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Microbiological Assessment Of Date Fruits Purchased From Owode Market, In Offa, Kwara State Nigeria.

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Abstract: *Hard and soft dates sold in nylon were purchased from owode market in Offa, Kwara state, Nigeria. The bacteria load ranged from* $4 \times 10^5 - 19 \times 10^5$ *and* $10 \times 10^5 - 20 \times 10^5$ *respectively. The bacteria isolated were Staphylococcus aureus, Streptococcus species, Proteus mirabilis, Enterobacter species, Escherichia coli and Salmonella species. The coliform count were between 3-9 in hard date fruits and 11-23 in soft date fruit respectively. Mould count were* $1.0 \times 10^2 - 2.8 \times 10^2$ *and* $4.8 \times 10^3 - 7.2 \times 10^3$ *while yeast count were* $1.6 \times 10^2 - 8.2 \times 10^3$ *and* $5 \times 10^4 - 16 \times 10^4$ *respectively. All these values were above the Saudi specification. Therefore these samples cannot be recommended for human consumption. A means of preserving dates fruit from microbial contamination and spoilage should be discovered, also storing in hygienic conditions is important before selling to the populace for consumption to prevent the spread of infection and diseases.*

Keywords: *Date palm(phoenix dactilyfera L.), Staphylococcus aureus, Escherichia coli, Salmonella species, mould yeast, contamination, pathogenic*

I. Introduction

The date palm (*Phoenix dactylifera* L.) is one of mankind's oldest cultivated plants. It has been used as food for 6000 years (W. M. Amer 1994). It could be used for generations to come due to its remarkable nutritional, health and economic value, in addition to its aesthetic and environmental benefits. Every part of the date palm is useful. Dates offer useful prospects for fighting hunger and diseases. Dates (*Phoenix dactylifera* L.) are mainly grown in Middle East and North African countries, with a worldwide annual production of about 6 million tones. Saudi Arabia produces yearly about 900 thousand tones and ranks as the third largest producer in the world (FAO, 2008). About 50% of the produce in Saudi Arabia is consumed locally as human food, only about 4% is exported, while the rest is mainly used as animal feed (Al Eid, 2010).

A date palm fruit is an important component of the diet in most of the hot arid and semi arid regions of the world. It is found to contain carbohydrates (total sugars 44% - 88%), fats (0.2% - 0.4%), proteins (2.3% - 5.6 %), fibers (6.4% - 11.5%), minerals and vitamins (W. M. Amer 1994).

Carbohydrates in dates are mostly in the form of fruc-tose and glucose, which are easily absorbed by the human body. Interestingly, dates contain high concentrations of protein when compared to other cultivars of fruits such as apples, oranges, bananas and grapes (0.3%, 0.7%, 1.00%, and 1.00% proteins, respectively).Showiman (S. S. Al-Showiman 1998).

Twenty three different amino acids were found in date's proteins, many of which are not found in the most popular fruits (M. Al-Farsi et al 2005). There are more than 2000 different varieties of fresh dates [1]. Many fresh varieties are available throughout 8 months of the year. Packed, dry dates keep well without the addition of preservatives for at least 8 months, the high sugar content acting as an effective preservative.

Microbial contamination, especially with molds, is a major obstacle facing international marketing of Saudi dates (Al Eid, 2010). Dates are fairly dry fruits, with water and sugar contents of 10-15% and 60-88% (on dry basis), respectively (Barreveld, 1993), hence they are generally regarded as stable to microbial spoilage. However some contaminants, especially osmotolerant yeasts and molds, may survive for longer times or even grow on the fruits. Microbial contaminants isolated from date fruits include yeasts, molds, lactic acid bacteria and some potential pathogens like *Staphylococcus aureus E. coli*, and *A. flavus/parasiticus* (Bolin et al., 1972; Abu-Zinada and Ali, 1982; El-Sherbeeny et al., 1985; Nussinovitch et al., 1989; Abdulsalam et al.,1991; Aido et al., 1996; Kader 2007; Hamad, 2008).

II. Materials And Method

Collection of Samples

Dates fruits (*Phoenix dactylifera* L.) were purchased from owode market in Offa, Kwara state of Nigeria. Hard and soft date palm fruit Samples were picked randomly from the seller, to assure a good representation. The samples were cleaned by removal of foreign matter and taken in Polyethylene bags with labels and brought the laboratory for analysis immediately.

Preparation of Date Fruits for Analysis

The sample collected were cleaned and pitted; 10g of hard and soft date were aseptically weighed into sterile stomacher bags and 90ml distilled water added. Samples were then homogenized for 10minutes and aliquot were used for microbiological analysis.

III. Microbiological Analysis

To determine total viable count, 1ml of aliquot of hard and soft dates were serially diluted in 9ml of distilled water respectively. 1ml aliquots from suitable dilution ($10^3 - 10^5$) were transferred aseptically into sterile Petri-dishes. To each dilution 10-15ml of melted and cooled to (45^0C), Nutrient were added; Inoculum was mixed well with the medium and allowed to solidify. The plates were then incubated at 37^0C for 24-48 hours.

Inoculum were streaked on nutrient agar, mannitol salt agar, Mac-Coney agar and Deoxy Cholate citrate agar (DCA). Incubated at 37^0C for 24-48hours. Pure culture were made by sub-culturing on nutrient agar plate, using the streaked plate method until a pure culture was obtained.(Monica cheesebrough, 1984). Morphological characteristics, Gram staining and biochemical test were used to confirm the bacteria isolated. (Monica cheesbriugh 1984).

The coliform test was performed by placing 9ml double strength Lactose broth in 3 test tubes and 9ml single strength in six-test-tubes, three tube containing double strength were inoculated with 10ml of the aliquot, three tubes containing single strength Lactose broth were inoculated with 1ml of aliquot, while the last 3 test-tubes were inoculated with 0.1ml of original stock culture, Durham tubes were inverted into these tubes and gas bubbles were removed to prevent false positive result. The mouth of the test-tubes were plugged with cotton wool, wrapped with foil and were incubated at 37^0C for 48hours; then change in colour and gas production were observed. The confirmed tests were done by inoculating Mac-Conkey agar plate with 1ml positive presumptive test- in tubes by streaking respectively. The plates were incubated at 37^0C for 24hours. Colonies from these were sub-cultured on Eosin methylene blue agar (the completed test), incubated at 37^0C for 24-48 hours.

Isolation of mould and yeast cell were done by stabbing Potatoe dextrose agar plate and Malt extract plate with an inoculum from aliquot grounded with grinder. Another set of plates were inoculated with 0.5 ml inoculum from (10^4-10^5) and spread by glass spreader, incubated at $27^0 \pm 1$ for 3-5 days. Growth were observed and recorded.

IV. Results And Discussion

The total bacterial viable count for Hard date fruit were between 8×10^5 - 19×10^5 Cfu/ml. The soft date fruits were 10×10^5- 20×10^5 Cfu/ml. This is shown in Table 1 below.

Table 1 Showing bacterial and Coliform count from Dates fruit Phoenix Dactylifera

BACTERIAL VIABLE COUNT			COLIFORM COUNT	
S/N	HARD	SOFT	HARD	SOFT
1	19×10^5	20×10^5	3	11
2	8×10^5	14×10^5	7	18
3	4×10^5	10×10^5	9	23

Table 2 Showing mould count and Yeast Viable count from dates fruits Phoenix Dactylifera

MOULD COUNT			YEAST VIABLE COUNT	
S/N	HARD	SOFT	HARD	SOFT
1	1.0×10^2	4.8×10^3	3.0×10^3	5×10^4
2	1.4×10^2	6.0×10^3	1.6×10^2	10×10^4
3	2.8×10^2	7.2×10^3	8.2×10^3	16×10^4

The bacteria counts were very high in both the hard and soft Date fruit Phoenix Dactylifera in table 1. The values obtained were much higher than the recommended value for food < 10^4 Cfu/ml.(Ossai ochonogor Samuel, 2012) This implies extreme contamination and potential health risk; these findings correlated with similar earlier study (Olukoya *et al* 1991; Mensah *et al* 2002; Yahoah Manu *et al* 2010). The high incidence of bacterial contamination are mainly due to unsanitary and largely unhygienic nature of selling conditions and environment which are good indicator of the state of environment in which the dates are being sold. Majority of these fruits are close to waste disposal point or dusty road or street with human and vehicular traffic which

encourage multiple contamination due to the deposition of bioaerosol on the exposed fruits also transfer from one hands and flies also lead to contamination (Yassin and Almouqatea 2010).This show the date fruits were highly contaminated, and therefore make the date fruit unfit for consumption. The total bacteria viable count usually indicates the general microbiological quality of date fruits (Moore *et al* 2001). The bacteria isolated were *Staphylococcus aurues, Staphylococcus species, Streptococcus species, Proteus mirabilis, Enterobacter species, Escherichia coli and Salmonella species.* All the bacteria isolated are pathogenic and can lead to serious diseases especially in the case of *Salonella, Staphylococcs and Escherichia coli* which are known pathogens that can cause serious infections damages to organs and can lead eventually to death. The coliform count were between 3-9 in hard date fruit and 11-23 in soft date fruit, though this values were below 100, recommended values for coliform count in foods(Ossai ochonogor Samuel, 2012), but it still confirm faecal contamination from the environment ,like from the aerosol in the environment or handling and the selling condition in the market. Coliform bacteria chiefly faecal coliform are enteric bacteria, whose natural habitat is the intestinal tracts of humans and animals (Pelczar *et al* 2005). They are faecal indicators, and their isolation in hard and soft dates fruits indicates the presence of faecal contamination from the unsanitary environment or via human handler (Pelczar *et al* 2005). The isolation of coliform bacteria in all the dates fruits samples makes these fruits hazardous for human consumption. The isolated enteric bacteria are known pathogens responsible for millions of cases of infectious gastro intestinal diseases and death in each year.

Yeast cells counts in hard dates fruits were between 1.6×10^2 to 8.2×10^3. on the other hand, date fruit with soft cotyledons have yeast count which ranged from 5×10^4 to 16×10^4 values obtained in this case were high and above recommended value, thereby making it unfit for human consumption. The limit for yeast contamination in date fruits according to the Saudi standard specification are in 2 out of 5 replicate tested from a sample the targeted limit is 10Cfu/g and no implicate should reach a load of 10^2 Cfu/g (SASO, 1999). The mould counts were $1 \times 10^2 - 2.8 \times 10^2$ in hard date and $4.8 \times 10^3 - 7.0 \times 10^3$ in soft dates (Table 2). This also is a high level of contamination, the limits for mould contamination in date fruits according to Saudi Standard Specifications are (these specifications include limits for moulds, Yeast and *E coli* only): in 2 out of 5 replicate tested from a sample the targeted limit is 10^2Cfu/g and no replicate should reach a load of 10^3Cfu/g (SASO, 1999). In hard date examined two out of the three samples (2,3) examined (Table 2) were therefore out of specification, also all the soft date were with load of 10^3Cfu/g because they contained more than 10^2Cfu/g, hence only 1 sample therefore in hard date met the requirement for limits of Saudi specification. The high level of mould contamination may be due to the period of harvesting, if it is dry-windy month contamination can occur; airborne mould spores can easily contaminate the fruits of the tall palm trees. Contamination can also occur from the market environment and through handling. Studies by (Siddig Hussein hamad 2012) show that most microbial contamination of date die with time if the fruits are packaged and stored at refrigeration temperature. Date fruits are also known to contain some antimicrobial components. For example, some varieties contain up to 2.5% tannis (Al-Hooti *et al* 1997; Myhara *et al* 2000) which have been reported to cause growth inhibition to many species of fungi and bacteria (Nelson *et al* 1997; Ishida *et al* 2006). Only some osmotolerant yeasts seem to be able to survive or grow in packaged date fruits stored under refrigeration conditions. The yeast cells and these mould isolated in most cases, causes food poisoning. Therefore, the date fruits are not good and dangerous for human consumption.

V. Conclusion

The result from present study shows that both the hard and soft dates purchased in nylon and open in wheel barrow from owode market in Offa, Kwara state were highly contaminated with pathogenic bacteria, mould and yeast. This makes it hazardous for human consumption. Therefore a way of storing and preserving date fruit should be considered before selling to the public for human consumption. A good method is by packaging and storing at low temperature in refrigerator.

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