Effect of storage temperatures on microbial load of some dates palm fruit sold in Saudi Arabia market

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Forty samples of four date palm fruit varieties (Rezizi, khasil, Sukri and Sefri) collected from date factories located in Riyadh and Al hasa regions in Saudi Arabia were examined for their total microbial load (Mesophilic aerobic count, Yeasts and Molds count, Spore-formers bacteria and Osmophilic yeast count). The effects of different storage temperatures (26, 4 and -19°C) for six months on their microbial population were also examined. The results showed that the microbial load of the date palm fruit was mixture of bacteria, yeasts and molds. The microbial load of Khasil variety was higher than the other varieties, whereas, the Sukri variety had the lowest. The aerobic mesophilic bacteria of samples stored at -19°C for 6 months decreased significantly (p < 0.05) while those stored at 4°C were slightly decreased, however, those stored at 26°C showed insignificant reduction. The yeasts and molds, spore-formers bacteria and osmophilic yeast counts were significantly decreased at -19°C storage compared to those stored at 26 and 4°C.

Key: words: Microbial load, storage temperature, dates palm fruit varieties.

INTRODUCTION

*Phoenix dactylifera* commonly known as the Date Palm is a palm in the genus *Phoenix*, extensively cultivated for its edible sweet fruit. Due to its long history of cultivation for fruit, its exact native distribution is unknown, but probably originated somewhere in the desert oases of northern Africa, and perhaps southwest Asia. With more than 10000 years of age it is one of the oldest trees in the world, it has been used as food for 6000 years (Amer, 1994). The production of dates, according to the recent FAO statistics in the Arab countries, is about 4332208 metric tons, which accounts to 71.69% of total the international production (FAO.STAT, 2006). Saudi Arabia ranked number one among the date producers and exporting countries in the world where it produces 7170 tons of date annually (Al-Showiman and Ba Osman, 1992). Saudi Arabia is also a genetic centre of date-palm trees and there are more than 400 different cultivars of fruiting date palm of economic value (Fayadh and Al-Showiman, 1990).

Temperature, oxygen and moisture content are the most important factors that influence the type of microbial growth and spoilage of food during storage. The microbial spoilage of date can be caused by yeasts, molds and bacteria, mainly yeast species of *Zygosaccharomyces* that are more tolerant of high sugar content. The deterioration of dates by fermentation and molds increase with increase of water content, therefore, the temperature of storage and water content are the major factors which affect the shelf life of dates (Rygg, 1956). The enumeration of the microorganisms causing spoilage of fresh dates could lead to storage process that prevents date deterioration. The aim of this study is to determine the initial microbial load of date fruit and to compare the effect of storage for 6 months at different temperature on the microbial load ( Tamr stage).

MATERIALS AND METHODS

Collection of samples

Random forty commercial retail samples with same production and expiry dates (Dates sealed in sterile polythene bags) each weighting 1 kg dates fruit at tamr stage of Rezizi, Khasil, Sukri and Sefri varieties were collected from date factories in Riyadh and Al hasa. Samples were divided into two parts. The first part of samples was immediately tested upon collection for Mesophilic aerobic count, Yeasts and Molds count, Spore-formers bacteria and Osmophilic yeast count. Second part of samples was stored at three different storage temperatures 4, -19 and room temperature (26°C) for six months. At the end of storage period, the microbiological analysis was carried out.
Enumeration of microbes

Ten grams of date samples were aseptically placed in a sterile stomacher bag and mixed with 90 ml sterile 0.1% peptone water (Oxoid CM9) using a stomacher lab-blender 400 (Seward Medical, London, UK) for 2 min. After a further serial dilution in 90 ml of 0.1% peptone water, samples were plated on different selective agar media for enumeration of microbes. Colonies were counted after proper incubation using colony counter (Model 3327- American media for enumeration of microbes. Colonies were counted after 2 min. After a further serial dilution in 90 ml of 0.1% peptone water, samples were plated on different selective agar media for enumeration of microbes. Colonies were counted after proper incubation using colony counter (Model 3327-American Optical, USA) then results were reported as colony forming units (cfu) per gram.

Aerobic mesophilic bacteria were enumerated on Plate Count Agar (Oxoid CM325) following the pour plate method and incubated in an inverted position at 32°C ± 1 for 48 h (APHA, 1992). Osmophiles yeasts were counted according to the method described by Zottola (1992). Yeasts and Molds were enumerated on acidified potato dextrose agar (Oxoid CM139) which was acidified to pH 3.5 ± 0.1 by addition of sterile 10% tartaric acid [Fluka – AG – Buchs. SG]. Plates were inverted and incubated at 25°C±1 for 3 - 7 days (Koburger and Marth, 1984). Mesophilic aerobic spore formers bacteria were determined using plate count agar (Oxoid CM325) in accordance with the pour plate method at 30°C for 72 h, after a heat treatment at 80°C for 10 min to destroy vegetative cells and activate the germination (ICMSF 1978).

Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. The statistical analysis was performed with SAS program (SAS, 1990) using analysis of variance (ANOVA) and means were separated by Duncan’s multiple range tests with a probability p ≤ 0.05 (Steele and Torri, 1990).

RESULTS AND DISCUSSION

Microbial load

The microbial load of the four varieties of the date’s palm fruit (Tamr stage) are shown in Table 1. The aerobic mesophilic bacteria count ranged from 2.55 ± 0.07d to 3.69 ± 0.03a (Khlas). The total osmophilic yeast count was found to be 2.39 ± 0.02c for (Sukri) and 3.07 ± 0.02b for (Sefri). The total yeasts and molds count ranged from 2.37 ± 0.06ab (Khlas) to 2.46 ± 0.05a (Sefri). The total sporeformers count was found to be 2.12 ± 0.08c (Khlas) to 2.26 ± 0.03b (Sefri). The result clearly show that the microbial load of the date palm fruit was mixture of bacteria, yeasts and molds. Furthermore, the results indicated that the microbial load (Total aerobic bacteria and yeasts and molds count) of Khlas variety was the highest while, Sukri variety had the lowest count, this could be due to its (Sukri variety) high content of sugar than the other varieties. This observation is in agreement with those reported by Hamad et al. (1982), Mohammed and Hossein (2005) in Saudi Arabia and Omogabi et al. (2007) in Nigeria. Moore et al. (2001) reported 530 cfu/g count of yeasts and molds in edible dates fruit collected from Al Hassa region in Saudi Arabia. Al Sheikh (2009) also isolated 16 genera of fungi from local varieties grown in Saudi Arabia. Ragab et al. (2001) in Egypt also reported from 310-3030cfu/g as range of total molds count in semi dry Saidy date.

Effect of storage temperatures

Figure 1 shows the effect of storage temperature on the aerobic mesophilic count of date palm varieties. The initial numbers of aerobic mesophilic for the four varieties ranged from 3.71 - 2.57 log cfu/g. The counts at 26°C storage were 3.68, 3.4, 2.54, 3.36 and 3.38, 3.15, 2.51, 2.8 for those stored at 4°C while those at -19°C were 3.34, 2.04, 2.23 and 2.94 for Rezizi, Khlas, Sukri and Sefri varieties, respectively. These results revealed that the samples stored at 4°C slightly decreased the population of aerobic mesophilic bacteria and those at ambient storage (26°C) showed insignificant changes. While, the microbial load of the frozen samples (-19°C) were decreased significantly.

Figure 2 shows the total count of yeasts and molds. Initial population of yeasts and molds for the date palm varieties was in the range of 1.85 - 3.26 log cfu/g. The counts ranged from 2.2 - 3.2. from 1.95 - 2.91 and from 1.6 - 2.8 log cfu/g for samples stored for 6 months at 26°C, 4°C and -19°C respectively. The results indicated that the yeasts and molds count had decreased significantly (p <0.05) when stored at -19°C for 6 months but no significant changes found on those stored at 26°C and 4°C temperatures.

The total mesophilic spore-formers count of date palm

Table 1. Microbial load of four varieties of date palm fruit (tamr stage) collected from Riyadh and Al Hasa factories.

<table>
<thead>
<tr>
<th>Date palm varieties</th>
<th>Aerobic mesophilic bacteria count</th>
<th>Total yeasts and molds count</th>
<th>Total osmophilic yeast counts</th>
<th>Total spore-formers count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rezizi</td>
<td>3.51 ± 0.07b</td>
<td>3.35 ± 0.04a</td>
<td>2.37 ± 0.06ab</td>
<td>2.12 ± 0.08c</td>
</tr>
<tr>
<td>Khlas</td>
<td>3.69 ± 0.03a</td>
<td>3.41 ± 0.04a</td>
<td>2.46 ± 0.05a</td>
<td>2.27 ± 0.01b</td>
</tr>
<tr>
<td>Sukri</td>
<td>2.55 ± 0.07d</td>
<td>2.39 ± 0.02c</td>
<td>2.40 ± 0.02ab</td>
<td>2.41 ± 0.01a</td>
</tr>
<tr>
<td>Sefri</td>
<td>3.25 ± 0.02c</td>
<td>3.07 ± 0.02b</td>
<td>2.25 ± 0.07b</td>
<td>2.26 ± 0.03b</td>
</tr>
</tbody>
</table>

Results are means of triplicate determinations. Values in a column followed by the same letters are not significantly different at p ≤ 0.05.
Figure 1. Total aerobi mesophilic count of date palm varieties (Tamar stage) stored at different temperatures for 6 months.

Figure 2. Total yeast and molds count of date palm varieties (Tamar stage) stored at different temperatures for 6 months.
fruit (Tamar stage) are shown in Figure 3. The initial count was 2.57, 2.4, 2.36 and 2.23-log cfu/g for Rezizi, Khlas, Sukri and Sefri varieties, respectively. The population of spore-formers for the samples stored for 6 months at 26°C were 2.57, 2.76, 2.3, 2.18 and 2.18, 2.71, 2.4, 2.4, for those stored at 4°C, while, those stored at -19°C were 2.15, 2.57, 1.9, 1.85 log cfu/g for the four varieties respectively. The results revealed that the storage for 6 months at 26°C and 4°C did not cause significant (P>0.05) changes on the microbial counts but, storage at -19°C showed significant decrease.

Figure 4 shows the effect of storage at different temperatures for 6 months.
temperatures on osmophile yeasts count. The initial counts were varied from 1.85 (Sukri) – 3.23 (Sefri) log cfu/g for the four varieties. After 6 months of storage no significant changes observed on the counts of yeasts for the date stored at 26 and 4 °C temperatures, whereas there was significant decrease at those stored at -19°C. In general, the results of microbial counts after 6 months of storage at 26, 4 and -19°C did not show significantly change indicating good stability of the products. These results are expected because of the physical and chemical properties of date palm fruit at tamr stage that characterized with high sugars content and low moisture content, in addition of packing in sterile polyethylene bags under vacuum and following good level of aseptic precautions during processing steps. In agreement with our observation Tafi and Fooladi (2006), stated that Iranian Shamsaei date at tamr stage is resistant to microbial spoilage because of its high sugar content and low moisture content. The reasons for the reduction in the microbial load of samples stored at -19°C could be attributed to that freezing storage causes temperature shock to some microbes, denaturation of cellular protein and metabolic injury to the viable cells (Fennma, 1985). The suitable storage temperature of dates palm fruit has been studied by many researchers. Rygg et al. (1956) found Deglet Noor dates can be stored at 0°C in good condition up to one year and more than one year when stored at -17.5°C but will not stand more than one month at 27°C. Similarly Bejamin et al. (1976) reported that the best storage temperature for Zadhi date fruit varieties ranges from -3 to 5°C.

**Conclusion**

In conclusion, the present research has shown that the storage of the date palm fruit at 26 and 4°C for 6 months did not cause any significant change on the microbial load, while storage at -19°C significantly reduced the microbial population, indicating that the dates can be stored for longer time and cold storage of dates at tamr stage was a successful method to preserve the dates. Moreover these results clearly indicate that storage at -19°C is useful for extending dates shelf life.

**REFERENCES**
