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Mould and yeast flora in fresh berries, grapes and citrus fruits

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Abstract

Fresh fruits are prone to fungal contamination in the field, during harvest, transport, marketing, and with the consumer. It is important to identify fungal contaminants in fresh fruits because some moulds can grow and produce mycotoxins on these commodities while certain yeasts and moulds can cause infections or allergies. In this study, 251 fresh fruit samples including several varieties of grapes, strawberries, blueberries, raspberries, blackberries, and various citrus fruits were surface-disinfected, incubated at room temperature for up to 14 days without supplemental media, and subsequently examined for mould and yeast growth. The level of contamination (percent of contaminated items/sample) varied depending on the type of fruit. All raspberry and blackberry samples were contaminated at levels ranging from 33% to 100%, whereas 95% of the blueberry samples supported mould growth at levels between 10% and 100% of the tested berries, and 97% of strawberry samples showed fungal growth on 33–100% of tested berries. The most common moulds isolated from these commodities were *Botrytis cinerea*, *Rhizopus* (in strawberries), *Alternaria*, *Penicillium*, *Cladosporium* and *Fusarium* followed by yeasts, *Trichoderma* and *Aureobasidium*. Thirty-five percent of the grape samples tested were contaminated and supported fungal growth; the levels of contamination ranged from 9% to 80%. The most common fungi spoiling grapes were *Alternaria*, *B. cinerea* and *Cladosporium*. Eighty-three percent of the citrus fruit samples showed fungal growth at levels ranging from 25% to 100% of tested fruits. The most common fungi in citrus fruits were *Alternaria*, *Cladosporium*, *Penicillium*, *Fusarium* and yeasts. Less common were *Trichoderma*, *Geotrichum* and *Rhizopus*. Published by Elsevier B.V.

Keywords: Moulds; Yeasts; Berries; Grapes; Citrus fruits

1. Introduction

Fruits contain high levels of sugars and other nutrients, and they possess an ideal water activity for microbial growth; their low pH makes them par-

ticularly susceptible to fungal spoilage, because a big part of the bacterial competition is eliminated since most bacteria prefer near neutral pH. Some fungi are plant pathogens and can start the spoilage from the field while others, although they could contaminate the fruits in the field, actually proliferate and cause substantial spoilage only after harvest when the main plant defenses are reduced or eliminated. Fungal spoilage of fruits will depend on cultivation, harvest-

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ing, handling, transport, and post-harvest storage and marketing conditions. Various means of controlling post-harvest microbial spoilage of fruits such as careful culling, storage at low temperatures or under controlled atmospheres and application of fungicides have been used (Beuchat, 1987; Ryall and Pentzer, 1982; Eckert and Ogawa, 1988). Refrigeration slows down fungal growth dramatically and prolongs the shelf life of fruits. Some fruits, however, are sensitive to low temperatures and they could suffer chilling injuries, therefore, become very susceptible to microbial spoilage (Ryall and Pentzer, 1982). On the other hand, many fungi can grow at low temperatures and cause substantial damage especially if the fruits are stored for extended periods of time. The use of synthetic fungicides could prevent spoilage to some degree but some fungi could become resistant to commonly used pesticides (Spotts and Cervantes, 1986). Also, attempts to reduce chemical contamination of the environment as well as health hazards associated with consumption of pesticide residues dictate the reduction of the use of such chemicals.

Post-harvest fruit spoilage results in significant economic losses. Additionally, if the spoiling fungi are toxigenic or pathogenic, they could pose a health risk for the consumer. Toxigenic fungi have been isolated from spoiling fruits in the past (Ryall and Pentzer, 1982; Stinson et al., 1981). Some of these moulds could produce mycotoxins while grown on fruits (Stinson et al., 1980, 1981) even during refrigeration (Tournas and Stack, 2001). Pathogenic fungi, on the other hand, could cause infections or allergies in susceptible individuals (Kurup, 2003; Lewis et al., 1975; Monso, 2004).

Restrictions or ban of certain fungicides and use of new ones in recent years may have changed the post-harvest fungal profiles of fruits. This study investigates the current fungal profiles of various fresh fruits sold in the Washington, DC Metro area in order to find out if toxigenic moulds and pathogenic moulds and yeasts are present and likely to grow on these commodities.

2. Materials and methods

Ripe, sound fruits including strawberries, blueberries, raspberries, blackberries, various types of grapes (red seedless, red seeded, green seedless, black seed-

less, and black seeded), navel, temple and Minneola oranges, tangerines, Sunkist and citron lemons, and limes were purchased from local supermarkets in the Washington, DC area. All berry samples were purchased in their individual intact packages weighing 6 oz (for raspberries, blackberries and blueberries) and 16 oz (for strawberries), grape samples were purchased in their individual plastic bags weighing approximately 24 oz each, and citrus fruit samples were drawn from bulk trays (8–10 fruits per sample). From each berry and grape sample 50 g were aseptically transferred into sterilized, cotton-plugged 1000 ml flasks. From each citrus fruit sample 3 or 4 healthy and ripe fruits were aseptically placed into individual pre-sterilized glass beakers covered with double aluminum foil. Subsequently, all samples were surface-disinfected in dilute commercial bleach (1:10 in sterile distilled water) for 2 min with constant stirring, drained and incubated at room temperature for up to 2 weeks. Moulds and yeasts grown on incubated fruits were isolated and purified on PDA. Subsequently, mould isolates were cultured on CYA, MEA and G25N agar at 5, 25 and 37 °C and identified according to methods described in “Fungi and Food Spoilage” (Pitt and Hocking, 1985). *Fusarium* isolates were identified using PDA and carnation leaf agar (CLA) as described in *Fusarium* Species, An Illustrated Manual for Identification” (Nelson et al., 1983).

3. Results and discussion

3.1. Berries

The overall fungal contamination of tested fruits is summarized in Table 1. One hundred percent of blackberry and raspberry, 97% of strawberry and 95% of blueberry samples showed some sort of fungal contamination. The contamination level (percent of contaminated berries per sample) differ among the various types of berries; the highest mean contamination level of 82% was observed in raspberries, closely followed by blackberries and strawberries, whereas the lowest of 38% occurred in blueberries (Table 1). The lower contamination level in blueberries was probably due to the fact that these fruits have smooth, hard skin, therefore, impermeable to most fungi. Blackberries, raspberries and strawberries, on the

Table 1

Mould and yeast contamination of fresh berries, grapes and citrus fruits

Fruits	Total no. of samples	Contaminated samples (%)	Contamination level ^a	
			Mean	Range
<i>Berries</i>				
Blackberries	9	100	80	33–100
Blueberries	22	95	38	0–100
Raspberries	20	100	82	50–100
Strawberries	39	97	79	0–100
<i>Grapes</i>				
Red seedless	19	32	12	0–70
Red seeded	8	38	12	0–50
Green seedless	20	35	15	0–80
Black seedless	5	60	12	0–33
Black seeded	17	29	5.3	0–33
<i>Citrus fruits</i>				
Minneola oranges	16	100	51	25–75
Navel oranges	11	82	35	0–67
Temple oranges	6	100	58	50–75
Tangerines	21	76	34	0–70
Sunkist lemons	21	71	30	0–75
Citron lemons	4	50	38	0–100
Limes	13	92	44	0–75

^a Percent of contaminated items per sample.

other hand, have significantly thinner, more susceptible to injury and breakage epidermis with numerous indentations and fiber-like protuberances where the various microorganisms can easily attach and invade the inner tissues of the fruits.

The most common fungi found in berries were *Botrytis cinerea*, *Alternaria*, *Cladosporium*, *Penicillium*, *Fusarium* and *Rhizopus*. Less common were *Trichoderma*, *Aureobasidium pullulans* and yeasts (Table 2). *B. cinerea* was by far the most common spoiler of berries contaminating 78% of blackberry, 55% of blueberry, 75% of raspberry and 77% of strawberry samples. This organism grew rapidly and consumed the fruit producing an unsightly watery (except on strawberries where the decay was dry) rot accompanied with an off odor. Devastating losses of berries due to *B. cinerea* have been reported in the past (Pitt and Hocking, 1985; Ryall and Pentzer, 1982; Splittstoesser, 1987). *Rhizopus* was found in 23% of strawberry, 11% of blackberries and 10% of raspberry samples (Table 2). Although this organism often started from one or two berries, quickly spread over

the neighboring healthy ones and consumed the whole sample within 3–4 days. *Rhizopus* possesses pectic enzymes (polygalacturonases), therefore, it easily breaks pectins producing a watery decay of the affected fruits with white coarse mycelium and sparse black sporangia completely covering the fruits. Splittstoesser (1987) had reported that *Rhizopus* caused watery, soft rot in strawberries, while Pitt and Hocking (1985) stated that *Rhizopus stolonifer* was the cause of a major rot in berries.

Table 2

Frequency of various moulds and yeasts on berries

Organism	Contaminated samples (%)	Contamination level ^a (range)
<i>Blackberries</i>		
<i>Botrytis cinerea</i>	78	0–100
<i>Cladosporium</i>	33	0–80
<i>Fusarium</i>	22	0–100
<i>Penicillium</i>	22	0–50
<i>Rhizopus</i>	11	0–50
No growth	0	–
<i>Blueberries</i>		
<i>Botrytis cinerea</i>	55	0–100
<i>Alternaria</i>	46	0–75
<i>Fusarium</i>	13	0–25
<i>Penicillium</i>	9	0–50
<i>Aureobasidium pullulans</i>	5	0–40
<i>Cladosporium</i>	5	0–20
<i>Trichoderma</i>	5	0–30
Yeasts	5	0–60
No growth	5	–
<i>Raspberries</i>		
<i>Botrytis cinerea</i>	75	0–100
<i>Fusarium</i>	25	0–50
<i>Cladosporium</i>	20	0–65
<i>Penicillium</i>	15	0–50
<i>Rhizopus</i>	10	0–90
Yeasts	5	0–65
No growth	0	–
<i>Strawberries</i>		
<i>Botrytis cinerea</i>	77	0–100
<i>Rhizopus</i>	23	0–100
<i>Penicillium</i>	10	0–67
<i>Fusarium</i>	8	0–75
<i>Alternaria</i>	8	0–67
<i>Cladosporium</i>	5	0–60
<i>Trichoderma</i>	3	0–50
Yeasts	3	0–75
No growth	3	–

^a Percent of contaminated items per sample.

Alternaria was found in 46% of blueberry and 8% of strawberry samples. White-grayish mycelium growing relatively slowly appeared after several days of incubation starting from the stem or bruised areas. This organism consumed up to 75% of the berries of the affected samples. Woolly *Alternaria* growth on blueberries was also reported by Splittstoesser (1987). The high *Alternaria* contamination of blueberries may be partially explained by the fact that faster-growing moulds such as *Rhizopus* spp. and *B. cinerea* were not always present in these fruits; therefore competition and prevalence of the latter organisms was non-existent. *Alternaria* growth on blueberries is especially problematic since this organism was capable of producing mycotoxins on this product (Stinson et al., 1980). *Fusarium* spp. were isolated from 22%, 13%, 25% and 8% of blackberry, blueberry, raspberry and strawberry samples, respectively. *Cladosporium* was found in 33% of blackberry, 20% of raspberry, 5% of blueberry and 5% of strawberry samples, whereas *Penicillium* spp. were present in all types of berries tested (Table 2). Invasion of overripe or damaged strawberries by *Penicillium* and *Cladosporium* spp. was also reported by Pitt and Hocking (1985).

Yeasts were only encountered in about 5% of blueberry and raspberry and in 3% of strawberry samples. Lower yeast incidence could partially be explained by the fact that these organisms cannot break the fruit epidermis and infect the inner fruit tissues; additionally, yeasts were probably more sensitive to chlorine, therefore, they were inactivated and/or washed off during the disinfection step.

The results of this research showed that a wide variety of fungi (mostly moulds) were capable of growing and spoiling the various types of berries; that is not surprising considering the fact that these commodities contain high levels of sugar and other nutrients and a water activity, ideal for fungal growth. Additionally, the low pH of these fruits eliminates the competition from many bacterial species, making it easier for fungi to grow and spoil the fruits.

3.2. Grapes

The highest percentage (60%) of contaminated grape samples was found in the black seedless variety and the lowest (29%) was present in the black seeded.

The levels of contamination (percent of contaminated berries per sample) ranged between 0–33% in black grapes and 0–80% in green seedless grapes. The mean contamination level, however, was relatively low ranging from 5% in black seeded and 15% in green seedless grapes (Table 1). Very low contamination level of black (especially of black seeded) grapes can probably be explained by the fact that these varieties possess very hard, difficult to break skin, whereas the higher sensitivity of the green seedless grapes to fungal contamination could be due to their thinner, easier to invade epidermis.

Fungal profiles of the various grape varieties are summarized in Table 3. Moulds commonly isolated from grapes were *B. cinerea*, *Alternaria* and *Clados-*

Table 3
Frequency of various moulds and yeasts on grapes

Organism	Contaminated samples (%)	Contamination level ^a (range)
<i>Black seedless</i>		
<i>Alternaria</i>	20	0–33
<i>Cladosporium</i>	20	0–10
<i>Ulocladium</i>	20	0–15
No growth	40	–
<i>Black seeded</i>		
<i>Alternaria</i>	23	0–33
<i>Cladosporium</i>	12	0–20
No growth	71	–
<i>Green seedless</i>		
<i>Botrytis cinerea</i>	10	0–80
<i>Penicillium</i>	10	0–70
<i>Aspergillus carbonarius</i>	5	0–65
<i>Alternaria</i>	5	0–10
<i>Fusarium</i>	5	0–16
Yeasts (<i>Rhodotorula</i>)	5	0–10
No growth	60	–
<i>Red seedless</i>		
<i>Alternaria</i>	16	0–20
<i>Botrytis cinerea</i>	11	0–70
<i>Aspergillus niger</i>	5	0–15
<i>Fusarium</i>	5	0–50
No growth	68	–
<i>Red seeded</i>		
<i>Alternaria</i>	13	0–50
<i>Cladosporium</i>	13	0–20
<i>Fusarium</i>	13	0–25
No growth	63	–

^a Percent of contaminated items per sample.

porium. Less common were *Fusarium*, *Penicillium*, *Aspergillus carbonarius*, *Aspergillus niger*, *Ulocladium* and yeasts. *Alternaria* was found in all types of grapes but it was more common in black grapes contaminating 20% of the black seedless and 24% of the black seeded samples. This organism was found less frequently in green and red grapes. The presence of *Alternaria* in grapes is of concern since this organism can grow and even produce toxins at refrigeration temperatures (Tournas and Stack, 2001). *B. cinerea* was present only in 10% of green and 11% of red seedless grape samples. Splittstoesser (1987) and Pitt and Hocking (1985) also reported that *Botrytis* caused spoilage of grapes. *Cladosporium* spp. grew in 20% of black seedless, 12% of black seeded and 13% of red seeded grape samples. *Cladosporium* spoilage of the three grape varieties that seemingly possess the hardest skin may indicate that *Cladosporium* spp. have the means to break the skin barrier, invade the inner fruit tissues and establish growth before other moulds have the chance to germinate. Since cladosporia are capable of growing at refrigeration temperatures, they could cause significant spoilage if the fruits are kept for extended periods of time. *Penicillium* grew on 10% of green seedless whereas *Ulocladium* was present in 20% of black seedless grape samples. Market disease of grapes caused by *Penicillium* was previously reported by Ryall and Pentzer (1982). Many *Penicillium* spp. can also grow at low temperatures and possibly produce mycotoxins on the decayed fruits.

A. carbonarius was found in 5% of green seedless whereas *A. niger* was isolated from 5% of red seedless grapes (Table 3). The contamination of grapes by *A. carbonarius* and *A. niger* and the production of ochratoxin A in grapes has been reported by Pitt (2000). Yeasts were found only in 5% of the green seedless grape samples, contaminating up to 10% of berries. De La Torre et al. (1999) reported that yeasts such as *Sporobolomyces roseus*, *Cryptococcus albidus*, *Rhodotorula rubra* and *Candida* were part of the natural microbiota of certain varieties of grapes.

The majority of the grape samples showed no fungal growth after 2 weeks of incubation (Table 3). Limited fungal growth on grapes can be explained by the fact that these commodities possess a very hard, smooth skin which protects the vulnerable, nutritional inner tissues from fungal invasion since most fungi

causing market disease are not capable of breaking the skin barrier. Additionally, grapes are sprayed with fungicides very near the harvest time; such pesticide residues remaining on the fruit during marketing protect against fungal spoilage.

3.3. Citrus fruits

A high percentage of the citrus fruits tested was contaminated and supported fungal growth after 2 weeks of incubation at room temperature. Contamination and spoilage affected 100% of Minneola and Temple orange, 82% of Navel orange, 76% of tangerine, 71% of Sunkist lemon, 50% of citron lemon, and 92% of lime samples. Although the majority of the samples showed fungal growth, the levels of contamination were not very high; mean levels of contamination in citrus fruits ranged from 30% (observed in Sunkist lemons) to 58% (found in Temple oranges) (Table 1). The fungal growth on these commodities was slow in the beginning, but by the end of the incubation period the affected fruits were often entirely consumed.

The most frequently encountered moulds were *Alternaria*, *Cladosporium*, *Penicillium* and *Fusarium*; *Trichoderma*, *Geotrichum*, *Rhizopus* and *A. niger* were isolated less often. Fungal spoilage of citrus fruits attributed to *Alternaria*, *Fusarium*, *Penicillium digitatum*, *Penicillium italicum*, *Aspergillus*, *Geotrichum* as well as to *Botrytis* was also reported by Splittstoesser (1987) whereas citrus fruit decays by *P. digitatum*, *Alternaria citri* and other moulds were described by Ritenour et al. (2003). *Alternaria* decays were mainly internal; only a light brown to black spot was sometimes visible at the button area, while the interior of the affected fruit was completely invaded and spoiled by the mould. Such decays are of great concern (especially if the fruits are used for juice); since the spoilage is not visible from the outside and the affected fruits cannot be culled out before juicing, some decayed fruits could be juiced along with the sound ones and contaminate the final product. Although most of the juice today is undergoing pasteurization before marketing and *Alternaria* will be killed during this process, if any mycotoxins were produced by the organism, they could remain active after the heat treatment and pose a health risk for the consumer.

Table 4
Frequency of various moulds and yeasts on citrus fruits

Organism	Contaminated samples (%)	Contamination level ^a (range)
<i>Oranges</i>		
Minneola		
<i>Alternaria</i>	50	0–67
<i>Cladosporium</i>	38	0–50
<i>Penicillium</i>	31	0–75
<i>Fusarium</i>	19	0–50
<i>Trichoderma</i>	19	0–75
<i>Geotrichum</i>	6	0–50
No growth	0	–
Navel Sunkist		
<i>Alternaria</i>	55	0–50
<i>Cladosporium</i>	36	0–67
<i>Geotrichum</i>	9	0–50
Yeasts	9	0–40
No growth	18	–
Temple		
<i>Cladosporium</i>	33	0–50
<i>Penicillium</i>	33	0–75
Yeasts	17	0–50
No growth	0	–
<i>Lemons</i>		
Sunkist		
<i>Alternaria</i>	29	0–75
<i>Cladosporium</i>	24	0–60
<i>Penicillium</i>	19	0–50
Yeasts	10	0–75
<i>Fusarium</i>	5	0–33
<i>Aspergillus niger</i>	5	0–60
No growth	29	–
Citron		
<i>Alternaria</i>	50	0–50
<i>Cladosporium</i>	50	0–100
No growth	50	–
<i>Limes</i>		
<i>Penicillium</i>	39	0–75
<i>Fusarium</i>	31	0–75
<i>Cladosporium</i>	23	0–50
<i>Trichoderma</i>	8	0–40
No growth	8	–
<i>Tangerines</i>		
<i>Alternaria</i>	24	0–50
<i>Cladosporium</i>	24	0–70
<i>Fusarium</i>	10	0–50
<i>Rhizopus</i>	5	0–25
Yeasts	24	0–50
No growth	24	–

^a Percent of contaminated items per sample.

Penicillium produced a soft rot. The decay was light-colored at first with sparse mycelium apparent, but after several days the lesions were covered with green or bluish conidia and had a strong off odor. *Geotrichum* caused a very soft rot with a sour odor with white mycelium and arthroconidia covering the affected area. Once *Geotrichum* colonization was established, the lesions expanded relatively fast. *Cladosporium*, on the other hand, grew at a slow rate and created dark olive spots on the fruits. Yeasts grew only on 9% of the Navel Sunkist and 17% of the Temple orange, 10% of Sunkist lemon, and on 24% of tangerine samples (Table 4). Yeasts apparently were not easily rinsed off the fruits during the disinfection step probably protected inside the tiny pits of the uneven outer surface of these fruits. The yeasts and moulds that grew on citrus fruits were not inhibited by the essential oils of the rind of these fruits. Delayed colony development of some fungi, however, could be due to a partial inhibition exerted by the volatile compounds found in the essential oils of the skin.

4. Conclusions

Several fungi including some from the mycotoxin-producing genera of *Penicillium*, *Alternaria*, *Fusarium* and *Aspergillus* were present and capable of growing on fresh fruits at room temperature. Among all fruits tested, berries had the highest levels of contamination; strawberries, raspberries and blackberries were the most susceptible probably due to the fact that their skins are soft, easily ruptured with numerous indentation and hair-like protuberances which allow most organisms to attach and proliferate. *B. cinerea* was by far the most common organism in berries. Thirty-five percent of the grape samples tested supported fungal growth. Lower fungal contamination and growth on grapes could partially be explained by the fact that these fruits are sprayed with fungicides very near the harvest time. Over 80% of citrus fruit samples were contaminated and supported fungal growth after 2 weeks at room temperature; growth on citrus fruits was slow, but often the mould had consumed the whole interior of the fruit before surface damage was apparent. *Alternaria*, *Cladosporium*, *Penicillium* and *Fusarium* were the predominant moulds on these commodities.

Some of the moulds found in the fruits tested during the course of this study (e.g. *Cladosporium*, *Alternaria* and *Penicillium*) are known to cause allergies when they are able to produce high numbers of conidia. Advanced spoilage of fruits by these moulds accompanied by heavy conidiation could have an adverse health effect on the personnel handling these commodities. Extra care should be taken during harvest, cleaning, sorting, packaging, transport, storage and marketing not to injure and further contaminate the fruits. Refrigeration is essential for keeping quality and extending shelf life, although several moulds and yeasts can grow at low temperatures at reduced rates. Fruits that are sensitive to low temperatures should be marketed quickly in order to avoid fungal spoilage and the potential of mycotoxin production and related health risks.

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