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## Bacterial Contamination of Date Fruits During Postharvest Handling

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**Abstract:** Microbial contamination of date fruits may happen during the different phases of their postharvest handling. In this work, six samples of dates belonging to the varieties Succary, Rothaneh, Barhi, Sebbaka, Umm-AIKhashab and Reshodiah were randomly collected from Buraydah City date marketplace during the seasons 2000/2001 and 2001/2002 and investigated for bacterial contamination using the aliquot obtained from three successive washings with sterilized distilled water for each of fresh, cooled (4°C) and frozen (-10°C) date samples. The results revealed the presence of a high count of aerobacteria as a result of the first washing for fresh dates ranging between  $56.7 \times 10^3$  (Sebbaka) and  $101.2 \times 10^3$  (Umm AIKhashab) CFU/g date in season 2000/2001. The percentage reduction in the total count of bacteria as a result of the first washing ranged between 84.5 and 98% in season 2000/2001. Likewise in season 2001/2002, the total count due to the first washing of fresh dates ranged between  $150 \times 10^3$  (Succary) and  $240 \times 10^3$  (Reshodiah), which resulted in a percentage reduction in the total count ranging between 90.5 and 99.5%. The presumptive test indicated the presence of coliform bacteria on the surface of some date varieties after 24-48 h. However, the IMViC reactions showed the presence of *Enterobacter aerogenes* rather than *Escherichia coli* which dismissed the likelihood of fecal contamination in the examined samples. On the other hand, *Salmonella enteritidis* inoculated to date surface failed to survive more than 9 days.

**Key words:** Date fruits, Coliform, aerobacteria, *Salmonella*, bacterial contamination

### INTRODUCTION

Dates (*Phoenix dactylifera* L.) have been considered as stable food for thousands of years in the desert areas of the world, and are regarded as a popular food commodity in Saudi Arabia. Most of the dates in the kingdom are consumed as such at their Rutab and at their Tamr stages of maturity. However, large quantities of surplus are either dried or kept in cold storage at low temperature and used later for consumption as needed.

Primitive methods of harvesting, curing and drying of dates on the farm and poor handling practices thereafter, beside the high moisture content of dates, contribute to the spoilage of dates and make them more vulnerable to microbial contamination<sup>[1,2]</sup>.

Microorganisms such as bacteria are expected to be present in considerable numbers on the surface of fresh and stored dates when delivered to the packing plants, retail markets and casual consumers.

To date, according to our information, no studies were carried out on the bacterial contamination of dates after harvesting and the consequent non hygienic handling by retailers and consumers. Moreover, dates are traditionally marketed in bags or buckets without

fumigation or even normal washing<sup>[3]</sup>. However, in this part of the world, it is generally believed that dates are not vulnerable to infestation by microbes. This belief finds support from the religious proverbs inaugurating the value of dates as a naturally sterile food<sup>[4,5]</sup>, this motivated a wide sector of the Saudi community to consume dates without washing them. Considering this situation, many of the dates consumers showed a rising concern regarding the imminent dangers that may result from consuming unwashed dates, and the hygienic fitness of unwashed dates for consumption has been questioned.

This research attempts to find out how clean the dates, sold in the market place, are with respect to bacterial contamination and how are they repelling infestation by microbes. To the authors' knowledge, this is the first research of its type in Saudi Arabia.

### MATERIALS AND METHODS

**Collection and preparation of date samples:** Depending on the popularity and abundance, six samples of fresh dates belonging to the varieties Succary, Rothaneh, Barhi, Sabbakeh, Umm AIKhashab and Reshodiah were obtained from the date marketplace in Buraydah City (Central Saudi

Arabia) during seasons 2000/2001 and 2001/2002. The samples were promptly transferred to the laboratory and were divided into three portions. The first portion was used promptly for bacterial contamination measurements, while the second and third portions were kept for six months in cold storage at 4°C and at -10°C, respectively. Bacterial counts in all date samples (fresh and stored samples) were performed by washing date surfaces and consequently the wash-water was used for the assessment of aerobacteria and coliform bacteria.

**Procedure for washing dates:** A portion of dates (500 g) of each variety was weighed, using three replicates, and transferred to a 2-liter volumetric flask containing one liter of sterilized distilled water. The flask was then shaken for 5 min using an electric reciprocating shaker. The resulting solution was labeled as the first washing. The same procedure of washing was repeated twice to obtain a second and a third washing. All solutions obtained were saved in the refrigerator at 4°C and thereafter used for assessing aerobacteria and coliform bacteria.

**Aerobacteria count:** Total colony count for aerobacteria was carried out by serial dilution and pour plate methods using Difco Plate Count Agar. Plates were incubated at 35°C for 48 h. Colonies were counted with the aid of a Gallenkamp Colony Counter<sup>[6]</sup>. Colony forming units (CFU) were calculated as CFU g<sup>-1</sup> dates.

**Coliform count:** The 3-tube procedure using lactose broth (Difco) was used for estimating the most probable number (MPN) of coliform organisms. Tubes were incubated at 37°C for 48 h and the MPN was obtained according to the Standard Methods for the Examination of Water and Wastewater<sup>[7]</sup>. The confirmed coliform test was done by culturing positive presumptive test tubes into brilliant green bile broth (Difco) and incubating at 37°C for 48 h. Differentiation of fecal coliform was done by plating positive tubes from the brilliant green bile broth cultures on eosin methylene blue agar. Colonies from these plates were then differentiated by the Indole, Methyl red, Voges-Proskauer, Citrate (IMViC) tests<sup>[8]</sup>.

**Survival of *Salmonella* on the date surface:** In the second season (2001/2002), samples of the dates belonging to the varieties Succary, Reshodiah, and Rothaneh were deliberately contaminated with *Salmonella enteritidis* provided by the Microbiological Laboratory, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine. *S. enteritidis* was grown in the selective media *Salmonella-Shigella* Agar (S.S. agar, Oxoid, Hampshire, England) at 37°C for 48 h. Cells were

harvested and washed in physiological saline solution (0.15 M Na Cl) and transferred to a 500-ml sterilized volumetric flask. The resuspended cells were brought to a final volume of 250 ml by adding sterilized distilled water. A portion of each variety of dates was immersed in the flask containing 57X10<sup>5</sup> cells ml<sup>-1</sup> of *S. enteritidis* for 30 seconds. Inoculated dates were then taken out and incubated at 25°C in the open air. *S. enteritidis* cells on the surface of dates were enumerated after 0, 1, 2, 4, 7, 9, and 11 days of incubation using the following procedure: A weighed portion of each variety of dates was transferred to a volumetric flask containing 100 ml of sterilized distilled water and mechanically shaken for 5 min. *S. enteritidis* cells in the resulting suspension were enumerated according to the standard method for the Examination of Water and Wastewater<sup>[7]</sup>.

## RESULTS AND DISCUSSION

Tables 1 through 3 summarize the counts of aerobacteria on the surface of six popular dates varieties in Al-Qassim region (Central Saudi Arabia) during seasons 2001/2002 and 2002/2003. It may be observed that the colony counts for the various treatments were higher in the first washing than in the second and the third washings where the counts declined sharply by more than six folds on the average. The mean total count of aerobacteria for all treatments in the two seasons ranged between 29X10<sup>3</sup> and 251X10<sup>3</sup> CFU g<sup>-1</sup> date for the first washing, between 2.91X10<sup>3</sup> and 27.1X10<sup>3</sup> CFU g<sup>-1</sup> date for the second washing and between 0.1X10<sup>3</sup> and 1.28X10<sup>3</sup> CFU g<sup>-1</sup> date for the third washing. The sharp decline in the total counts after the first washing was clearly reflected in the high percentage reduction in aerobacteria count in all treatments for both seasons (Table 4).

The obtained results indicated the presence of coliform bacteria in some of the date varieties under study namely, Succary, Sebbaka and Umm Alkashab (Table 5). However, the IMViC tests revealed that the coliform bacteria found in these dates belong to *Enterobacter aerogenes* rather than *Escherichia coli*. This finding indicated that the coliform were of non-fecal origin. *E. aerogenes* is normally found in soil, dust, and animal wastes, which may mean that contamination with pathogenic bacteria is not evident in the date varieties used in this study. It is possible that the low presence of coliforms relative to aerobacteria is due to the antagonistic antimicrobial actions of some compounds such as tanins found on the surface of dates<sup>[9,10]</sup>.

The primary implication of these results is that bacteria can survive on the surface of dates but may significantly be removed by washing the dates with tap

Table 1: Total count of aerobacteria ( $X10^3$  CFU  $g^{-1}$  date) on the surface of fresh dates in seasons 2000/2001 (season I) and 2001/2002 (season II) as affected by washing with distilled water

Date variety	First washing				Second washing				Third washing			
	Season I		Season II		Season I		Season II		Season I		Season II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Succary	84	46-149	150	111-187	7.40	5.3-9.0	8.0	5.7-11.5	0.31	0.24-0.42	0.82	0.53-1.2
Rothaneh	101	73-155	182	132-234	12.20	6.2-20.2	9.4	7.2-11.7	0.49	0.40-0.66	0.81	0.54-0.96
Barhi	66	57-73	212	187-235	8.00	6.0-9.3	10.8	9.4-12.3	0.31	0.26-0.35	0.76	0.51-1.2
Sabbakeh	57	51-63	251	215-300	8.80	7.9-10.5	27.0	25.1-30.3	0.29	0.26-0.32	1.1	0.98-1.15
Umm Alkashab	101	56-173	237	177-274	12.40	5.9-16.5	22.4	17.7-27.4	0.39	0.24-0.53	1.28	1.1-1.3
Reshodiah	88	74-115	240	209-249	5.80	5.6-6	10.5	8.7-11.9	0.24	0.18-0.28	0.81	0.71-0.89
SD	18.5		34.4		2.7		9.7		0.10		0.2	

Table 2: Total count of aerobacteria ( $X10^3$  CFU  $g^{-1}$  date) on the surface of dates stored at 4°C for 6 months in seasons 2000/2001 (season I) and 2001/2002 (season II) as affected by washing with sterilized distilled water

Date variety	First washing				Second washing				Third washing			
	Season I		Season II		Season I		Season II		Season I		Season II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Succary	48	41-59	50	45-58	5.1	4.4-5.7	3.8	3.6-4.0	0.31	0.26-0.32	0.34	0.30-0.40
Rothaneh	69	55-95	88	67-90	8.3	5.3-11.8	4.7	3.5-5.8	0.32	0.21-0.36	0.30	0.27-0.33
Barhi	50	40-60	64	56-73	5.4	4.9-6.1	4.3	4.0-4.8	0.21	0.12-0.18	0.31	0.25-0.35
Sabbakeh	58	45-52	70	60-88	7.3	6.1-9.3	4.8	4.4-5.2	0.22	0.16-0.23	0.26	0.22-0.30
Umm Alkashab	88	47-142	95	79-106	8.9	4.5-10.1	6.2	5.0-7.0	0.30	0.29-0.32	0.50	0.42-0.58
Reshodiah	76	62-88	70	62-83	4.8	4.6-5.2	4.4	3.2-5.5	0.21	0.19-0.21	0.28	0.25-0.33
SD	20.2		14.9		1.8		0.18		0.10		0.08	

Table 3: Total count of aerobacteria ( $X10^3$  CFU  $g^{-1}$  date) on the surface of dates stored at -10°C for 6 months in seasons 2000/2001 (season I) and 2001/2002 (season II) as affected by washing with sterilized distilled water

Date variety	First washing				Second washing				Third washing			
	Season I		Season II		Season I		Season II		Season I		Season II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Succary	40	38-48	30	25-35	4.9	4.4-5.6	3.37	3.2-3.6	0.22	0.16-0.26	0.18	0.16-0.23
Rothaneh	58	42-85	54	50-60	4.3	2.8-5.9	4.6	4.3-4.9	0.25	0.11-0.40	0.23	0.16-0.32
Barhi	43	36-48	29	24-32	4.5	3.9-5.5	2.93	2.8-3.1	0.10	0.10-0.11	0.17	0.16-0.19
Sabbakeh	42	40-47	47	36-60	5.2	4.6-5.9	3.63	3.4-3.9	0.22	0.13-0.27	0.14	0.12-0.16
Umm Alkashab	70	44-104	82	60-87	5.3	3.6-6.1	5.4	4.2-6.0	0.26	0.24-0.30	0.24	0.17-0.28
Reshodiah	62	50-80	63	59-70	3.5	3.1-3.9	4.7	3.5-6.0	0.13	0.13-0.16	0.13	0.11-0.15
SD	12.5		20.5		0.7		0.9		0.1		0.1	

Table 4: Reduction in total count of bacteria (%) due to washing with sterilized distilled water in seasons 2000/2001 (season I) and 2001/2002 (season II)

Date variety	Fresh date				Cooled date				Frozen dates			
	Season I		Season II		Season I		Season II		Season I		Season II	
	First washing	Second washing	First washing	Second washing	First washing	Second Washing	First washing	Second Washing	First washing	Second Washing	First washing	Second Washing
Succary	91.2	95.8	94.7	89.8	89.4	94.1	92.4	91.1	87.8	96.3	87.6	94.1
Rothaneh	87.9	96.0	94.8	91.4	87.9	96.4	93.9	93.6	92.6	94.7	91.5	94.5
Barhi	87.9	96.1	99.5	92.6	89.1	96.3	93.2	92.7	89.4	96.2	89.8	96.6
Sabbakeh	84.5	96.7	93.3	95.9	87.4	97.1	93.1	94.6	87.7	97.3	92.2	93.9
Umm Alkashab	98.1	96.9	90.5	94.3	89.9	96.6	93.4	91.9	92.4	95.5	93.4	95.2
Reshodiah	93.4	95.9	95.6	92.3	93.7	95.6	93.8	93.6	94.3	96.3	92.5	97.2
SD	4.8	0.4	2.9	3.0	5.2	1.1	0.5	1.2	2.8	0.9	2.1	1.4

water before consumption since it may sometimes involve harmful strains. Even though the identification of bacterial genera and species was beyond the scope of this work, genera belonging to *Arthrobacter*, *Bacillus* and *aerococcus*, which are typically found in soil and air in addition to *Micrococcus* frequently found in soil and animal skin and *Lactobacillus* which is widely distributed in nature may be speculated to be present on the date

surfaces<sup>[8]</sup>. These microorganisms could be deposited and survive on the date surfaces during harvesting, drying, handling, and transport, specially under warm temperature conditions and dusty winds prevailing in Central Saudi Arabia. We are not certain whether the speculated genera are accompanied by harmful ones, however, work is underway to identify the genera.

Table 5: Presence of coliform bacteria in six varieties of dates in season 2001/2002†

Date variety	Coliform bacteria	IMViC Tests‡	
		<i>E. coli</i>	<i>E. aerogenes</i>
Succary	+	Nil	+
Rothaneh	Nil	Nil	Nil
Barhi	Nil	Nil	Nil
Sabbakeh	+	Nil	+
Umm Alkashab	+	Nil	+
Reshodiah	Nil	Nil	Nil

† Coliform was only detected in the season of 2001/2002 (Season II)

‡ IMViC tests were used to differentiate between *E. coli* and *E. aerogenes*

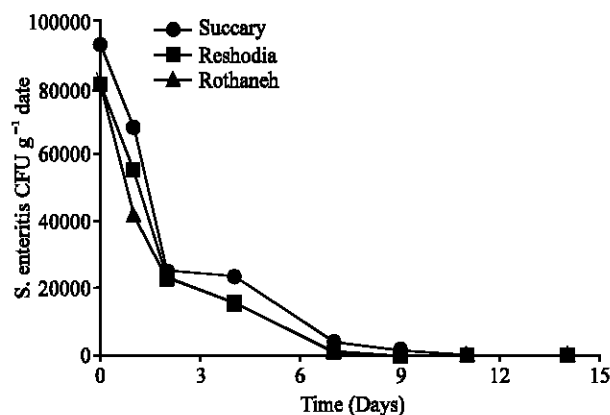


Fig. 1: Survival of *Salmonella enteritidis* on the surface of inoculated dates

To examine the longevity of survival of microbial pathogens on the surface of dates, *Salmonella enteritidis* was used as an indicator microorganism and was inoculated to dates and incubated under laboratory conditions. The results obtained, as shown in Fig. 1, revealed that there was a sharp decline in the number of *S. enteritidis* cells with advancement of incubation days. After 2 days of incubation, only 29% of the initial *S. enteritidis* cells survived and the cells vanished completely by the 9th day of incubation. This high rate of mortality of cells could be attributed to various reasons including the presence of tanins on the surface of dates<sup>[9]</sup>. Previous evidence in the literature reported similar results for *Vibrio cholerae* inoculated to the surface of Iraqi dates<sup>[5]</sup>.

In conclusion, it may be inferred from the present results that aerobacteria are capable of surviving on the surface of dates but may be greatly removed by washing dates with tap water before consumption. *S. enteritidis* failed to survive on the surface of dates for more than 9 days, which may indicate the occurrence of antagonistic antimicrobial reactions on the surface of dates.

It is recommended that dates must be washed with clean tap water prior to consumption in order to remove any concerns related to the lack of hygiene accompanying the postharvest handling of dates.

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