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The microbial quality of processed date fruits collected from a factory in Al-Hofuf City, Kingdom of Saudi Arabia

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Abstract

The microbial contamination of samples of processed date fruits collected from a factory in Al-Hofuf City, Kingdom of Saudi Arabia, was measured immediately after processing and then after refrigerated storage at 4-7°C for 2, 4 and 6 months. Freshly processed samples were found contaminated with potential spoilage microorganisms including mesophilic aerobic bacteria, molds, and yeasts. The amount of contamination in general decreased steadily with storage time. The main contaminants of freshly processed fruits were mesophilic aerobic bacteria and the mold *Aspergillus niger*. The yeasts *Zygosaccharomyces mellis, Debaryomyces hansenii, Candida lipolytica*, and *Torulaspora delbrueckii* were detected in stored samples up to 6 months. Coliforms, fecal coliforms and nonpathogenic *Escherichia coli* strains were detected in freshly processed samples and in samples stored for 2 months. The potential pathogens *Staphylococcus aureus* and *Aspergillus flavus/parasiticus* were detected in the freshly processed samples only.

Key words: Date fruits, Microbial contamination, Cold storage, Spoilage, Pathogens

Introduction

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). 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).

This study was undertaken to investigate the microbial contamination of processed date fruits collected from a factory in Al-Hofuf City, Kingdom of Saudi Arabia. Tests were performed on samples immediately after processing, and on samples stored at 4-7°C for 2, 4, and 6 months.

Materials and Methods

Samples

Samples were collected from a date processing factory in Al-Hofuf City, Saudi Arabia. The processing line consists of a conveyer belt on which the dates are first rinsed with water to prevent clumping, and then rinsed with chlorinated water, then with water again, before drying with hot air, and finally packaging under partial vacuum in sealed high density polyethylene containers as unpitted pressed fruits. A total of 40 samples (each sample 1 kg package) were collected, 10 samples from lots processed on the same day (samples 1-10), and then each time other 10 samples taken after 2, 4, and 6 months of storage at 4-7°C in cold stores (samples 11-20, 21-30 and 31-40, respectively), which is the storage practice followed in this factory. The samples were taken to the laboratory and analyzed on the same day.

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Microbiological analysis

Five replicates were tested from each sample as required by the Saudi Standard Specifications for Foods, so that 200 replicates were tested from the 40 date fruit samples collected. The samples were aseptically destoned using sterile forceps and microbial loads calculated for the flesh**.** To test for aerobic mesophilic bacteria and coliforms, the samples (10g) were weighed into sterile stomacher bags, 90 ml sterile peptone water (Oxoid, CM0009) added, homogenized in a stomacher (Lab-Blender 400, Seward Medical, England) for 45 seconds and then serial dilutions prepared. Using the pour plate method (1 ml inoculum size), aerobic mesophilic bacteria were counted on Plate Count Agar dishes (PCA Oxoid, CM0325) incubated at 30˚C for 2 to 3 days. Enumeration of *E. coli* and coliform bacteria was done using the most probable number (MPN) method. Lauryl Tryptose Broth (LST, Oxoid, CM0451) incubated at 35°C for 24-48 h was used for the presumptive test for coliforms, fecal coliforms, and *E. coli.* Brilliant Green Bile 2% Broth (BGLB, Oxoid, CM0263) incubated at 35°C for 48 h for the confirmed test of coliforms, EC broth (Oxoid, CM0853) incubated at 45.5°C for 24-48 h for the confirmed test of fecal coliforms and *E. coli*, and Levine's Eosin-Methylene Blue Agar (L-EMB Agar, Oxoid, CM0069) incubated at 35°C for 18-24 h for the completed test of *E. coli.* Five suspicious colonies from each L-EMB plate were transferred to PCA slants, incubated for 18-24 h at 35°C and used for further identification. The further identification of *E. coli* was performed using the api 20 E method, and the tested isolates identified using the api 20 E analytical profile index (bioMérieux sa, France). The pathogenecity of *E. coli* isolates was tested serologically (DENKA SEIKEN CO. LTD, REF. 295347). To enumerate yeasts, moulds, *A. flavus/parasiticus* and *Staphylococcus aureus,* 20 ml sterile peptone water were added to 10g samples (1:3 dilution), to account for low loads (further dilutions performed when necessary). Yeasts and molds were counted on Potato Dextrose Agar medium (PDA Oxoid, CM0139) to which 100 mg/l chloramphenicol (SR0078, Oxoid) were added to suppress bacterial growth. In both tests the spread plate method was used and 0.5 ml sample aliquots were added to the dishes. The dishes were left in the upright position for about 15 minutes until inoculum was absorbed by agar. Yeasts were incubated at 30°C for 3 days, and molds at 20-30°C for 3 to 7 days. Representative isolates (10 isolates from each colony form) were made from yeasts found in counts around 10^2 cfu/g in samples stored at 4-7°C for 2, 4 and 6 months (potential spoilage organisms). The isolates were purified by repeated streaking on PDA plates and then stored in slopes in the refrigerator for identification. Lactic acid bacteria (LAB) were enumerated using De Man, Rogosa, and Sharpe for lactobacilli (MRS Agar, Oxoid, CM0361) and M_{17} Agar media for streptococci and lactococci (Oxoid, CM0785). The plates were incubated in anaerobic jars with anaerobic gas generating kits (Oxoid, BR0038) for 2 to 3 days at 30°C. *Staphylococcus aureus* was enumerated on *Staphylococcus* medium No. 110 (CM0145, Oxoid) incubated at 35-37°C for 24-48 hours and identified using the Staphylase Test (DR0595, Oxoid). *Aspergillus flavus/parasiticus* was detected and enumerated on *Aspergillus flavus-parasiticus* agar (AFPA, CM0731, Oxoid) incubated at 30°C for 48 hours.

Moisture content was determined by difference after heating about five gram samples overnight in an oven at 105°C.

Identification of yeasts

Identification of yeasts was done according to the methods described by Barnett et al. (2000). First, microscopical examination of the appearance of non-filamentous vegetative cells grown in shake flasks in malt extract broth (CM0057) for 2 days at 25°C, and microscopical examination for filamentous growth using the slide culture technique was performed. The isolates were then examined for glucose fermentation in Durham tubes and for sporulation on; a. malt-yeast-glucose-peptone (YM agar), b. Gorodkowa agar, c. McClary acetate agar, d. Malt extract agar, the dishes incubated at 25°C and examined after 3 days for up to 6 weeks. After that, the appropriate subsequent tests according to the identification keys 1, 2, 3, and 4 were performed. Using these results, the isolates were then identified after the above mentioned identification keys.

Identification of molds

Molds were identified from the macroscopic morphology of the colony and the microscopic morphology of the hyphae, conidia and conidiophores of cultures grown on Czapek Dox Aar (Oxoid, CM0097) dishes incubated at 20-30°C for 3-7 days (Larone, 1995).

Results and Discussion

Contamination of the fruits with potential spoilage microorganisms

Contamination of freshly processed samples

The moisture content of all samples, freshly processed and stored, was in the range 13-15% (results not shown). As can be seen in Table 1, all

of the 10 samples analyzed on the same day of processing were contaminated with mesophilic aerobic bacteria and molds; while yeasts contamination was detected in 5 samples (loads for each sample were averages of 5 replicates). The loads of mesophilic aerobic bacteria in three samples were in the order 10^5 cfu/g, in other three samples in the order 10^4 cfu/g and in four samples in the order 10^3 cfu/g. This relatively high amount of contamination is similar to values we detected in raw date fruits (results not shown), indicating that the washing process practiced in this factory didn't result in a significant reduction in the microbial load.

Contamination with molds was in the order 10^3 cfu/g in three samples, 10^2 cfu/g in five, and less than 10^2 cfu/g in two samples. This is also a high level of contamination. The limits for mold contamination in date fruits according to the Saudi Standard Specifications are (these specifications include limits for molds, yeasts and *E. coli* only): in 2 out of 5 replicates tested from a sample the targeted limit is 10^2 cfu/g and no replicate should reach a load of 10^3 cfu/g (SASO 1999). The three samples with loads 10^3 cfu/g (samples 4, 6, and 7 in Table 1) were therefore out of specification. In addition, samples 1, 5, and 9 were also out of specification because 3 to 4 replicates of each sample contained more than 10^2 cfu/g (results not shown). Hence only four samples met the requirements for limits of mold contamination. About 95% of the mold contaminants were identified as *Aspergillus niger*. The high level of mold contamination can be attributed to the fact that dates are harvested in the dry windy months of July – September. Airborne mold spores can easily contaminate the fruits of the tall palm trees.

Five out of the 10 samples were found contaminated with yeasts. One sample contained 1.4x102 cfu/g, and the other 4 samples 19-76 cfu/g (Table 1). The limits for yeast contamination in date fruits according to the Saudi Standard Specifications are: in 2 out of 5 replicates tested from a sample the targeted limit is 10 cfu/g and no replicate should reach a load of 10^2 cfu/g (SASO 1999). Accordingly, sample 9 was out of specification, and also sample 6 was out of specification because 3 out of its 5 replicates tested contained more than 10 cfu/g (results not shown). Contamination with lactic acid bacteria was minimal. It was detected in only two samples at low concentrations (Table 1).

From the above results it appears that only four out of the 10 samples tested met the Saudi requirements for microbiological limits in date fruits, and that mold contamination was the main problem.

Table 1. Contamination of date fruit samples with yeasts, molds, and mesophilic aerobic bacteria, analyzed on the same day of processing (packaging). Results of each sample are averages of 5 replicates.

Sample No.	Yeasts (cfu/g)	Molds (cfu/g)	Mesophilic aerobic bacteria (cfu/g)	LAB (cfu/g)
1	38	$9.0x10^2$	$1.4x10^3$	n.d.
2	n.d.	52	$1.3x10^5$	n.d.
3	24	$2.1x10^2$	$6.0x10^5$	73
4	n.d.	$1.0x10^3$	$1.5x10^5$	62
5	n.d.	$8.8x10^2$	$6.4x10^4$	n.d.
6	76	$5.2x10^3$	$3.4x10^4$	n.d.
7	19	$4.2x10^3$	$2.0x10^3$	n.d.
8	n.d.	$1.5x10^2$	$9.9x10^3$	n.d.
9	$1.4x10^2$	$7.1x10^2$	$1.1x10^4$	n.d.
10	n.d.	87	4.2x103	n.d.

n.d. = not detected, LAB = lactic acid bacteria

Contamination of samples stored for 2 months after processing

The microbial loads of the samples stored at 4-7°C for 2 months (samples 11-20) were much less than those of the samples analyzed immediately after processing. Four samples were found contaminated with mesophilic aerobic bacteria at loads in the order 10^2 cfu/g, other four samples contained less than 10^2 cfu/g, and two samples were free of detectable contamination with these types of bacteria. This amount of contamination is less than that of the freshly processed samples by about 2-3 log cycles (Tables 1 and 2). It is apparent that the mesophilic aerobic bacterial contaminants were quite sensitive to the conditions of low temperature storage, low water activity and high sugar content in the fruits and the low level of oxygen in the

packages so that the majority of them couldn't survive.

All samples were found contaminated with molds, but the amounts of contamination were also generally less than those of the freshly processed ones. One sample contained mold contamination in the order 10^3 cfu/g, four samples in the order 10^2 cfu/g, and five samples less than 10^2 cfu/g. Sample 18 (Table 2), which contained $1.4x10^3$ cfu/g, was out of Saudi specification, and also samples 17 and 20 were out of specification because more than two replicates from each sample contained more than 10^2 cfu/g mold contamination (results not shown). Molds, which are generally more tolerant to low water activity levels than bacteria, seem to be more persistent in the conditions prevailing in the package. Again about 90% of the molds were identified as *A. niger*.

Yeast contamination was detected in 5 samples. Sample 19 (Table 2) was out of specification because one of its replicates tested contained $1.2x10^2$ cfu/g, and also sample 17 was out of specification because 3 of its replicates contained more than 10 cfu/g (results not shown). The yeast strain detected in sample 19 was identified as *Zygosaccharomyces mellis* (Table 6). This is an osmotolerant yeast showing growth on 50 and 60% glucose, hence it seems that it was able to survive and grow on the date fruits under the prevailing storage conditions. *Z. mellis* is also psychrophilic yeast with maximum growth temperature for some strains of about 30°C (Barnett et al., 2000). This yeast was reported as a spoilage organism of date fruits at the rutab stage (Hamad 2008). Lactic acid bacteria were detected in only three samples at low concentration.

Table 2. Contamination of processed date fruit samples stored at 4-7°C for 2 months with yeasts, molds, and mesophilic aerobic bacteria. Results of each sample are averages of 5 replicates.

Sample No.	Yeasts (cfu/g)	Molds (cfu/g)	Mesophilic aerobic bacteria (cfu/g)	LAB (cfu/g)
11	n.d.	60	n.d.	n.d.
12	n.d.	94	$4.9x10^2$	n.d.
13	n.d.	$2.0x10^2$	95	n.d.
14	33	79	$1.3x10^2$	n.d.
15	25	$1.1x10^2$	89	58
16	n.d.	83	$2.6x10^2$	n.d.
17	79	$7.4x10^2$	86	83
18	17	$1.4x10^3$	n.d.	n.d.
19	$1.2x10^2$	58	$2.5x10^2$	n.d.
20	n.d.	$6.8x10^2$	76	61

n.d. = not detected, LAB = lactic acid bacteria

Contamination of samples stored for 4 months after processing

The amounts of contamination with mesophilic aerobic bacteria and molds in the samples stored for 4 months at 4-7°C (samples 21-30) were still lower than those of the samples stored for 2 months (Table 3). Only two samples contained loads of mesophilic aerobic bacteria in the order 10^2 cfu/g, 5 samples contained less than 10^2 cfu/g, and 3 samples were free of detectable contamination. With respect to contamination with molds, only one sample was out of specification because three of its replicates tested contained more than 10^2 cfu/g (results not shown). Six samples contained less than 10^2 cfu/g and 3 were free of detectable mould contamination. In case of yeasts it seems that some osmophilic and psychrotrophic strains were able to grow in the packages. Samples 24 and 28 (Table 3) contained $2.1x10^2$ and $1.6x10^2$ cfu/g yeasts, respectively and hence were out of specification. Two samples contained 16 and 21 cfu/g while 6 samples were free of detectable contamination with yeasts. The yeast found in sample 24 was *Z. mellis*, and that in sample 28 was *Candida lipolytica* (the asexual state of *Yarrowia lipolytica* (Barnett et al., 2000). The later yeast is osmotolerant showing growth at 50% glucose (Table 6) and is also a psychrotrophic organism (Deak, 2008). No lactic acid bacteria were detected in any sample.

Table 3. Contamination of processed date fruit samples stored at 4-7°C for 4 months with yeasts, molds, and mesophilic aerobic bacteria. Results of each sample are averages of 5 replicates.

Sample No.	Yeasts (cfu/g)	Molds (cfu/g)	Mesophilic aerobic bacteria (cfu/g)	LAB (cfu/g)
21	n.d.	n.d.	78	n.d.
22	n.d.	59	n.d.	n.d.
23	n.d.	64	$1.1x10^2$	n.d.
24	$2.1x10^2$	n.d.	53	n.d.
25	21	94	85	n.d.
26	n.d.	n.d.	$2.7x10^2$	n.d.
27	16	82	93	n.d.
28	$1.6x10^2$	75	n.d.	n.d.
29	n.d.	$1.4x10^2$	n.d.	n.d.
30	n.d.	88	69	n.d.

n.d. = not detected, LAB = lactic acid bacteria

Contamination of samples stored for 6 months after processing

The 10 samples stored at 4-7°C for 6 months (samples 31-40) contained still lower microbial contamination, except for one sample (Table 4). Only one sample contained mesophilic aerobic bacteria in the order 10^2 cfu/g, 5 samples contained less than 10^2 cfu/g and 4 were free of detectable contamination. In case of molds, 5 samples contained less than 10^2 cfu/g, 5 were free of detectable contamination and none of them was out of Saudi specification. The only one sample, namely sample 40, which was out of specification was contaminated with yeasts at $4.1x10^3$ cfu/g. The contamination was a mixed population of *Debaryomyces hansenii* and *Torulaspora delbrueckii* at about 2:1 ratio. The two strains were osmotolerant at 50% and 60% glucose (Table 6) and are also known to be psychrotrophic (Deak, 2008). All 5 replicates of this sample contained more than 10^2 cfu/g yeasts, which indicate that the yeasts were growing in the package. No contamination with lactic acid bacteria was detected.

These results indicate that most microbial contaminants of dates 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% tannins (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.

Table 4. Contamination of processed date fruit samples stored at 4-7°C for 6 months with yeasts, molds, and mesophilic aerobic bacteria. Results of each sample are averages of 5 replicates.

Sample No.	Yeasts (cfu/g)	Molds (cfu/g)	Mesophilic aerobic bacteria (cfu/g)	LAB (cfu/g)
31	n.d.	n.d.	69	n.d.
32	n.d.	77	n.d.	n.d.
33	23	n.d.	n.d.	n.d.
34	n.d.	68	78	n.d.
35	n.d.	n.d.	n.d.	n.d.
36	n.d.	n.d.	86	n.d.
37	n.d.	79	75	n.d.
38	n.d.	n.d.	94	n.d.
39	n.d.	83	$1.7x10^2$	n.d.
40	$4.1x10^3$	64	n.d.	n.d.

n.d. = not detected, LAB = lactic acid bacteria

Contamination with potential pathogenic microorganisms

As can be seen in Table 5, potential pathogens were detected in some freshly processed samples (samples 1-10) and in some samples stored for 2 months at 4-7°C (samples 11-20), but none in the samples stored for 4 and 6 months (samples 21-40). Coliforms were found in 3 out of the 10 freshly processed samples and in 2 out of the 10 samples stored for 2 months (Table 5). Sample 8 showed the

highest level of contamination with coliforms, where 3 of the 5 replicates contained up to 150 MPN/g. Two of the 5 replicates in samples 1 and 6 also contained coliforms, and in samples 18 and 20 only one replicate contained coliform. Fecal coliforms were detected in 2 replicates of samples 1 and 8 and in one replicate of samples 6 and 18. *E. coli* was detected in 2 replicates of sample 8 and in one replicate in each of samples 1, 6, and 18. Thirteen out of 20 isolates (isolates 1-5, 10, 11, 13 and 16-20) made from the contaminated samples were identified as *E. coli* 1 and 7 isolates (isolates 6-9, 12, 14 and 15) as *E. coli* 2 (Table 7). Isolates 1 to 5 (from sample 1) gave identical numerical profile of 5144552 and the quality of identification as *E. coli* 1 was good with %id = 97.7 and T value = 1.0. Isolates 6-9 (from sample 6) gave identical numerical profile of 4044102 and the quality of identification as *E. coli* 2 was good with %id = 99.8 and T value = 0.98. Isolates 10, 11, 13 and 17 (from samples 6, 8 and 18), gave identical numerical profile of 1044552 and the quality of identification as *E. coli* 1 was good with %id = 69.3 and T value = 0.86. Isolates 12, 14 and 15 (from sample 8) gave identical numerical profile of 0044112. Discrimination from *Shigella* spp. was low, and the %id and T value for *E. coli* 2 were 70.1 and 0.95, respectively. Isolates 16 and 18-20 (from sample 18) gave identical numerical profile of 5044572 and the quality of identification as *E. coli* 1 was very good with %id = 99.2 and T value = 0.91. The pathogenicity tests showed that none of these isolates belonged to the 43 pathogenic strains described in the test kit. Still, sample 8 will be regarded out of Saudi specification which requires that the load of a maximum of 2 out of 5 replicates of a sample of date fruits should not exceed 10 cfu/g *E. coli* (SASO, 1999).

Samples 4, 7, and 9 were found contaminated with *S. aureus*. Contamination was relatively high in sample 4 where 3 of the 5 replicates contained loads up to $2.2x10^2$ cfu/g. Two of the replicates of sample 7 were contaminated with *S. aureus*, while only one replicate of sample 9 was contaminated with the bacterium. The potential aflatoxin producer *A. flavus/parasiticus* was detected in only one replicate of samples 3 and 10. These results indicate that pathogenic microorganisms don't grow or survive for long times in dates stored at refrigeration temperature.

Table 5. Contamination of date fruit samples with potential pathogenic microorganisms. Five replicates were tested for each sample, and the results shown are loads of individual replicates. Samples 1-10 analyzed immediately after processing and samples 18 and 20 analyzed after 2 months storage at 4-7°C.

Sample No.	Coliforms (MPN/g)	Fecal coliforms (MPN/g)	*E. coli* (MPN/g)	*Staph. aureus* (cfu/g)	*A. fl./para.* (cfu/g)
1	43/93*	9/21	21	n.d.	n.d.
3	n.d.	n.d.	n.d.	n.d.	65
4	n.d.	n.d.	n.d.	97/130/220	n.d.
5	n.d.	n.d.	n.d.	n.d.	n.d.
6	21/43	15	15	n.d.	n.d.
7	n.d.	n.d.	n.d.	135/165	n.d.
8	43/93/150	75/75	39/43	n.d.	n.d.
9	n.d.	n.d.	n.d.	79	n.d.
10	n.d.	n.d.	n.d.	n.d.	73
18	15	15	15	n.d.	n.d.
20	21	n.d.	n.d.	n.d.	n.d.

*Each digit represents the microbial load of one replicate of a sample, results of replicates containing no detectable loads are not shown. n.d. = not detected

Table 6. Profiles of aerobic assimilation of different carbon sources and ability to grow at 50% and 60% glucose concentration for yeasts isolated from date fruit samples.

	Isolates identified as			
Carbon source	*Z. mellis*	*D. hansenii*	*C. lipolytica*	*T. delbrueckii*
D-galactose	-	+	±	±
D-glucosamine	-	±	-	-
D-xylose	-	+	-	-
Sucrose	±	+	-	±
Maltose	-	+	-	±
A, α-Trehalose	-	+	-	+
Me α-D-glucoside	-	-	-	-
Cellobiose	-	±	-	-

Salicin	-	-	-	-
Arbutin	-	-	-	-
Raffinose	-	+	-	-
Melezitose	-	±	-	-
Glycerol	+	+	+	-
Ribitol	-	+	-	-
Xylitol	±	+	-	-
D-glucitol	+	+	+	+
D-mannitol	+	+	+	-
D-glucono-1,5-lactone	-	-	+	-
2-keto-D-gluconate	-	+	-	-
D-gluconate	+	±	±	+
DL-lactate	-	-	-	-
Succinate	-	+	+	-
Citrate	-	±	+	-
Ethanol	-	-	+	+
Propane 1,2 diol	-	-	-	-
Butane 2,3 diol	-	-	-	-
D-galactonate	-	-	-	-
50% D-Glucose	+	+	+	+
60% D-Glucose	+	+	-	+

Tests conducted according to Barnett *et al*.5; -, no growth; +, growth; ±, some isolates gave growth others didn't.

Table 7. Profiles of the api 20 E biochemical tests for *E. coli* strains isolated from date fruit samples. Isolates 1-5, 10, 11, 13, and 16-20 (numerical profiles 5144552, 5044572 and 1044552) identified as *E. coli* 1 and isolates 6-9, 12, 14 and 15 (numerical profiles 0044112 and 4044102) as *E. coli* 2.

Test name	Isolates																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
B-galactosidase	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	+	+	+	+	+
Arginine dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+
Ornithine decarboxylase	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryptophan deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	+	+	+	+	+
Rhamnose	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
Melibiose	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	+	+	+	+	+
Amygdaline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Conclusion

All date fruit samples tested immediately after processing were found contaminated with mesophilic aerobic bacteria and molds. Some samples were also found contaminated with yeasts, coliforms, and some potential pathogens like *E. coli, Staph. aureus*, and *A. flavus/parasiticus*. The amount of contamination decreased with time in samples stored at refrigeration temperature, indicating that most contaminants were not able to survive the conditions of low temperature, low water activity and high sugar concentration prevailing in the fruit packages. The contaminants may also have been affected by antimicrobial

compounds such as tannins which are probably present in the date fruits. The yeasts *Zygosaccharomyces mellis, Candida lipolytica*, *Debaryomyces hansenii* and *Torulaspora delbrueckii* were detected in some samples stored for 2-6 months, indicating that they were able to survive and/or grow in the samples. In general, it can be said that when date fruits are packaged and stored at refrigeration temperature, they will be safe of most spoilage and pathogenic microorganisms.

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