Comparison of phenolic-compounds, antioxidant activity and microbial quality of two varieties of fresh and frozen date-palm fruit during 8 months storage in freezer

Ghasempour Jahromi A¹, Shahdadi F²* and Nasabpour R³

¹. Faculty Member, Islamic Azad University, Jiroft Branch, Jiroft, Iran
². Food Science and Technology, University of Jiroft, Iran
³. Laboratory Expert, Islamic Azad University, Jiroft

Corresponding author: Shahdadi F

ABSTRACT: Date-palm fruit is an important horticultural fruit in Iran with a high nutritive value. In this research the effects of freezing process on phenolic-compounds content, antioxidant properties and microbial quality of two common date varieties, namely, Mazafati and Kaluteh. They were frozen and stored for 1, 2, 4, 6 and 8 months at -18 °C. The phenolic content was measured by the Folin–Ciocalteau method, the antioxidant activity by DPPH method. Standard plate count agar and Potato Dextrose Agar (PDA) media were used for total count and molds and yeast determination, respectively. The Mazafati variety had a higher contents of phenolic compounds (677.3 mg gallic acid/100 g dry weight) and a higher antioxidant activity (22.45% radical scavenging DPPH) than the Kaluteh variety (573.6 mg gallic acid/100 g dry weight and 18.86% radical scavenging DPPH, respectively). The freezing process causes an increase in phenolic compounds and antioxidant activity in comparison with fresh dates. The highest and lowest contents of phenolic and antioxidant activity were found in the frozen date (8 months) (892.12 mg gallic acid/100 g dry weight and 41.33% radical scavenging DPPH, respectively) and fresh date samples, respectively. Freezing process and keeping date fruits at a temperature of -18 °C for a long period (1, 2, 4, 6 and 8 months) also led to decreases in microbial activity.

Keywords: Date-palm fruit, freezing process, Phenolic compound, Antioxidant activity, microbial quality

INTRODUCTION

In general, frozen foods have an excellent safety record. it is extremely rare for a foodborne illness to be traced back to a frozen food. Freezing preserves food by either stopping microbes (bacteria, fungi etc.) from multiplying or halting the foods own enzyme activity that would otherwise cause the food to rot (Gill, 2002). Most pathogens don’t multiply at freezer temperature and many of them perish because their enzymes don’t work properly to maintain normal cell activity. Also, pathogens need water to grow and freezing turns the available water into solid ice crystals. The slower the freezing process the larger the crystals become and the more cells they damage. A temperature of –18°C is easily achieved by a home freezer and effectively prevents the growth of these moulds and stops other microorganisms from multiplying (Lund, 2000).

The fruit of the date palm (Phoenix dactylifera) is an important commercial crop in the Middle Eastern countries. Date fruits are still considered by many people in this part of the world as a staple food (Sawaya . 1982). Date palm is a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium and a significant amount of calcium (Anwar-Shinwary, 1987). The nutritional and biochemical aspects of date fruits were reported by
many workers (Al-Farsi, 2005; Myhara, 2000). They are rich in simple sugars such as glucose and fructose (65–80 %), and a good source of fibres and some essential minerals, but low in fat and protein with no starch (Myhara . 2000). Besides nutritional value, date fruits are rich in phenolic compounds that have in vitro antioxidant and antimutagenic properties (Vayalil 2005; Osman . 2012; Gao, 2011). Recently, several studies have reported such activity of date fruits from Algeria (Mansouri . 2005), Kuwait (Vayalil 2002), Oman (Al-Farsi . 2005) and the USA (Vinson . 2005); These studies showed that fresh and dried dates varied quantitatively and qualitatively in their phenolic acids content. Such variations are a reflection of the diversity of date cultivars. More information about the antioxidant activity of various date cultivars at different maturity stages and the relationship of such activity with chemical constituents is needed. Biglari . (2009) showed that total phenolic and total flavonoids of dates increased during long-term cold storage (4 °C) followed by an additional one week storage at 18 °C.

Oraei . (2011) Effect of Gamma irradiation and frozen storage on microbial quality of Rainbow trout fillet were studied. Their results showed that Gamma irradiation and increasing of frozen storage time had significant effects on the reduction of microorganism's population. The total count showed that all samples maintained acceptable microbiological quality until the end of the fifth month of frozen storage. The aim of the present study was Comparison of phenolic compounds, antioxidant activity and microbiological quality of two varieties of fresh and frozen date-palm fruit during 8 months storage in freezer.

MATERIALS AND METHODS

2.1. Plant materials
Two varieties of date were used in this study, Jiroft Kaluteh date and Mazafati dates that are grown mostly in Kerman Province of Iran. The dates were obtained from Jiroft distribution centre at tamr stage.

2.2. Chemicals and reagents
2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium carbonate, Folin–Ciocalteu reagent, methanol, were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

2.3. Freezing of dates
Fresh matured date palm fruits were freezed at -18°C in a household freezer (Samsung, England). Then frozen dates were kept in a cold storage at -18° C for 8 months. Samples were analyzed for phenolic compounds, antioxidant and microbiological quality at the first day of frozen storage as 2-month sampling intervals for 8 months.

2.4. Extraction of antioxidants from date fruit
The flesh part of date (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dry-blended for 3 min with a domesticated blender (Panasonic, Penang, Malaysia). The extraction solvent was 300 ml methanol–water (4:1 v/v), and extraction carried out at ambient temperature (20 °C) for 8 h using a laboratory shaker. The ratio of methanol and water which lead to the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by and biglari . (2009). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the extracts were dried by a freeze dryer and dried sample constituents stored at 4 °C until use (Arabshahi-Delouee and Urooj, 2007).

2.6. Estimation of total phenolic compounds
Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard, and Singleton 1977). Briefly, 20 μl of extract solution were mixed with 300 μl of Na2CO3 solution (20 %), then 1.16 ml of distilled water and 100 μl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation were obtained from calibration curve:

\[
A = 0.98 C + 9.321 \times 0.001 \\
R^2 = 0.9965
\]

Where A is the absorbance and C is concentration as gallic acid equivalents (μg/ml).
2.7. DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Blois (1958) method. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 50–400 μg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

\[
\text{DPPH scavenging activity(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100}
\]

2.8. Microbial activity

For the microbiological analysis 10 g of date palm fruit was removed with a sterile scalpel and minced under aseptic conditions. Then it was homogenized for 2 minutes with 90 ml of 0.1% (w/v) sterile peptone water (Merck, Germany) using a lab-blender 400 stomacher (Seward medical, UK). Subsequent dilutions were prepared by mixing a 1-ml sample with 9 ml of sterile peptone water. For determination of total bacterial count, 1 ml of appropriate dilutions were poured-plated with melted plate count agar (PCA) (Merck, Germany) and then were incubated at 35-37°C for 48 h. Total yeasts and molds were enumerated on potato dextrose agar (Merck, Germany after incubation at 25°C for 3–5 days (Kristo, 2008).

2.9. Statistical analysis

All these experiments were replicated three times (from 3 different batches of samples), and the average values are reported. The effect of freezing process on phenolic compounds, antioxidant activity and microbial quality of two date varieties were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan’s test at 5 % significant level using the SAS software (2008) program.

RESULTS AND DISCUSSION

3.1. Total phenolic compounds

![Figure 1. Effect of storage at -18 °C on total phenolic compound of two date palm fruits varieties.](image)

Each observation is a mean ± SD of 3 replications. In each figures means with same superscripts had no significant difference with each other (P >0.05)

As can be seen from Fig. 1, date varieties and storage at freezing condition had significant effect on (P <0.05) total phenolic content. By increasing in freezing storage time, total phenolic compound increased. No result was reported about the effect of freezing process on total phenolic compounds but Biglari . (2009) showed that total phenolic and total flavonoids of dates increased during long-term cold storage (4 °C) followed by an additional one week storage at 18 °C.
2.2. DPPH radical scavenging activity

The results of DPPH radical scavenging activity of extracts affected by ripening stages were shown in Fig. 2. Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman . 2003). The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto . 2000).

As it can be seen from Fig. 2, frozen date at end of storage that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by 6, 4, 2, 1 months and fresh date. No significant differences (P <0.05) were found between DPPH radical scavenging of frozen date at 4, 6 and 8 months of storage. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Gao ., 2000a, b; Jimenez-Escrig . 2001; Arabshahi Delouee and Urooj 2007). Figure 2 also showed that mazafati date had higher DPPH (%) than kaluteh variety in all storage time (p <0.05).

![Figure 2. Effect of storage at -18 °C on DPPH radical scavenging of two date palm fruits varieties.](image)

2.3. Microbial activity

Microbiological analysis of the frozen dates showed that the total microbial population was far below the standard requirement for frozen vegetables (Table 1, 2). The standard frozen vegetables required a minimum total plate count value of $10^5$ colonies/ml.

The results of microbial activity of Mazafaty and kaluteh frozen dates were shown in tables 1 and 2. This tables showed that total plate count and yeast and molds decreased by increasing storage and. These results were similar to results of Mansouri Jajai (1996).

Al-jasser (2012) evaluated Effect of cooling and freezing temperatures on microbial and chemical properties of chicken meat during storage. The data showed that total viable count of bacteria were significantly decreased when samples were stored at -10°C, -18°C and -18°C with vacuum package.

<table>
<thead>
<tr>
<th>Storage times (months)</th>
<th>Total plate counts (log cfu/g)</th>
<th>Yeast and molds (log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh dates</td>
<td>5.39b</td>
<td>3.33a</td>
</tr>
<tr>
<td>1</td>
<td>4.27a</td>
<td>4.38b</td>
</tr>
<tr>
<td>2</td>
<td>3.85c</td>
<td>4.12d</td>
</tr>
<tr>
<td>4</td>
<td>3.71e</td>
<td>3.60f</td>
</tr>
<tr>
<td>6</td>
<td>3.35g</td>
<td>3.18h</td>
</tr>
<tr>
<td>8</td>
<td>2.85i</td>
<td>2.43j</td>
</tr>
</tbody>
</table>

Each observation is a mean ± SD of 3 replications. In each column means with same superscripts had no significant difference with each other (P >0.05)

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<td>Fresh dates</td>
<td>5.79a</td>
<td>5.21a</td>
</tr>
<tr>
<td>1</td>
<td>5.65b</td>
<td>4.91c</td>
</tr>
<tr>
<td>2</td>
<td>4.46d</td>
<td>4.27e</td>
</tr>
<tr>
<td>4</td>
<td>3.85f</td>
<td>4.10g</td>
</tr>
<tr>
<td>6</td>
<td>3.26h</td>
<td>3.71i</td>
</tr>
<tr>
<td>8</td>
<td>2.56i</td>
<td>2.92j</td>
</tr>
</tbody>
</table>
Each observation is a mean ± SD of 3 replications. In each column means with same superscripts had no significant difference with each other (P >0.05)

Some microbial activities and total phenolic content of Kerman dates during storage times at -18 °C were determined and presented in this paper. Two date variety had significant difference in case of antioxidant activity and total phenolic content (p <0.05) and mazafati variety had higher than kaluteh in these case. In both varieties antioxidant activities and total phenolic content increased by storage at freezing condition. Results of this study showed that microbial activities varied during storage at -18 °C and decreased by increase of storage time.

REFERENCES

Biglari F, AlKarkhi AFM and Alawi A. 2012. Effect of cooling and freezing temperatures on microbial and chemical properties of chicken breast, bacon, spinach, cheese, and fish were decreased to 100 colonies/ ml after storage at -18°C for 240-300 days. Beside L. monocytogenes, other microbes resistant to low storage temperatures are Bifidobacterium bifidum that is still survive at -25°C (Kebary 1996) and Lactobacillus acidophilus at temperature of -30°C (Foschino 1992; Gianfranceschi and Aurelli 1996).

CONCLUSION

The antioxidant and microbial activities and total phenolic content of Kerman dates during storage times at -18 °C were determined and presented in this paper. Two date variety had significant difference in case of antioxidant activity and total phenolic content (p <0.05) and mazafati variety had higher than kaluteh in these case. In both varieties antioxidant activities and total phenolic content increased by storage at freezing condition. Results of this study showed that microbial activities varied during storage at -18 °C and decreased by increase of storage time.

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