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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 due to postharvest processing and preservation handling. This study was carried out to determine the microbial loads and survey the different preservation methods of Dates fruit. Six date fruits were obtained from two different markets (Kpakungu and Mobil) with a random selection of date fruit sellers using different or similar preservation methods. Samples were taken to the laboratory and subjected to a test to determine bacterial and fungal loads. The major bacteria isolated were (Bacillus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli). To help safeguard the well-being of end users and consumers of fruits and vegetables, proper postharvest processing and preservation methods should be in check by farmers/producers as well as buyers to reduce the alarming rate of contamination of mycotoxins and the presence of health-challenging microbes on fruits consumed directly or indirectly. Fruit vegetable sellers and consumers should ensure that the produce is more hygienic and fit for consumption and improve 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 a significant 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 is a source of several minerals, vitamins, carbohydrates, and fibre and is consumed regularly. Annual production in the Middle East was estimated at 5.1 million metric tonnes, and world production has been estimated at 7.2 million metric tonne s 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 a few exceptions on Earth (Swanson *et al*., 2022), including sterilized surfaces. Hence, human activities cannot be wholly separated from those of microbes. Thus, many pathogenic microbes have found their way into fresh fruits and vegetables, 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* to produce yoghurt. The growing demand for fresh fruits and vegetables has necessitated more significant 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 pathogenic microbes, making the safety of consumers uncertain. Consumption of fresh fruits and vegetables increases as consumers strive to eat healthier diets. Production, handling, and packing processes may predispose certain produce to contamination with food-borne pathogens. Thus, a suitable preservation method is one of the most critical inputs contributing to fruit production because it increases 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 managing food safety issues, the latter of which includes contamination with mycotoxic fungi and contamination with human food-borne pathogens. Kurtzman (1987) reported that mould growth in foods consumed directly could result in direct exposure to mycotoxins, which are very harmful to humans.

Given the ongoing concern about the microbiological contamination of fresh fruits and vegetables, particularly date fruits, this research was initiated with the aim of identifying the types of microbial loads present, determining the most suitable storage method for date fruits, and comparing the microbial loads on date fruits under different postharvest storage methods. This would provide valuable insights into the best practices for preserving the safety and quality of date fruits.

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 media (Sabouroud Dextrose Agar (SDA) Nutrient Agar), Conical flasks (500ml), Test tubes, a Source of flame, a Syringe and needles (10ml), an Autoclave, Petri dishes, Distilled water (grams), a Pipette, an Oven, an Incubator, a Fungi Hood, salt, and water.

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

1. Polyethene covering storage method at Kpakungu market
2. Powder application storage method one at Kpakungun market
3. Powder application storage method two at Kpakungun market
4. Polyethene and sack plus clothing storage method at Mobil market
5. Sack clothing bags one storage method at Mobil market
6. Sack clothing bags two storage methods at the Mobil market

Media Preparation: Serial dilution was used to enumerate bacterial and fungal loads, which is the step-wise dilution of a substance in solution and could be used to get more manageable results. Two different media were used: Nutrient Agar (NA) and Saboraud Dextrose Agar (SDA). 28g of Nutrient agar was dissolved in 1000 ml of distilled water, and 65g of Saboraud dextrose agar was also dissolved in 1000 ml of distilled water. After dispensing into distilled 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 bacteria and fungi were inoculated.

Preparation of Diluent: Serial dilution was carried out on the samples by mixing 1mL of the sample into the first test tube with a micropipette. The sample aliquot was retaken from the first test tube. This was repeated until the last tube was achieved. 1 ml of the diluted sample was taken and dispensed into the sterile Petri dish, and about 20 ml 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 24 hours, while that containing the Macconkey agar was cultured at 370C for 24 hrs. The resulting growth of the cultures was counted to the colony-forming unit per ml (CFU/ml). Nine mL of distilled water was dispensed into a test tube, i.e. six test tubes per sample and cork with foil paper, and were autoclaved for 15 minutes at 1210C, and the test tubes were cooled at room temperature.

Inoculation: Two test tubes to be used for serial dilution were arranged in the test tubes rack per sample, and 1g of the Dates fruit sample was introduced into the first test tube and was labelled as 10^1 and 1ml was taken from 10^1 and was introduced into the second test tube and labelled 102 and shaken. One mL out of 10^2 was taken into the Petri dish in an aseptic order for bacterial inoculation and another 1 ml 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 Petri dishes for bacteria and Saboraud Dextrose Agar (SDA) into the Petri dishes for fungi and were allowed to jell.

Counting of Viable Colony: The developed colony was counted by counting the cells using a colony click-counter machine and a pen. The number of colonies obtained was multiplied by the dilution factor, e.g., six colonies (6×105colony forming unit). It is expressed as 6×105cfu/g.
Statistical Tool: All collected data were subjected to a statistical Analysis of Variance (ANOVA) test using the Duncan Multiple Range Test (DMRT), with the mean separated at the 5% level of significance.

RESULTS AND DISCUSSION

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

The effect of the storage method on the bacteria load present on the fruit from various locations is shown in Table 1. Polyethene with a sack plus clothing storage method at the mobile market had more bacteria in the fruit. The lowest count was recorded in the sample with clothing alone. The reason for the higher number under Polyethene with sack plus clothing storage method at Mobil market may not be unconnected with the fact that nylon does encourage heat build-up, and with heat build-up, bacterial presence is favoured. This is in line with the observations of Gil *et al*. (2015), who asserted that the use of postharvest handling mediums can encourage contamination.

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	14.33×10^{6}cd
Powder application K.market	23.67×10^{6}bc
Powder application K.market	14.00×10^{6}cd
Polythene/sack clothing bag M.market	46.00×10^{6}a
Sack clothing bags 1 M.market	28.00×10^{6}b
Sack clothing bags 2 M.market	6.00×10^{6}d
SE $\pm$	3.24

Significant Variations in Fungi Loads on Fruits Due to Different Storage Methods Sourced from the Market

Table 2 presents the impact of the storage method on the fungi load found on fruits from various locations. The fungi count varied significantly, with the use of Sack Clothing Bag 2 resulting in the lowest population of fungi. This can be attributed to the sack material's higher aeration due to more holes, which discourages fungal growth.

Polyethene and sack plus clothing storage methods at the Mobil market had date fruits with higher fungi counts. The reason for this may not be unconnected with the heat produced when things are stored in polythene materials. In addition, most of these materials are hardly washed but subjected to continual usage, thus encouraging microbial build-up. This is in line with the conclusion of Acheampong (2015), who stated that containers used in washing vegetables by farmers and fruits and vegetable vendors are not mostly washed after use. 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	34.67×10^6b
Powder application K.market	18.69×10^6cd
Powder application K.market	25.33×10^6bc
Polythene/ Sack clothing bag M.market	49.33×10^6a
Sack clothing bags 1 M.market	22.00×10^6bc
Sack clothing bags 2 M.market	8.33×10^6d
SE ±	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 the reduction of microbial loads on the fruits. This is because similar preservation methods from the same location produced different results. For example, Sack Clothing 1 did not encourage the presence of *Bacillus aureus* and *Bacillus subtili* in the Mobil market location, but with Sack Clothing 2 at the Mobil market, these two bacterial loads were found. One of the isolated bacteria, *Bacillus cereus,* is an example of Gram-positive bacteria which is responsible for causing intoxication 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

The comparison of bacterial loads found on date fruits from markets and those treated with Salt Solution after Purchase, as detailed in Table 4, highlights the crucial role of consumers in ensuring food safety. After treating the date fruits with a salt solution, the fungi load was significantly reduced, making them safer for consumption. This underscores the need for consumers to take responsibility for treating produce purchased from the market before consumption to prevent food poisoning. Acheampong (2015) emphasized the importance of this, particularly for farmers, to ensure the repeated disinfection of containers before selling to consumers. By following this advice, consumers can significantly reduce their exposure to contaminants.

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	34.67×10^6b
Powder application K.market	18.67×10^6cd
Powder application K.market	25.33×10^6bc
Polythene/S.clothing bag M.market	49.33×10^6a
Sack clothing bags 1 M.market	32.00×10^6b
Sack clothing bags 2 M.market	8.33×10^6d
0.5% salt concentration/oven dry	25.33×10^6bc
1.0% salt concentration/oven dry	8.00×10^6d
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. polyethene covering, powder application, and sack clothing, there was no storage in which bacterial presence was not noticed, though the prevalence varied. The reasons for the high microbial load could be due to the postharvest handling from which these sellers source their produce. Proper treatment of fruits before consumption will also make produce consumption safer. A light salt solution for washing before consumption is recommended as a proven method of processing and handling date fruits.

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