Investigation of Enteric Gram-Negative Bacilli Contamination in Mazafati Date

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Abstract

Food safety is related to the existence and level of food hazards used. The World Health Organization considers diseases caused by the consumption of contaminated food as the most important and serious problem of today's world. Therefore, appropriate sanitary measures are necessary to avoid risks to human health. Dates are one of the major Iranian agricultural products that have significant and effective role in Iran's non-oil export in addition to domestic consumption. Hundred 500-gram packages of date were randomly sampled and tested from the date distribution centers. After appropriate dilution, bacteriological tests were performed. Coliform count of the solid medium (VRB), Enterobacteriaceae count of solid medium (VRBD), E.coli from liquid medium LS as well as confirmatory tests for the detection of enterococcus, bromocresol liquid medium-purple azide broth culture as well as KF solid medium for confirmatory tests were used. After reviewing the results, only 11% of the samples had coliform contamination. There were no E.coli, Enterobacteriaceae and enterococcus contamination in the samples. This fact confirms the antibacterial effects of date; however it may require further research in this area.

Key words: Date, coliform, E.coli, Enterobacteriaceae, enterococcus

1 Introduction

Development of science and technology followed by industrial advancement has caused some pollutants to enter the industry. This has led to some health problems in foods. Thus, Monitoring food hygiene has become the main task of health professionals (Shiralipor et al. 2012) [16]. Food safety risks to the community is one of the main problems due to dramatic changes in lifestyle and eating habits in recent decades and lack of adequate training in this area. World Health Organization considers diseases caused by consumption of contaminated food as the most important and serious problems in today's world. On the one hand, food International trades and overseas journeys bring a significant social and economic benefit,
but on the other hand, it also facilitates the spread of diseases throughout the world. Dietary habits have undergone a major transformation in many countries over the past two decades and new food production, preparation and distribution techniques have developed as the outcome. Therefore, appropriate hygienic measures are necessary to avoid risk to human health and economic consequences of food spoilage, damage and diseases caused by food (Shiralipor et al. 2012) [16]. Date should be controlled from the health viewpoint as one of the most fundamental and widely used products in Iran. Palm belongs to the Palmae family. With scientific name of phoenixdactyliferaL. It is a monocot plant from the family of palms consisting of about 200 genera and 4000 species. Palm tree is a dioecious and permanent tree. After five years of planting, it gives fruit until it dies. Differences between species are in the shape of stems, leaves, flowers and fruits. Date is a single, elongated, cylindrical tip fruit with stigma terminal. Weight of dates varies from about 2 to 60 grams, length from 18 to 110 mm and its width from 18 to 32 mm depending on the variety and growing conditions (Ashraf Jahani, 2002) [2]. Mazafati date is known as one of the most important dates in the provinces of Kerman, Sistan and Baluchestan and also the city of Bam. These wet dates are the third Iranian produced dates. Taking the rate of planting into account, its yield is very high and approximately 800 million tons of dates are exported to other countries, annually (Ashraf Jahani, 2002) [2]. Reduction of microbial contamination of this product is necessary in order to maintain and create export markets for these products to successfully compete with other producing countries. Microbial contamination brings about lower quality dates at the time of harvest, improper transport and storage, inadequate cleaning, disinfection and non-normative lack of moisture adjustment. This issue has led to date spoilage in high tonnages and is causing a lot of damage to producers. Enterobacteriaceae are a group of enteric gram-negative bacilli whose only natural habitat is intestine of humans and animals. There are several genera in this family. These organisms are facultative aerobic. They can ferment variety of carbohydrates. They are versatile having pritish and sedentary Flagella (Jawetz et al. 2005) [11]. In this study, coliforms, E.coli, enterococcus, Enterobacteriaceae and evaluation of the contamination of Mazafati date were assessed in different types of date group.

2 Materials and Methods

Hundred 500-gram date packages were purchased from various date distribution centers in Tehran, then sampled and tested. Cochran's formula was accordingly used to estimate the minimum sample required for the test given the absence of previous similar studies.

Theories:
Confidence coefficient: 95%, $z = 1.96$, $d = 0.1$
Error amount: $p - q = 0.5$
Cochran formula: $n = \frac{z^2 pq}{d^2}$

Microbiological tests including enumeration of Enterobacteriaceae, enumeration of coliform, detection of E. coli, and detection of intestinal enterococcus were carried out on samples after transferring the samples to a microbiology lab in the city of Karaj at the Standard Research Institute. Initial suspension and decimal dilutions were prepared in order to do the test and then Ringer's dilutor solution was utilized for the preparation of the initial suspension. 500-milliliter distilled water was added to each Ringer pill. It was held at 25 °C temperature after sterilization (ISO 6887-1, 1999 [5]; ISO, 6887-4 2003).

Materials and methods performed for each biological test was as follows:
2.1. Enumeration of coliforms
Crystal violet solid medium was utilized for total coliform count which manifested crystal violet neutral red bile lactose agar using pour plate technique. 1 ml of primary suspension was then moved into each of two sterile plates (duplicate) by sterile pipette. The above-mentioned operation was performed in other decimal dilutions by another sterile pipette. About 12 ml to 15 ml of VRBL culture medium was added to each plate with temperatures up to 44°C - 47°C. About 4 ml of the VRBL medium was poured again to prevent the growth of colonies and create semi-aerobic conditions after the complete closure of the medium. The plate was incubated upside down in the temperature of 37°C ± 1°C up to 24 h ± 2 h after closing the medium. Observation of purple-red colonies is indicative of the coliform (with minimum 0.5) sometimes with reddish halos caused by the deposition of bile. These coliform colonies do not require confirmatory test (ISO 4832, 2006) [7].

2.2. Enumeration of Enterobacteriaceae
Violet red bile glucose agar (VRBG) solid medium was utilized for enumeration of Enterobacteriaceae using pour plate technique. The test stages are similar to preparation of plates for the enumeration of coliforms. About 12 ml to 15 ml of VRBG culture medium was added to each plate with temperatures up to 44°C - 47°C. Other stages of the test are the same as coliforms test. Observation of pink, red or purple (with or without aura sediment) colonies is indicative of Enterobacteriaceae after the incubation period (ISO 21528-2, 2004).

2.3. Detection of E.coli
Lauryl sulfate broth with double-strength medium, LS+ as well as EC broth confirmatory medium and peptone water indole free to detect E.coli. Inoculated tubes were put at temperatures 37 °C ±1°C for 24 h ± 2h. After the incubation period and in the case of gas or turbidity in the tubes, it is harvested by culture circles. It was then inoculated in EC broth and was incubated in water or an incubator set at 44 °C for 24 h ± 2 h. After the incubation period of the tubes and if visible gas or turbidity is observed, it was inoculated at the tube of peptone water preheated to 44 °C for 24 h ±2 h. After incubation period, 0.5 ml of indole reagent was added to the tubes of peptone water. Then they were mixed and examined well and after 1 min a red color in the alcoholic phase indicated the indole presence (ISO 7251, 2005) [9].

2.4. Detection of enteric enterococcus
1 ml of primary suspension was added to 10 ml bromocresol liquid medium-purple azide broth and was incubated at the temperature of 37°C ±1°C for 24h ± 2h. Linear culture was performed on KF streptococcus agar and then the cultured plates were incubated at the temperature of 37° C ± 1°C for 24 h ± 2 h. Identified enteric colonies are seen at reduction of 2, 3 and 5 triphenyltetrazolium chloride and alteration of formazan into red, purple, pink and chestnut (ISIR No. 2198, 2008)

3 Results
Among the 100 samples tested, none of them were contaminated with E.coli, Enterobacteriaceae and enterococcus. Only 11% of the samples had coliform contamination. The results of the colony counts on contaminated plates with coliforms are illustrated in the table 1. Total coliform (N) per gram of sample was calculated according to the following formula:
\[ N = \frac{\sum a + \sum a'}{V(n_1 + 0.1n_2)} d \]

N: number of coliforms per gram
V: volume of test inoculated per plate
\( n_1 \): number of containers counted in the first dilution plate
\( n_2 \): number of containers counted in the second dilution plate
\( \sum a \): Total number of indexed colonies on all plates
\( \sum a' \): Total number of non-indexed and approved colonies on all plates
Since the non-indexed colonies were not observed in this test, the amount of \( \sum a' \) was considered zero. The amount of N is expressed on the basis of cfu/g.

Table 1: Number of colonies counted on plates contaminated with coliforms

<table>
<thead>
<tr>
<th>Sample</th>
<th>Primary Dilution 10^1</th>
<th>Secondary Dilution 10^2</th>
<th>Total number of colonies obtained from plates in a gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate I - Plate II (number of colonies counted)</td>
<td>Plate I - Plate II (number of colonies counted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14-7</td>
<td>0-1</td>
<td>10×10^1</td>
</tr>
<tr>
<td>2</td>
<td>0-1</td>
<td>0-0</td>
<td>0.4×10^1</td>
</tr>
<tr>
<td>3</td>
<td>0-3</td>
<td>0-0</td>
<td>1.3×10^1</td>
</tr>
<tr>
<td>4</td>
<td>17-13</td>
<td>0-0</td>
<td>13.6×10^1</td>
</tr>
<tr>
<td>5</td>
<td>4-10</td>
<td>0-0</td>
<td>6.3×10^1</td>
</tr>
<tr>
<td>6</td>
<td>0-1</td>
<td>0-0</td>
<td>0.4×10^1</td>
</tr>
<tr>
<td>7</td>
<td>0-2</td>
<td>0-0</td>
<td>0.9×10^1</td>
</tr>
<tr>
<td>8</td>
<td>1-1</td>
<td>0-1</td>
<td>1.3×10^1</td>
</tr>
<tr>
<td>9</td>
<td>0-1</td>
<td>0-0</td>
<td>0.4×10^1</td>
</tr>
<tr>
<td>10</td>
<td>1-1</td>
<td>0-0</td>
<td>0.9×10^1</td>
</tr>
<tr>
<td>11</td>
<td>0-1</td>
<td>0-0</td>
<td>0.4×10^1</td>
</tr>
</tbody>
</table>

4 Discussion

Harmful microorganisms causing spoilage in dates are classified into yeasts, fungi and bacteria. The most damaging yeast is the one that can favorably grow and multiply in concentrated sugar solutions (Ashraf Jahani, 2002) [2].

Fungi are rated behind yeasts in damaging and spoiling palms. Some fungi are potentially able to cause abundant date damages (Ashraf Jahani, 2002) [2], and may create a great deal of waste. This phenomenon has been observed especially when dates are exposed to rainy weather or when humidity is in higher levels (Ashraf Jahani, 2002) [2]. Growth of fungi on the dates can be identified by the fungi in row, Shedding spores and create change in the color or flavor of dates. Microorganisms normally spread on the date before it gets wrinkled or dried, since they are unable to grow on dried and wrinkled date.

The role and level of importance of bacteria in wasting dates is not yet known (Ashraf Jahani, 2002) [2]. Due to the high sugar content and low water activity (AW= < 0.9), Bacteria are not capable of growing on palm.

The level of date contamination by some enteric gram-negative bacilli was assessed in this study. None of the cases indicated contamination to Enterobacteriaceae, E.coli and enterococcus; however, coliform contamination was observed in a small number of samples. Seemingly this is an indication for anti-
bacterial characteristic of dates (Abuharfeil et al. 1999 [1]; Hammed and Sallal, 2002 [3]; Sabah et al. 2007 [12]; Sadikin et al. 2008 [13]; Sayyedi et al. 2006 [14]; Shariati et al. 2010 [15]). In this regard, several studies have been conducted to confirm the anti-bacterial properties of dates. One study conducted in Yasouj University of Medical Sciences indicated that consumption of the date as a food is effective in preventing tooth decay producing bacteria. This is probably due to the inhibitory effects on this complication (Sayyedi et al. 2006) [14]. There are also some reports on the positive effects of date extract in preventing the microorganism growth such as streptococcus (Abuharfeil et al. 1999) [1]. Also, there was another study indicating the anti-viral effect of date palm kernel extract against phagocytic Pseudomonas aeruginosa and proving that the extract can be a good deterrent against this phage (Sabah et al. 2007) [12]. Further studies have also been accomplished in this area. For instance, Sadikin et al. [13] studied about certain effects of Phoenix dactylifera extract against 6 mutant strains of Streptococcus and sensitivity of all strains towards it (Sadikin et al. 2008) [13]. In another study by Hammed and Sallal on the effects of date different concentrations to inhibit Streptococcus pyogenes the same result was obtained (Hammed and Sallal, 2002) [3]. After conducting different tests on the growth of 9 strains of staphylococcus aureus, it was proved that ethanolic and stoni extract of date kernel has good inhibitory effect against the growth of Staphylococcus aureus strains (Shariati et al. 2010) [15]. Having considered the studies carried out on the antibacterial properties of date and the results of this study and the absence of enteric gram-negative bacilli in cultured samples, it can be concluded that date has anti-bacterial effects (Abuharfeil et al. 1999 [1]; Hammed and Sallal, 2002 [3]; Sabah et al. 2007 [12]; Sadikin et al. 2008 [13]; Sayyedi et al. 2006 [14]; Shariati et al. 2010 [15]). This effective characteristic will be nominated as an alternative to current less effective or even ineffective medications as well as treatment of infectious diseases. More research is needed in this area to achieve stronger definitive results. Future research also should describe the type and amount of antibacterial substances, difference of local dates in terms of bacterial resistance and recommendations for the use of certain materials or products for anti-bacterial drug manufacturing facilities.

Acknowledgment

All the tests of this research were carried out in the Research Group of Microbiology, Department of Food Science; Standard Research Institute of the IRAN. This is to acknowledge and thank all the technicians and managers of this group.

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