution factor e.g six colony (6×105colony forming unit). It is expressed as 6×105 cfu/g.

**Statistical Tool:** All collected data were subjected to statistical Analysis of Variance (ANOVA) test using Duncan Multiple Range Test (DMRT), mean separated at 5% level of significance.

**RESULTS AND DISCUSSION**

**Difference in Bacterial Loads Found on Fruits with Different Storage Methods Sourced from the Market**

The effect of storage method on bacteria load present on the fruit from various locations are shown in Table 1. Polythene with sack plus clothing storage method at Mobil market had higher presence of bacterial on the fruit. The lowest count was recorded in the sample with clothing alone. The reason for higher number under Polythene with sack plus clothing storage method at Mobil market may not be unconnected with the fact that nylon do encourage heat build-up, and with heat build-up, bacterial presence are favoured. This is in line with observations of Gil *et al*. (2015) where he asserted that post-harvest handling medium can encourage contaminations

**Table 1: Difference in Bacterial Loads Found on Fruits with Different Storage Methods Sourced from the Market**

|  |  |
| --- | --- |
| **Storage Method and Location** | **Bacteria** (cfu/g) |
|  |  |
| Polythene covering K.market | 14.33 x 106cd |
| Powder applicationK.market | 23.67 x 106bc |
| Powder applicationK.market | 14.00 x 106cd |
| Polythene/sack clothing bagM.market | 46.00 x 106a |
| Sack clothing bags 1M.market | 28.00 x 106b |
| Sack clothing bags 2M.market | 6.00 x 106d |
| SE $\pm $ | 3.24 |

**Differences in Fungi Loads Found on Fruits with Different Storage Methods Sourced from the Market**

The effect of storage method on fungi load present on the fruit from various locations are shown in Table 2. The fungi count was significantly different. The use of Sack clothing bag 2 produced lowest population of fungi. Sack material, has more holes and encourage better aeration.

The use of Polythene and sack plus clothing storage method at Mobil market had date fruit with higher fungi count. The reason adduced for this may not be unconnected with heat produced when things are stored in the polythene materials. In addition to this, most of these materials are hardly washed, but subjected to continual usage and thus encourage microbial build up. This is in line with conclusion of Acheampong (2015) where he stated that “containers used in washing vegetables by farmers as well as fruits and vegetable vendors are not mostly washed after use, and even if washed, the water is used for several cycles allowing for cross-contamination of microbes with the recently washed ones since they are put in the same water as the first cycle” .

**Table 2: Differences in Fungi Loads Found on Fruits with Different Storage Methods Sourced from the Market**

|  |  |
| --- | --- |
| **Storage Method and Location** |  **Fungi (**cfu/g) |
|  |  |
| Polythene covering K.market | 34.67 x 106b |
| Powder applicationK.market | 18.69 x 106cd |
| Powder applicationK.market | 25.33 x 106bc |
| Polythene/ Sack clothing bagM.market | 49.33 x 106a |
| Sack clothing bags 1M.market | 22.00 x 106bc |
| Sack clothing bags 2M.market | 8.33 x 106d |
| SE $\pm $ | 4.51 |

**KEY:** K.market (kpakungun market). M.market (Mobil market)

**Cross Sectional view of Bacterial Isolated from Different Storage Methods and Locations**

Cross Sectional view of Bacterial Isolated from Different Storage Methods and Locations are shown in Table 3. From the chat, it can be concluded that the method of preservation may not be the reason for reduction of microbial loads on the fruits. This is because similar preservation methods from same location produced different results. For example, Sack clothing 1 did not encourage the presence of *Bacillus aureus* and *Bacillus subtili* in Mobil market location but with, Sack clothing 2 @ mobil market, these two bacterial loads were found. One of the isolated bacterial, *Bacillus cereus* is an example of Gram-positive bacteria which is responsible for causing intoxications in food (Bhunia, 2018)

**Table 3: Cross Sectional view of Bacterial Isolated from Different Storage Methods and Locations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **METHOD OF** **STORAGE**  | ***Bacillus*** ***aureus***  | ***Bacillus*** ***subtili***  | ***Pseudomonas*** ***aeroginosa***  | ***Staph*** ***aureus***  | ***E.coli***  |
| **Polythene covering** K-Market  | - - | ++ | - - | - - | ++ |
| **Powder application** K-Market  | ++ | ++ | ++ | - - | - - |
| **Powder application** K-Market  | - - | - - | ++ | ++ | - - |
| **Polythene/S.clothing** **bag** M-Market  | ++ | ++ | - - | - - | ++ |
| **Sack clothing bags 1** M-Market  | - - | - - | ++ | - - | ++ |
| **Sack clothing bags 2** M-Market  | ++ | ++ | - - | - - | ++ |

KEY:(++) means Samples with Storage Method has Bacteria. (- -) means Samples with Storage Method has no Bacteria. S. Clothing) means Sack Clothing Method. K. market means Kpakungun Market. M. market means Mobil Market.

**Comparison of Bacterial Loads Found on Date Fruits from Markets and those treated with Salt Solution after Purchase.**

Comparison of bacterial loads found on date fruits from markets and those treated with Salt Solution after Purchase is shown in Table 4. After treating the date fruits with salt solution, the fungi load on them were reduced and safer for consumption. This emphasizes the need for consumers to treat produce purchased from market before consumption to avoid food poisoning. Acheampong, (2015) asserted the importance of this especially to farmers to ensure proper disinfection of containers over and over before being used to sell to consumers. With this, consumers will have lesser contaminants to deal with.

**Table 4: Comparison of Bacterial Loads Found on Date Fruits from Markets and those Treated with Salt Solution after Purchase**

|  |  |
| --- | --- |
| **Method of Storage**  | **Fungi (**cfu/g) |
| **Polythene covering** K.market  | 34.67 x 106b  |
| **Powder application** K.market  | 18.67 x 106cd  |
| **Powder application** K.market  | 25.33 x 106bc  |
| **Polythene/S.clothing bag** M.market  | 49.33 x 106a  |
| **Sack clothing bags 1** M.market  | 32.00 x 106b  |
| **Sack clothing bags 2** M.market  | 8.33 x 106d  |
| 0.5% salt concentration/oven dry  |  25.33 x 106bc  |
| 1.0% salt concentration/oven dry  |  8.00 x 106d  |
| SE±  | 4.00  |

**CONCLUSION AND RECOMMENDATIONS**

The presence of bacterial load on date fruits from Minna has been confirmed. From the storage method i.e. polythene covering, powder application and sack clothing’s there is no storage in which bacterial presence was not noticed though the prevalence varies. The reasons for high microbial load could be as a result of the post-harvest handling from where these sellers source their produce from. These support the claim of Navarro, (2006) where he reported that field infestations of date fruits pose a serious contamination problem during the Postharvest life of dates and decrease quality. Proper treatment of fruits before consumption will also help in making produce consumption safer. The use of light salt solution for washing before consumption is recommended as a proven processing handling of date fruits.

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