Fungal microflora in dried persimmon fruits

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RESEARCH ARTICLE

Abstract

Persimmon fruit due to its short shelf life is often consumed as fresh during the season, but it is also consumed as a dried fruit. In recent years, dried persimmon fruits with mouldy appearance as a whole are sold by small-scale local enterprises. In this study, the fungal profile of both fruit surface and inner parts of the whole dried persimmon fruit was investigated. Mould and yeast counts and osmophilic count were determined by using acidified potato dextrose agar and malt extract agar containing 40% sucrose respectively. Mould isolates were identified considering their cultural and morphological properties. Two different sampling methods were applied and no significant differences were found for osmophilic yeast counts, yeast counts and mould counts except osmophilic mould counts. In this study, it was observed that 95% of the samples were contaminated with moulds and the number of moulds was in the range of <1–4.34 log colony forming units per gram (cfu/g). Seventeen different genera of moulds were isolated from dried persimmon fruits, and the dominant microflora of the analysed samples were *Rhizopus* spp., *Penicillium* spp. and *Aspergillus* spp.

Keywords: dried persimmon fruit; fungal microflora; osmophilic yeast

1. Introduction

Persimmon fruit (*Diospyros kaki* L.) has a tough, glossy, orange-reddish skin and a sweet, juicy and yellow-orange flesh when ripe (Tous and Ferguson, 1996). Persimmon is cultivated in warm regions of the world, such as China, Japan and Korea, but is also gaining popularity in the Mediterranean countries such as Turkey (Akbulut *et al.,* 2008; Nicoleti *et al.,* 2007; Plaza *et al.,* 2012). Persimmon fruit is rich in nutrients such as vitamin C (0.7 mg/g of pulp) and pro-vitamin A (0.65 mg/g of pulp), and is a good source of calcium (0.09 mg/g of pulp) and iron (0.002 mg/g of pulp) (Tous and Furguson, 1996). Furthermore, the fruit is a good source of carotenoids, which may have a role in cancer prevention by acting as free radical scavengers or antioxidants (Tee, 1992), in preventing cardiovascular diseases (Gaziona and Hennekens, 1993) and in treating chronic diseases such as photosensitivity diseases (Matthews-Roth, 1993).

Persimmon fruit is generally cultivated in two varieties: astringent and non-astringent. The heart-shaped Hachiya is the most common variety of astringent persimmon. Non-astringent persimmon is consumed as a hard fruit, but it remains edible when it becomes soft.Astringent persimmon contains high levels of soluble tannins and is unpalatably astringent if eaten before it is completely softened (Akbulut *et al.,* 2008). Persimmon fruit reaches its best quality at the end of pre-climacteric stage when it has maximum sugar content and an appealing orange colour. However, as soon as climacteric stage starts, the fruit softens rapidly within a couple of days (Harima *et al.,* 2003). The shelf life of persimmon fruit is extended and its industrial exploitation increases by producing dried persimmon fruits. Drying process improves fruit's shelf life and reduces physiological, microbial and enzymatic degradation (Gardeli *et al.,* 2009). In recent years, dehydrated fruit and pulp have received attention because of certain advantages. The dehydrated fruit is easily obtainable, retains its natural characteristics, has reduced transportation cost, and is safe due to its appropriate moisture level required to prevent the growth of mould, which deteriorates freshness of fruits (Marques *et al.,* 2006). The drying process reduces costs of packaging, storage and transportation because of loss of weight and volume and easiness of handling and further processing (Chauhan and Srivastava, 2009; Duan *et al.,* 2010).

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There are several factors such as the nature of food, drying method, drying conditions and pre-treatment prior to drying, which affect the quality of dried products (Rahman *et al.,* 2009; Sablani, 2006). Microbial growth is prevented or retarded in dried products if a sufficient number of pathogenic microorganisms are present after the drying process, but this may pose threat to consumers. Microbial growth is inhibited once the product is in a desiccated state, but vegetative cells and spores can remain viable for months (Beuchat *et al.*, 2013; Bourdoux *et al.*, 2016). Moulds are common contaminants of agricultural products. Mould contamination in food not only results in spoilage or economic losses but is a serious concern for human and animal health since it may produce toxic metabolites called mycotoxins (Park *et al.*, 2003). Mycotoxin formation can take place at any of the stages of growing, harvesting, transporting, drying and during the storage of dried fruit and vegetable products. Natural occurrence of mycotoxins and fungal contamination of dried fruits have been investigated in many countries by different authors (Aksoy *et al*., 2007; Battilani *et al.,* 2003; Bayman *et al.,* 2002; Herry and Lemetayer, 1992; MacDonald *et al.,* 1999; Meyvaci *et al.,* 2005; Möller and Nyberg, 2003; Zohri and Abdel-Gawad, 1993). Persimmon fruits are dried mostly using sun-drying method; thus, there are major concerns regarding potential mixing of foreign substances, colour changes, mould growth and other damages during the drying process (Choi *et al.*, 2017). As dry fruit is normally consumed directly, there is a need to verify the microbiological quality of these products (Iamanaka *et al.*, 2007). The objective of this study is to determine mould and yeast counts in dried persimmon fruits and to identify fungal microflora in these sold as a whole in markets.

2. Materials and methods

Preparation of samples

In this study, 40 different samples of dried whole persimmons were purchased in their original intact packets from market and small-scale local enterprises in İzmir, Turkey, and analysed for fungal contamination. Each sample contains 5–6 dried whole persimmons. Two sampling methods were used for preparing dilution for microbiological counts. In the first sampling method (rinse method), three dried whole persimmon fruits (60–70 g) from each part were aseptically placed in a stomacher bag containing 100-ml diluent and shaken for 1 min. In the second sampling method (homogenisation method), 10-g samples were taken from the external surfaces of dried persimmon fruits. These samples were homogenised with a stomacher for 1 min after addition of 90-ml diluent. For both methods, appropriate decimal dilutions were prepared by using 0.1% peptone water (PW, pH 7.0–7.4) and 20% sucrose solution (1:10) for mould--yeast counts and osmophilic counts respectively.

Yeast and mould counts

Yeast and mould counts were done by plating of dilutions to acidified potato dextrose agar (PDA, Oxoid, pH 5.4–5.8). All plates were incubated at 25 °C for 3–5 days. At the end of the incubation period, mould and yeast counts were done separately and results were expressed as colony forming units per gram (cfu/g) or colony forming units per fruit (cfu/fruit) for homogenisation and rinse methods respectively.

Osmophilic count

Malt Extract Agar (MEA, Merck, pH 5.4–5.8) containing 40% sucrose was used to determine osmophilic mould and yeast counts. After the inoculation of plates using pour plate technique, they were incubated at 25 °C for 3–5 days. At the end of incubation period, mould and yeast colonies were counted separately (Pitt and Hocking, 2009).

Identification of moulds

Mould identification was performed according to the identification keys provided in Pitt and Hocking (2009). Moulds grown on incubated plates were isolated and purified on slant PDA and further sub-cultured for microscopic observation and identification. Ninety-two mould isolates were selected from different colonies on petri dishes using a stereoscopic microscope (Olympus SZ61, Tokyo, Japan). Selected mould isolates were inoculated with a single culture at three points on Czapek yeast extract agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar (G25N). Plates were incubated at 25 °C for 7 days and colonies were identified up to genus level according to the methods described in Pitt and Hocking (2009). Colony size, density, colouration, production of aerial mycelia and conidia, and surface and reverse colony pigmentation were examined with this method (Skaar and Stenwig, 1996).

Preparation of fungal slide cultures

Fungal slide cultures of selected mould isolates were prepared to determine morphological characteristics microscopically. For the preparation of slide cultures, melted PDA media of about 0.1 ml dropped onto sterile glass slide and each fungal isolate to be identified was inoculated onto edges of this solidified agar. After that, a sterile cover slip was placed to the top of solidified agar containing inoculated mold. The prepared slide culture was placed into a sterile Petri dish containing moistened cotton plugs in order to maintain required humidity. Plates were incubated at 25 °C for 7 days. Moulds grown on glass slides were examined microscopically. The fungal slide cultures were prepared to

observe the microscopic morphology more clearly (Prakash and Bhargava, 2016).

Statistical analyses

Differences between total mould and yeast counts were analysed by paired sample *t*-test and two independent samples *t*-test using IBM SPSS 20 Statistical Package; statistical significance was defined as $P < 0.05$. The Error bars in the given figures correspond to mean standard errors.

3. Results and discussion

The results of the analysis done by homogenisation and rinse methods are given in Tables 1 and 2 respectively. In the homogenisation method, the total number of mould and yeast on dried persimmons ranged from <1 to >5.48 log cfu/g, and 95% of dried persimmon fruits showed mould and yeast contamination. According to the microbiological criteria recommended for dried or frozen fruits, the maximum limit of mould and yeast is 4–5 log cfu/g (Turkish Food Codex, 2011). In the present study, of the 40 dried persimmon fruit samples taken, one (2.5%) was found above M-value (number of microorganisms separating marginally acceptable quality from unacceptable quality), four samples (10%) were found between m and M values, and the values of other 35 (87.5%) samples were smaller than the m-value (number of microorganisms separating good quality from marginally acceptable quality). The results showed that 12.5% of dried persimmon fruits were potential health hazard because of mould and yeasts. There are very limited data available on the microbiological quality of dried persimmon fruits. In the present study, moulds were dominant microflora of dried persimmon fruits as 90% of the samples were contaminated with moulds. On the other hand, yeast count was below the detection limit of <1.0 log cfu/g in 45% of the samples. In only one sample, yeast count was found above 5.48 log cfu/g. In comparison to the results obtained in this study, Park *et al.* (2003) obtained lower levels of mould count in dried persimmon fruits that ranged between 2.0 and 3.26 log cfu/g.

Dried fruits with low water activity and high sugar content are favourable medium for xerotolerant moulds. In the homogenisation method, osmophilic yeast count and osmophilic mould count were about the same or higher than yeast and mould counts in 75% and 70% of the samples respectively. Total osmophilic mould and yeast counts were significantly higher than the total mould and yeast counts by using homogenisation method ($P < 0.05$). On the other hand, no significant differences were found by using the rinse method ($P > 0.05$).

In the rinse method determining microflora on fruit surfaces, the mould and yeast counts were in the range of <1.52 to >7.0 log cfu/fruit. Total mould and yeast counts were below the detection limit of 1.52 log cfu/fruit in

Table 1. Frequency of yeast and mould count per gram of dried persimmon fruits (homogenisation method).

Fungi	Range (log cfu/g)	No. of samples in the indicated interval (log cfu/g)				
		<2	2–3	3–4	4–5	>5
Mould and yeast count	<1–>5.48	12	17	7	3	1
Mould count	<1–4.34	17	17	5	1	-
Yeasts count	<1–>5.48	21	12	5	1	1
Osmophilic count	<1–>5.48	8	14	16	1	1
Osmophilic mould count	<1–3.81	14	18	8	-	-
Osmophilic yeast count	<1–>5.48	21	7	10	1	1

Table 2. Frequency of yeast and mould count on the surface of dried persimmon fruits (rinse method).

Fungi	Range (log cfu/fruit)	No. of samples in the indicated interval (log cfu/fruit)				
		<2	2–3	3–4	4–5	>5
Mould and yeast count	<1.52–>7.00	8	5	15	8	4
Mould count	<1.52–6.15	9	8	15	7	1
Yeast count	<1.52–>7.00	18	5	10	4	3
Osmophilic count	<1.52–>7.00	7	6	13	10	4
Osmophilic mould count	<1.52–5.85	10	8	15	5	2
Osmophilic yeast count	<1.52–>7.00	19	4	9	6	2

eight samples. The mould and yeast counts were above 4 log cfu/fruit in 12 of the 40 samples. Yeast count was below the detection limit of <1.52 log cfu/fruit in 45% of the samples; on the other hand, the number was above 4.0 log cfu/fruit in 17.5% of the samples.

The rinse method was used to determine mould and yeast counts on the surface of whole fruits and to identify the moulds present on fruit surface. The surface of dried persimmon fruits had a mouldy appearance because of diffused sugar. In many studies, the rinse method was generally applied to determine microbial count of fruits and vegetables. In the present study, osmophilic counts and total mould and yeast counts were significantly higher in the rinse method compared with the homogenisation method ($P < 0.05$) (Figure 1). In the rinse method, samples were taken from the entire surface of fruit, while in the homogenisation method, only 10 g of sample was taken for analysis. The rinse method is more appropriate for determining mycoflora in fruits.

According to statistical analysis, results show no significant difference between two sampling methods ($P > 0.05$) for osmophilic yeast count and separate counts of mould and yeasts. On the other hand, significant differences were observed by homogenisation and rinse methods for osmophilic mould counts ($P > 0.05$). Burnett and Beuchat (2001) indicate that the type of processing method, washing in peptone water, stomaching or homogenising, does not have significant influence on the number of *Salmonella* spp. recovered from raw fruits and vegetables.

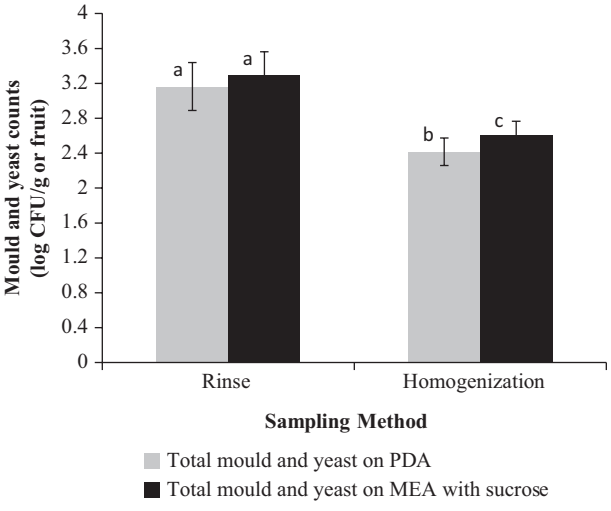

Figure 1. Mean values of yeast and mold counts of dried persimmon fruits obtained with different sampling methods using PDA or MEA with sucrose. Different letters indicate significant mean difference according to statistical analysis (*P < 0.05*). The error bars in the figures correspond to the mean standard errors.

Persimmon fruits were peeled and the mycoflora present on fruit surfaces was removed before the sun-drying process. Izumi *et al.* (2008a) obtained on average 4.1 log cfu/g of mould count in peeled persimmon fruits; on the other hand, mould count was below the detection limit of <3 log cfu/g for fruit flesh. Murakami *et al.* (2012) also indicated that mould counts of enzyme-peeled persimmon slices were below the detection limit of 3 log cfu/g. During the sun-drying process, drying fruits are re-contaminated with a variety of moulds and yeasts. Because of long drying periods of traditionally sun-dried foods, contaminated moulds produce mycotoxins by finding a growth medium. Microbial counts and diversity increase after coming in contact with plastic harvesting baskets and containers, which may be a source of contamination during harvesting. Therefore, to reduce microbial contamination in farms, agricultural water and harvesting equipment should focus on control points (Izumi *et al.,* 2008b).

In the present study, it was observed that mycoflora of dried persimmon fruits contained 17 genera of mould at different contamination levels (Figure 2). The most common mould identified in dried persimmon fruits was *Rhizopus* spp., which has isolated from 28 of 40 samples (70%). *Rhizopus* spp. is very common air contaminant found in most environments and possibly in processing plant areas (Tournas *et al.,* 2006). Beside *Rhizopus* spp., the microflora of dried persimmon fruits mainly consisted of *Penicillium* spp. (40%), *Aspergillus* spp. (22.5%), *Cladosporium* spp. (15%) and *Alternaria* spp. (10%). Other mould genera which include *Ulacladium* spp., *Chrysonilia* spp., *Endomyces* spp., *Chaetomium* spp. and *Paecilomyces* spp. were isolated at a frequency of 5%, while *Moniella* spp., *Basipetrospora* spp., *Monascus* spp., *Mucor* spp., *Trichothecium* spp., *Byssochlamys* spp. and *Geotrichum* spp. were isolated at a low frequency of 2.5% of dried persimmon fruits. Generally, microorganisms

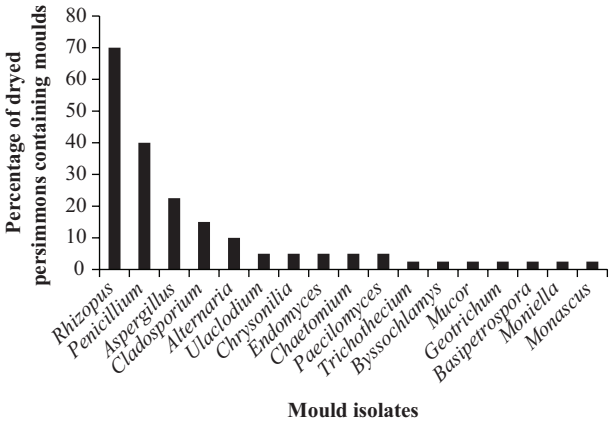

Figure 2. Frequency and distribution of moulds isolated from dried persimmon fruits.

found at low frequencies are considered random contaminants (Tournas *et al.,* 2006). High frequency of *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp. are a concern since these microorganisms are known to produce a wide spectrum of highly toxic metabolites. Bok-Hee *et al.* (2012) reported that *Penicillium* spp., identified as a major causative microorganism, accounted for the highest percentage of contamination in dried persimmons. *Cladosporium* spp. and *Aspergillus* spp. were also isolated. Park *et al.* (2003) performed mycoflora detection of nine dried persimmon fruits. Identified mould proportions were found as: *Alternaria* 38%, *Aspergillus* 2%, *Cladosporium* 16%, *Fusarium* 3%, *Mucor* 4%, *Penicillium* 2%, *Rhizopus* 6% and unknown 29%. There is little information about the mycobiota present on dried persimmon fruits. On the other hand, post-harvest diseases of fresh persimmon fruits were also studied. According to a previous study (Palou *et al.,* 2015), *Penicillium expansum*, *Cladosporium cladosporioides*, *Botrytis cinerea*, *Rhizopus* spp. and *Trichoderma* spp. were the most frequently isolated fungi in fresh persimmon fruits. Aktaruzzaman *et al.* (2018) indicated that fungus *B. cinerea* was the causal agent of disease ('grey mould') found in fresh persimmon fruit. On the other hand, in our study, a low frequency of *Botrytis* spp. was isolated in dried persimmon fruits. It was indicated that the most frequent disease found in persimmons was alternaria black spot caused by *Alternaria alternata.* Other post-harvest pathogens such as *P. expansum* and *C. cladosporioides* were also isolated from infected persimmons after storage at 20–25 °C for up to 9 weeks (Palou *et al.,* 2015).

In the present study, 95% of the samples were contaminated with moulds, and 17 different genera of moulds were isolated from dried persimmon fruits. Isolation of a wide range of mould genera increases the risk of presence of mycotoxins. Wang *et al.* (2018) found many types of mycotoxins, including Aflatoxin B_2, Alternaria toxins, Ochratoxins (OTA, OTB) and Beauvericin (BEA) in dried persimmon fruits. Dried fruits are thought to be resistant to microbial spoilage because of their low water activity and moisture content, and high acidity and sugar content. Nevertheless, moulds are the most important microorganisms in dried fruits in terms of spoilage. Even if a small part of surface is infected by a mould, it may grow quickly within a short period. In addition, the number of infected fruits increases rapidly if the drying process is not performed correctly (Montville and Matthews, 2008; Vinson *et al.,* 2008).

4. Conclusions

Mycological quality and the fungal profiles of dried persimmon fruits available in the market were evaluated. It was observed that dried persimmon fruits contained 17 different genera of moulds and 95% of the samples were contaminated with moulds and yeasts at different levels. For this reason, it is necessary to carry out the hurdle technique to prevent fungal growth that may occur during harvesting, processing and storage of the dried fruits. Our study shows that further research is needed to determine the mycotoxin production potential of isolates during the processing of persimmons.

Conflict of interest

The authors declare no conflicts of interest with respect to research, authorship and/or publication of this article.

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Compliance with ethical standards

This article followed all ethical standards for a research without direct contact with human or animal subjects.

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