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Microbial Evaluation and Control of Microbes in Commercially Available Date *(Phoenix dactylifera Lynn.)* Fruits

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Abstract

Commercially available date fruit samples in different forms were collected and tested for the initial microbial load. Various types of bacterial and fungal species were observed from the test results. Food borne pathogens like *S. aureus* was identified in collected samples. Pet Jar Seedless and Polythene Seeded packs contained more amounts of microbes. So samples from these types are taken and subjected to physical control parameters like refrigeration, deep freezing, heat treatment and irradiation. The radiation and hot air oven treatments contain different time intervals and packed aseptically and stored in room temperature. Samples also kept in refrigerator and in deep freezer for cold treatments. Then the samples kept as undisturbed for 30 days. Based on 15th and 30th day microbial counts from samples, it is observed that deep freezing and refrigeration has more positive effect to control the level of microbes and it is concluded that refrigeration is the best way to control microbes in date (*Phoenix. dactylifera. Lynn.*) fruits.

Keywords: Dates; Microbes; Control; Radiation; Freezing; Hot air oven

Introduction

Date fruits (*Phoenix dactylifera Lynn.)* is one of the oldest fruits in the world. It has been used from 6000 Years ago [1]. It is grown in many countries worldwide, majorly Algeria, China, Egypt, Iran, Iraq, Pakistan, Saudi Arabia, Sudan and United Arab Emirates (UAE) [2]. The fruit weights from 4.60 g to 11.62 g. 100 g of date flesh provide 73.5 g of carbohydrates, 2.3 g of proteins, 1.5 g of ash and 0.2 g of fat. It also contains minerals like calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper manganese and selenium [3]. Dates fruits are generally resistant to microbial contamination due to their high sugar contents but they are affected by various fungal species, insect infestations and bacterial contaminants [4]. It reduces the texture, taste and shelf life of dates. Majorly lactic acid bacteria and acetic acid bacteria colonize in higher number. A numerous fungal species also involved in the contamination of date fruits. Fungal contamination is a direct relationship with both the physical initial dates and environmental conditions of the premises including the storage temperature and humidity can alter the organoleptic parameters of dates, and consequently decrease the market value. So control parameters like sun drying, chemical fumigation, modified atmospheric packaging, radiation, deep freezing, refrigeration, Water washing, oil coating were followed to prevent the entry of microbes (Table 1).

Materials and Methods

Sample collection

A total of seven different varieties of dates samples were collected which are commercially available. They are vacuum packed, date's syrup, loosely available, both seeded and seedless in polythene packed, pet jar packed. They were covered and kept in aseptic conditions. In laminar chamber, it was opened in sterile condition for processing.

Total microbial analysis

Each was measured 10 grams and mixed with 90 ml of distilled sterile water and mixed well. Then the samples are serially diluted up to 10^{-7} dilutions in sterile distilled water. For bacterial enumeration, 10^{-4} and 10^{-6} dilutions were taken and for fungal enumerations, 10^{-3} and 10^{-5} dilutions were taken. For bacterial count Plate count agar is used and for fungal count Rose Bengal agar supplemented with chloramphenicol is used. Pour plate technique performed for inoculating the samples into media. The plate count agar plates were incubated at 37°C for 24 hours and fungal plates were incubated at 27°C for 5 days. For each dilution three replicas were made and after proper incubation, the colonies counted on colony counter and statistically analyzed [5].

Possible pathogen detection

Some of the colonies grown in plate count agar doubted as pathogens were studied microscopically and biochemically. Based on the confirmative tests they confirmed as pathogens. So samples from all the collected date fruits were analysed for the presence of common food borne pathogens.

Pathogens analysis

To detect the common food pathogens like *Staphylococcus aureus, Bacillus cereus, Salmonella, Shigella, E. coli* and other coliforms contamination samples from dilution 10^{-2} is taken and inoculated into specific selective media and incubated. For *Staphylococcus*

Kingdom	Plantae
Order	Arecales
Family	Arecaeceae
Genus	*Phoenix*
Species	*dactylifera*
Common name	Date palm fruit, Dates

Table 1: Classification of date plant.

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aureus Mannitol Salt Agar, *Bacillus cereus* Bacillus HiVeg Medium, *Salmonella* and *Shigella* SS agar, *E. coli* Eosin Methylene Blue agar and for Coliforms Violet Bile Red Agar is used. After incubation based on growth, the samples analyzed for the presence of pathogens.

Control parameters

To control the microbial load, physical parameters like deep freezing, refrigeration, hot air oven and radiation was followed in different time intervals. The physical parameters followed in different time intervals to check the effectiveness and to find out the optimum contact time of particular parameter. For heat treatment in hot air oven, 10 g of sample kept in 100°C for 1 min, 2 mins and 3 mins. For each time interval, three replicas were made and packed aseptically. Then they are serially diluted and plated for initial microbial load. After 15th and 30th day, the samples was plated and checked for total microbial load by that day. In radiation, the samples kept at 240 nm radiations of UV rays from 15 mins, 30 mins and 45 mins. In each time interval, the samples taken and aseptically packed and analyzed immediately for initial load and in 15th and 30th day. Likewise samples kept in 4°C and -19°C also analyzed in 15th and 30th day.

Statistical analysis

The colonies were counted by using digital colony counter and the counting's was applied on the following formula to get a neutral value from the replicas [6].

$$\text{Number of colonies} = \frac{\sum C}{(N_1 + 0.1N_2)\,D}$$

Where,

$\sum C$ = Total number of colonies,

N_1 = Number of dishes obtained in first dilution,

N_2 = Number of dishes obtained in second dilution,

D = Dilution factor corresponding to first dilution.

Results and Discussion

Total microbial load

The total number of colonies was represented in Table 2. The load of both bacteria and fungi are higher than the normal permitted level of not more than 10,000 bacterial count in 1 gram of food and yeast and mould should be absent in 0.1 g of food as per Prevention of food adulteration rules, India, 1956. To the maximum, in seedless samples in pet jar packed, 8.75×10^5 cfu/g bacterial loads observed and in fungal load, the maximum number of 16.36×10^3 cfu/g was observed in locally packed dates. The observed results were matched with previous researches from Salah et al. [7] and Raimi [8]. In plating, colonies with various types of morphology were observed. In fungi, most of the colonies resembled *Aspergillus* species. They were observed under light microscope and confirmed. The high number of microbial load may occurred due to poor post-harvest handling, storage temperature variance, damages in the skin of fruit, mycorhyzzal association relationship etc. [9].

Possible pathogen detection

Some colonies were taken and observed under light microscope. Their structure resembled alike *Staphylococcus* species and *Bacillus* species. So biochemical tests were performed like IMViC, catalase and oxidase. Based on the test results, the colonies were identified as *Staphylococcus aureus* and *Bacillus cereus*.

Enteric pathogen detection

Because of the presence of pathogens detected in some of the samples, tests performed to check out the detection of enteric pathogens in all the food samples. The results were represented in Table 3. Presence of *Staphylococcus aureus* observed in polythene seedless, pet jar seeded and in locally packed samples. *Staphylococcus* is a serious food borne pathogen. It is presented in human skin as normal flora. So through improper post-harvest handling, it gains enter into the food. It produces heat resistant enterotoxin. It produces symptoms like nausea, vomiting, abdominal cramps (which are usually quite severe), diarrhoea, sweating, headache, prostration, and sometimes a fall in body temperature generally lasting from 24 to 48 hours, and the mortality rate is very low or nil. The usual treatment for healthy persons consists of bed rest and maintenance of fluid balance [10]. *Bacillus* species was observed in pet jar seedless and locally packed samples. Members of the *B. cereus* group are ubiquitously distributed in the environment, mainly because of their spore-forming capabilities. Thus *B. cereus* can easily contaminate various types of foods, especially products of plant origin. It produces diarrhoea, gastroenteritis, emetic syndrome, abdominal pain etc., Its mortality rate is very low but deaths also observed due to bodily complications which occurred due to the toxicity of *Bacillus* [11]. However, no other common food pathogens like *Salmonella, Shigella,* Coliforms or *E. coli* found in samples.

Control parameters

Control parameters like Cold Storage, Heat treatment and radiation was followed and the effectiveness was checked on initially, 15th day and 30th day. They are represented in Figures 1a and 1b.

Cold storage: Chilling and freezing are successful methods for food preserving because chemical reactions and microbial are heat dependent. So, by keeping the food sample under low temperature conditions ceases these reactions so that the shelf life of food prolonged. Though psychrotrophs can grow in chilled foods they do so only relatively slowly so that the onset of spoilage is delayed. In this respect temperature changes within the chill temperature range can have pronounced effects [12]. Figures 1c and 1d show that there were

S.No.	Type	Bacteria (× 105 cfu/g)	Fungi (× 103 cfu/g)
1	Polythene seeded	6.42	7.27
2	Polythene seedless	5.48	7.88
3	Pet jar seeded	4.54	12.42
4	Pet jar seedless	8.75	8.79
5	Vacuum	1.64	11.21
6	Local packed	6.21	16.36
7	Syrup	1.67	2.12

Table 2: Stock load.

S.no.	Type of Sample	MSA ($\times 10^{-2}$ cfu/g)	EMB ($\times 10^{-2}$ cfu/g)	VBRA ($\times 10^{-2}$ cfu/g)	SSA ($\times 10^{-2}$ cfu/g)	Bacillus Hi Veg Medium ($\times 10^{-2}$ cfu/g)
1	Polythene seeded	0	0	0	0	0
2	Polythene seedless	2	0	0	0	0
3	Petjar seeded	0	0	0	0	0
4	Petjar seedless	1	0	0	0	3
5	Vacuum	0	0	0	0	0
6	Local packed	2	0	0	0	2
7	Syrup	0	0	0	0	0

Table 3: Pathogen testing on stock sample.

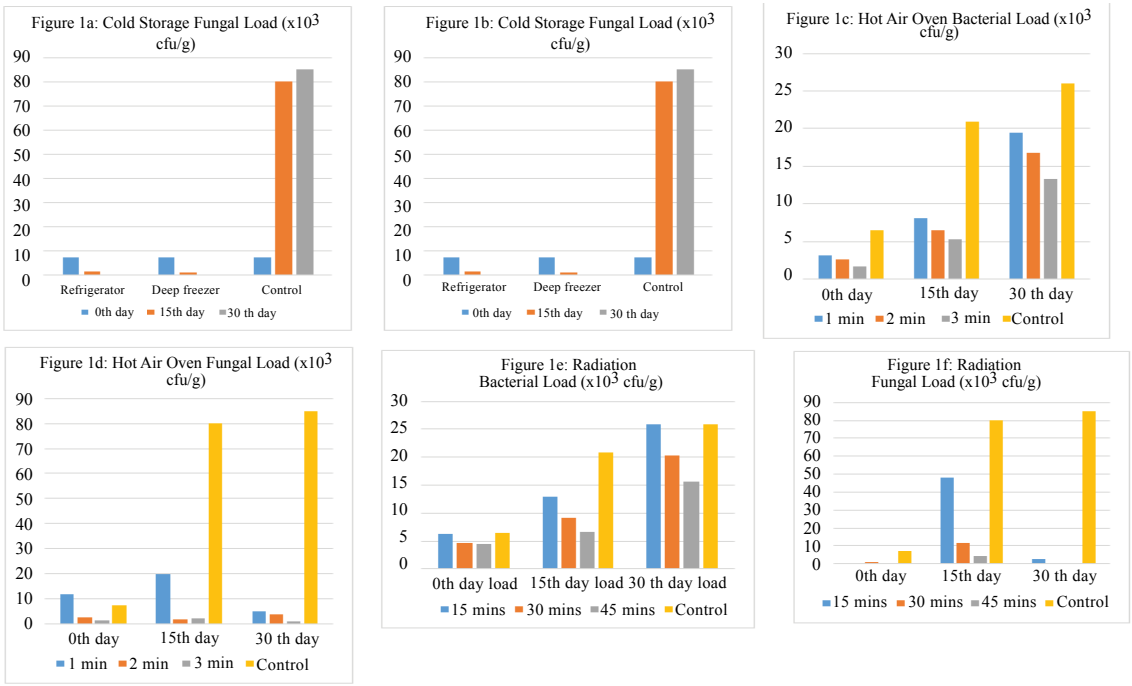

Figure 1: Various control parameters of the study.

very low growth of microbes, which are in negligible level observed. The results observed were matched with Al-Sahib [3]. After 30th day, pathogens also killed from stored samples.

Heat treatment: Most microorganisms can be killed at 100°C. Fungal spores are slightly resistant than the normal vegetative cells so that they can be killed at below 100°C [12]. From the results, it was observed that most of the microbial load was killed by heating but due to high moisture content still remained at the centre of the fruit; the endospores of bacteria gained withstanding and got growth when subjected to suitable environment again. But maximum fungal spores which were presented on the surface of fruit got lysed due to high temperature. No sensory changes observed till the end of storage time and the fruit's taste remained same.

Radiation: Microorganisms are more susceptible of UV rays. They kill the microbes by forming thymine dimers at 200 nm to 280 nm. Since most of the date contaminants present in the surface UV rays is used for preservation. Irradiation, like heat, kills microbial cells and destroys their spores at a predictable rate that is basically dependent on dose level, exposure time, and microbial type [13]. Its effectiveness on microbial control in food has been studied well since the beginning. Figures 1e and 1f showed that the radiation is more effective on fungal sterilization because of its surface presence. Because of the presence of bacteria in the endocorpic region, the UV rays are unable to penetrate inside the fruit so that the bacteria gains growth [14,15]. But the microbial growth was merely controlled than the fruit samples kept as controls.

Conclusion

Since radiation and heat treatment are more effective, due to less penetration power of UV and high moisture content of the fruit, these methods becomes ineffective. By storing the date fruits in such a very low temperature of 4°C or -19°C the metabolic activities of microbes become arrested so that the fruits remain same for a long time. During the research, the pathogens also identified from the samples so that the public are advised to be more cautious while buying the fruit. The future work may carry for detecting the survived organisms in such harsh conditions where the physical parameters used.

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