American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Food Technology

ISSN 1557-4571
DOI: 10.3923/ajft.2017.295.300

Research Article
Fungal Contamination and Invertase Activity in Dates and Date Products in Saudi Arabia

Laila Ahmed Nasser

Department of Biology, College of Sciences, Princess Nourah bint Abdul Rahman University , Riyadh, Saudi Arabia

Abstract

Background and Objective: The production of enzymes by fungi is the most likely source of contamination of substrates leading to spoilage in dates and date products. One of these enzymes is invertase, which takes a role in the ripening and maturation of the date fruit and date fruit products and eventually degradation and spoilage. This study was conducted to determine the fungal mycoflora in fresh and dried dates and investigate the production of invertase enzyme in date fruit and date fruit products. **Materials and Methods:** The mycoflora present in 40 samples of date fruit and date products was investigated using the dilution-plate and the direct plating techniques. Fungal isolates were counted and identified microscopically using the fungal taxonomy. Invertase activity was tested on liquid 20% sucrose-czapek's medium and was determined in the filtrates using the fehling's solution. **Results:** Twenty-seven fungal species and one variety representing 13 fungal genera were isolated and identified. The most common isolates included *Aspergillus*, *Cladosporium*, *Penicillium, Neurospora* and *Rhizopus*. *Aspergillus* was the most prevalent isolated fungi, followed by *Penicillium* and *Cladosporium*. The total fungal count was less in dates than on date products. Invertase activity was found in 48 isolates. **Conclusion:** There is a high prevalence of saprophytic fungi in date fruits and date fruit products. There is also a moderate to strong invertase activity in 8 out of 9 fungal isolates. It is highly recommended that extra precautionary measures must be adopted in consumption of ripened dates particularly those near spoilage due to its probable high saphrophytic fungal contents.

Key words: *Phoenix dactylifera*, invertase, spoilage, mycoflora, saprophytes

Citation: Laila Ahmed Nasser, 2017. Fungal contamination and invertase activity in dates and date products in Saudi Arabia. Am. J. Food Technol., 12: 295-300.

Corresponding Author: Laila Ahmed Nasser, Department of Biology, College of Sciences, Princess Nourah bint Abdul Rahman University, Riyadh, Saudi Arabia

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The fruits of the date palm tree (*Phoenix dactylifera* L.) are among the most important horticultural crops in Saudi Arabia as well as in Arab countries. They are consumed as fresh fruit or in their dried form. Dates contain a high percentage of carbohydrate (44-88% sugar), fat (0.2-0.5%), protein (2.3-5.6%), 15 salts and minerals, vitamins and a high percentage (6.4-11.5%) of dietary fiber[1].

At appropriate temperature and moisture content, moulds can germinate and resultant hyphae can infect the inner tissue of fresh or dried date fruits[2]. In fact, endogenous yeasts and filamentous fungi (such as *Cladosporium cladosporioides* and *Sporobolomyces roseus*) were found in as much as 530 colony forming units (cfu g^{-1}) of fruit[2]. In another study, *Aspergillus niger* was abundantly found in dates grown in Morocco[3]. Species of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. ochraceus*, *Penicillium chrysogenum* and *Rhizopus stolonifer* were also isolated in dried fruits including dates in some studies[4]. Aflatoxins ranging from 2.90-4.96 μg kg^{-1} dates and date products were also seen[5]. Ochratoxin A was also found in some date fruit samples tested in Iran[4,6].

With the variety of these fungi, they are most likely the source of contamination of the substrates leading to spoilage. The production of enzymes by fungi capable of degrading the food ingredients may cause deterioration and spoilage of these substrates. Thus, preventing the growth (germination and proliferation) of moulds on these fruits seems to be the logical solution to the problem of spoilage[7]. Sucrose is known as the main sugar and the principal component of date and date products. The invertase enzyme (sucrose) is responsible for the hydrolysis of sucrose. Any information about the effects of this enzyme and its role in the degradation and spoilage of date fruits and date products is highly sought not only by farmers but also the food industry, since this information will tremendously serve the date industry in the Arab region. This study determined the fungal mycoflora in fresh and dried dates and investigated the production of invertase enzyme in dates and date products.

MATERIALS AND METHODS

Collection of samples: Dates and date products were gathered from different retail markets in Riyadh, Saudi Arabia in the last quarter of 2016. Date products are products whose main ingredient is dates but is supplemented with any one of the following: Coconut, peanut, sesame, almond, chocolate or cream. Collected samples were transferred to the laboratory and were kept in 3-5°C temperature until fungal examination was done.

Fungal examination and identification: Dilution-plate method and direct-plating technique were used to isolate the fungi using 20% (w/v) sucrose-czapek's agar medium[8,9]. Chloramphenicol (20 μg mL^{-1}) and Rose-Bengal (30 ppm) were used as bacteriostatic agents. Four plates consisting of 2 plates each for the isolation method were used for each sample and were incubated at 28°C. The developing fungi were counted per gram or 8 segments of dates and were identified microscopically. Identification of isolated fungal species was carried out using the taxonomic keys[8].

Screening for invertase enzyme: The hydrolysis of sucrose by the invertase enzyme was tested on liquid 20% sucrose-czapek's medium. After incubation of the fungal isolates at 28°C for 10 days, the sucrose (invertase activity) was determined in the filtrates using the fehling's solution (Sigma Aldrich-Merck, Germany). A positive result was indicated by a yellow, green or brown precipitate. The degree of hydrolysis was interpreted as weak (+1), moderate (+2) and strong (+3).

RESULTS

There were a total of 40 samples of dates and date products collected. Of the samples tested, 27 fungal species and one variety representing 13 fungal genera were isolated and identified. The most common isolates included *Aspergillus*, *Cladosporium*, *Penicillium, Neurospora* and *Rhizopus* (Table 1, 2).

Aspergillus was the most prevalent isolated fungi, which was seen in 85 and 90% of the samples constituting 84.4 and 94.0% of the total fungi in dates and date products as shown in Table 1. Of this, 7 species and 1 variety were identified including *A. flavus*, *A. fumigatus* and *A. niger* which occurred in 35-85% and 25-80% of the samples. Other species of *Aspergillus* were found to be isolated less frequently. Next to *Aspergillus* when it comes to frequency of isolated fungal species was *Penicillium*. *Penicillium* accounted for 40 and 45% of the isolated samples (4.1 and 2.4% of the total fungal isolates, respectively). Among the 7 isolated *Penicillium* species, *P. chrysogenum* and *P. corylophilum* were the most frequent and rarely were *P. citrinum*, *P. crustosum*, *P. duclauxii*, *P. oxalicum* and *P. purpurogenum*. *Cladosporium* (represented by *C. cladosporioides* and *C. herbarum*) accounted for 40 and 45% of the samples and 5.5 and 2.7% of

Table 1: Total counts (TC) per gram, number of cases of isolation (NCI) and occurrence remarks (OR) of mycoflora isolated from date fruits using dilution-plate method

	Dates		Date products	
Fungal genera and species	TC	NCI or OR	TC	NCI or OR
Acremonium strictum	-	-	50	1R
Alternaria alternata	50	1R	-	-
Aspergillus				
A. alutaceus	50	1R	-	-
A. flavipes	-	-	50	1R
A. flavus	650	7M	800	8M
A. flavus var. columnaris	250	2R	-	-
A. fumigatus	700	7M	650	5L
A. niger	6700	17H	24200	16H
A. sydowii	50	1R	50	1R
A. terreus	-	-	300	3L
Cladosporium				
C. cladosporioides	400	5L	750	8M
C. herbarum	150	3L	-	-
Drechslera spicifera	50	1R	-	-
Emericella nidulans	50	1R	-	-
Eurotium				
E. amstelodami	-	-	100	2R
E. rubrum	50	1R	-	-
Fusarium solani	100	1R	-	-
Paecilomyces variotii	100	1R	-	-
Penicillium				
P. chrysogenum	150	3L	150	3L
P. citrinum	50	1R	50	1R
P. corylophilum	150	3L	200	3L
P. crustosum	50	1R	-	-
P. duclauxii	-	-	50	1R
P. oxalicum	-	-	50	1R
P. purpurogenum	-	-	50	1R
Rhizopus stolonifer	200	2R	50	1R
Syncephalastrum racemosum	-	-	50	1R
Total count	9950		27600	
Number of genera =12	10		7	
Number of species = 26 + 1 var	18+1		17	

OR = Occurrence remarks: H-high occurrence, from 12-20, M-moderate occurrence from 6-11, L-low occurrence from 3-5 and R-rare occurrence from 1-2 cases

Table 2: Total counts (TC), number of cases of isolation (NCI) and occurrence remarks (OR) of mycoflora isolated from date fruits using plating technique

	Dates		Date products	
Fungal genera and species	TC	NCI or OR	TC	NCI or OR
Aspergillus				
A. alutaceus	1	1R	-	-
A. flavipes	34	17H	46	12H
A. fumigatus	2	2R	1	1R
A. niger	158	17H	153	20H
A. terreus	3	2R	5	3L
Neurospora crassa	3	3L	7	5L
Penicillium				
P. chrysogenum	4	3L	12	4L
P. corylophilum	20	9M	29	11M
Rhizopus stolonifer	3	3L	12	5L
Total count	228		265	
Number of genera = 4	4		4	
Number of species = 9	9		8	

OR = occurrence remarks: H-high occurrence, from 12-20, M-moderate occurrence from 6-11, L-low occurrence from 3-5 and R-rare occurrence from 1-2 cases

the isolated total fungi. Some other fungal species were identified as single representatives including *Acremonium strictum*, *Alternaria alternata*, *Drechslera spicifera*, *Emericella nidulans,* *Eurotium amstelodami*,

Table 3: Ability of common fungal species isolated from date samples tested for production of invertase

			Degree of production		
Fungal species	NIT	NPI	Weak	Moderate	Strong
Acremoni umstrictum	1	1	-	1	-
Alternaria alternata	1	1	-	1	-
Aspergillus					
A. alutaceus	1	1	-	1	-
A. flavus	7	7	2	3	2
A. flavus var. columnaris	1	1	-	1	-
A. fumigatus	4	4	1	2	1
A. japonicus	1	1	-	1	-
A. niger	6	5	1	4	-
A. sydowii	1	1	-	1	-
A. terreus	1	1	-	-	1
A. versicolor	1	1	-	-	1
Cladosporium					
C. cladosporioides	6	6	1	4	1
C. herbarum	2	2	-	2	-
Drechslera spicifera	1	1	-	1	-
Eurotium rubrum	2	2	-	1	1
Fusarium solani	1	1	-	1	-
Paecilomyces variotii	1	1	-	1	-
Penicillium					
P. chrysogenum	3	3	1	1	1
P. corylophilum	4	4	-	3	1
P. crustosum	1	1	-	1	-
P. duclauxii	1	1	-	-	1
P. purpurogenum	1	1	-	1	-
Rhizopusstolonifer	1	1	-	1	-
Total isolates	49	48	6	32	10

NIT: Number of isolates, NPI: Number of positive isolates

Eurotium rubrum, *Paccilomyces variotii*, *Rhizopus stolonifer* and *Syncephalastrum racemosum*. Using the plating technique, 9 species representing 4 genera were isolated from dates and date products. The total count was less in dates (228 colonies/160 segments) than on date products (265 colonies). Similarly, *Aspergillus and Penicillium* were the most common isolated fungal species (Table 2).

Upon testing for the production of invertase enzyme, 48 isolates (98.0%) were able to hydrolyse sucrose. There were 32 isolates which showed moderate invertase activity including the fungal species *A. flavus*, *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. herbarum* and *P. corylophilum*. On the other hand, 10 isolates showed strong invertase activity, whereas the remaining 6 isolates showed weak invertase activity as shown in Table 3.

DISCUSSION

This study highlights the different mycoflora that were isolated from dates and date products that were sampled from markets in Riyadh, Saudi Arabia in the last quarter of 2016. In this study, out of the 40 samples that were tested, 27 fungal species and one variety representing 13 fungal genera were isolated and identified, including *Aspergillus*, *Cladosporium*, *Neurospora*, *Penicillium* and *Rhizopus*. Similar to the findings from previous studies, the *Aspergillus* species was the predominating fungal isolates from dates and date products[2,10,11]. This study showed that *Aspergillus* species, led by *A. flavus* constituted 85% of the fungi isolated from date fruits and 90% of date products. The prevalence rate in this study is comparatively higher compared to the prevalence of *Aspergillus* reported by Al-Bulushi *et al*.[12] (around 60%) but is lower in prevalence compared to that reported by Al-Asmari *et al*.[13] (37%).

Aspergillus and *Penicillium* are known potentially hazardous fungal genera to humans. *Aspergillus* can cause lung disease and other more serious infections in humans[14,15]. *Penicillium chrysogenum* in particular has an invasive character that causes intestinal invasion and disseminated disease in humans[16]. Furthermore, one study showed that almost 40% of different date varieties contain aflatoxins[5]. The growth of *Aspergillus flavus* on dates can result in aflatoxin contamination that would make them unsafe for human consumption and unmarketable[17].

Another highlight of this study is that 98.0% of the fungal isolates were able to hydrolyse sucrose, 10 with strong

invertase activity and 32 isolates with moderate invertase activity. Only six isolates showed weak invertase activity. This finding is similar to a previous study that accounted up to 87% of the isolated fungal species were able hydrolyse sucrose. Lately, *Aspergillus niger* and *Alternaria* species were found to be the agents of spoilage of fresh date fruits during storage and packaging, together with the effect of temperature and water activity, has a high sugar enzyme activity compared to present study results[18]. The same study reported that invertase enzyme production greatly varied among different species and isolates of the same species[19]. The activity of invertase and its role in the ripening and the increase in sugar content of dates has been substantiated in several research studies[18,19]. This study suggests that the higher the activity of the invertase enzyme, the higher will be its reducing sugar content, in agreement with the findings of Sidhu *et al.*[20] and Ahmad[21]. The current study also showed that *Aspergillus niger* produces high levels of invertase under culture conditions similar to the study of Ahmad[21]. This means that there coexist a beneficial effect between *A. niger* and the ripening of the date palm fruit[21]. In contrast, in one study it was found that increasing concentrations of glucose and fructose from the hydrolysis of sucrose stimulated the growth of fruits (particularly mangoes), however the levels of enzymes such as invertase enzyme was found to be high upon infection and spoilage[22]. The total carbohydrate content in the fruits decreased upon spoilage, total reducing sugars increased and the levels of glucose increased upon spoilage[22]. Several other studies support this claim on the enzymatic changes (invertase production) on other experimented fruits during ripening and its relationship to spoilage, as also suggested by the current study[23].

CONCLUSION AND FUTURE RECOMMENDATIONS

Aspergillus, *Penicillium*, *Cladosporium* and several other saprophytic fungi are common isolates from dates and more prevalent in date products. The high prevalence of these saprophytic fungi together with the high sucrose content which is hydrolyzed by the invertase enzyme takes the pivotal role in the ripening and maturation of the date fruit and also probably the spoilage of the date fruit and date products. Therefore, it is highly recommended that extra precautionary measures must be adopted in consumption of ripened dates particularly those near spoilage due to its probable high saprophytic fungal contents.

SIGNIFICANCE STATEMENT

This study discovers the different mycoflora that were isolated from fresh and dried date fruit and from date products that can be beneficial for authorities and manufacturers in the food business to improve the quality and safety of food processing, storage and distribution. Furthermore, this study highlighted the role of the high sucrose content together with the high invertase enzymes that play a major role in the spoilage and degradation of the date fruit and date products. This study discovers the interaction between the pros and cons of invertase enzyme in the timely ripening of the date fruit and the over-ripening that leads to spoilage of the fruit.

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