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Short communication

Moulds and yeasts in fruit salads and fruit juices

V.H. Tournas[a,*], J. Heeres[b], L. Burgess[b]

[a]Center for Food Safety and Applied Nutrition (HFS-315), Food and Drug Administration, College Park, MD 20740, USA
[b]JIFSAN/University of Maryland, College Park, MD 20740, USA

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Abstract

Thirty-eight fruit salad samples including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, and 65 pasteurized fruit juice samples (apple, carrot, grapefruit, grape and orange juices, apple cider, and soy milk) were purchased from local supermarkets in the Washington, DC area and tested for fungal contamination. The majority of fruit salad samples (97%) were contaminated with yeasts at levels ranging from <2.0 to $9.72\log_{10}$ of colony forming units per gram (cfu/g). Frequently encountered yeasts were *Pichia* spp., *Candida pulcherrima*, *C. lambica*, *C. sake*, *Rhodotorula* spp., and *Debaryomyces polymorphus.* Low numbers of *Penicillium* spp. were found in pineapple salads, whereas *Cladosporium* spp. were present in mixed fruit and cut strawberry salads. Twenty-two per cent of the fruit juice samples tested showed fungal contamination. Yeasts were the predominant contaminants ranging from <1.0 to $6.83\log_{10}$ cfu/ml. Yeasts commonly found in fruit juices were *C. lambica*, *C. sake*, and *Rhodotorula rubra. Geotrichum* spp. and low numbers of *Penicillium* and *Fusarium* spp. (1.70 and $1.60\log_{10}$ cfu/ml, respectively) were present in grapefruit juice.

Keywords: Moulds; Yeasts; Fruit salads; Fruit juices

1. Introduction

An increase in the consumption of minimally processed fruits (in the form of peeled, cut and packaged fruit salads) and fruit juices has been observed in recent years. Fruit salads are not heat-processed and contain no preservatives; therefore, they can be easily spoiled by micro-organisms. Many organisms, in particular acid-loving or acid-tolerant bacteria and fungi (yeasts and moulds), can use fruit as substrate and cause spoilage, producing off flavors and odors, discoloration of the product, and if the contaminating micro-organisms are pathogens could also cause human illness. Toxigenic fungi, on the other hand, under favorable conditions could produce mycotoxin in fruit products such as juice (Varma and Verma, 1987). Some reports of foodborne illnesses due to bacterial or viral contamination of fruit salads exist in the literature (Gaylor et al., 1995; CDC, 1991; White et al., 1986). No significant research on fungal contamination and spoilage of fruit salads has been reported in the literature so far.

Before pasteurization, fruit juices contain a microbial load representative of the organisms normally found on fruits during harvest plus contaminants added post-harvest (during transport, storage and processing). Pasteurization will rid juice of pathogens and other heat-sensitive microbes; therefore, it will reduce the microbial load substantially and extend the shelf-life of the product. If the original load is too high and/or the pasteurization process is inadequate, some micro-organisms will survive and subsequently will cause spoilage and possibly illness if the surviving organisms are virulent. Many reports of bacterial growth in fruit juices exist in the literature, but most of the ones describing human illness due to contaminated juice are dealing with un-pasteurized juice (Krause et al., 2001; Parish, 1998; Cook et al., 1998; Besser et al., 1993; CDC, 1996). Some investigations regarding fungal contamination of pasteurized fruit juice are also available (Mendoza et al., 1982; Kurtzman et al., 2001; Recca and Mrak, 1952). Most of these reports have shown yeasts to be the predominant fungi involved in juice

*Corresponding author. Tel.: +1 301 436 1963; fax: +1 301 436 2644.
E-mail address: vtournas@cfsan.fda.gov (V.H. Tournas).

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spoilage (Hatcher et al., 2000; Parish and Higgins, 1989). Yeast spoilage of fruit juice can result in formation of haze, production of CO_2 and off-odors, and changes in color (Grinbaum et al., 1994). This study was designed to investigate the mycological quality of packaged fruit salads and pasteurized fruit juices sold in the Washington, DC Metro area.

2. Materials and methods

2.1. Materials

A random set of samples including various fruit salads such as cantaloupe, citrus fruits, honeydew, pineapple, cut strawberry and mixed fruit salads, and pasteurized fruit juice samples (apple cider, apple, carrot, grapefruit, grape and orange juice, and soy milk) were purchased from local supermarkets in the Washington, DC area and tested for fungal contamination. All fruit salads were purchased in their original, intact packages; fruit juice samples were obtained in their original sealed containers. All samples were purchased, transported and maintained at refrigeration temperature (4 °C) until they were analysed. Analysis took place within 24 h from the time of purchase.

2.2. Mycological analysis

All samples were analysed before their use-by-date. Fruit salad samples were tested as follows: Fifty grams from each sample were aseptically removed and transferred to a sterile blender jar. Subsequently, each sample was blended in 450 ml of 0.1% peptone-water (1 g bacteriological peptone, 1 l distilled water) for 45 s. Serial dilutions of the homogenate (in 0.1% peptone-water) were plated in duplicate DRBC agar (10 g glucose, 5 g bacteriological peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g agar, 0.025 g Rose Bengal, 0.002 g Dichloran, 0.1 g chloramphenicol, 1 l distilled water) plates (0.1 ml/plate) and plates were incubated for 5 days at 25 °C. Fruit juices were analysed as follows: Fifty milliliters from each sample were added to 450 ml sterile peptone-water (0.1%) and mixed thoroughly by shaking. Serial dilutions were made and cultured as described above. Un-diluted juices were also cultured (0.1 ml/plate) and examined for fungal contamination.

Colonies were counted and counts were expressed as colony forming units (cfu) per gram or per ml; mould isolates were purified on potato dextrose agar (PDA) (DIFCO, Detroit, Michigan, USA) and further subcultured for microscopic examination and identification. Yeasts were identified using the ID 32C biochemical kit (Biomerieux, France). Mould identification was performed according to the methods described in 'Fungi and Food Spoilage' (Pitt and Hocking, 1999) and in '*Fusarium* species: An Illustrated Manual for Identification' (Nelson et al., 1983).

3. Results and discussion

The results of this study are summarized in Tables 1 and 2. Table 1 shows the mycological profiles and frequencies of fungal (yeast and mould) contamination in various fruit salads. The highest mean counts (9.53 $\log_{10}$ cfu/g) were observed in citrus chunks whereas the lowest (4.36 $\log_{10}$ cfu/g) were found in cut strawberries. Cantaloupe, citrus chunks, pineapple, watermelon and mixed fruit salads had mean counts higher than 6.0 $\log_{10}$ cfu/g. Overall, MY levels in salads ranged between <2.0 and 9.72 $\log_{10}$ cfu/g.

Yeasts were the predominant fungi found in fruit salads and they were isolated from all types of salads tested. Overall, yeast counts ranged between <2.0 and 9.72 $\log_{10}$ cfu/g. These organisms were isolated from 100% of cantaloupe, honeydew, pineapple, cut strawberry, watermelon, cantaloupe/honeydew and mixed fruit, and from 67% of citrus fruit salads (Table 1). The most common yeasts found in fruit salads were *Pichia* spp., *Rhodotorula* spp., *Candida pulcherrima, C. lambica, C. sake* and *Debaryomyces polymorphus* (Table 1). Generally, fresh, sound fruits have been reported to contain much lower yeast contaminants than above described. Tournas (unpublished data, 2000) found that fresh, good-quality grapes of several varieties and various citrus fruits showed yeast contamination of less than 3.0 $\log_{10}$ cfu/g, whereas Marshall and Walkley (1951) postulated that high-quality apples contained yeast populations < 3.0 $\log_{10}$ cfu/apple. Much higher levels of contamination in minimally processed fruits, like fruit salads, would be an indication of either the use of low-quality fruit (heavily contaminated and or partially spoiled) or the lack of adherence to good manufacturing practices (GMPs) during preparation and marketing of these products. Non-hygienic practices during preparation of the salads could add substantial numbers of micro-organisms especially yeasts and bacteria. Most yeasts and some moulds have difficulty entering the skin of many intact fruits, but they can sustain a luxurious growth once they enter the inner fruit tissues. Peeling and cutting the fruit eliminates the skin barrier and creates a large surface area for such organisms to grow. Some fungi found in minimally processed fruits can grow at refrigeration temperatures, therefore proliferate and reach high numbers during marketing. The longer the marketing period, the higher the numbers of contaminating fungi will be. Abuses of storage temperatures during transport and marketing will also increase the numbers of contaminating yeasts and moulds since these organisms grow at much higher rates at temperatures above refrigeration. Often additional contamination can originate in unclean, packaging trays and other materials that come in contact with the fruit salads.

Filamentous fungi were only isolated from pineapple chunks, cut strawberries and mixed fruit pieces. Small numbers of *Penicillium* spp. were isolated from 60% of pineapple chunks samples whereas *Cladosporium* spp. were found in 100% of cut strawberry salads and in 38% of

Table 1
Mould and yeast contamination of fruit salads

Product	Number of samples	MY $\log_{10}$ cfu/g		Frequency (% contaminated samples)	Yeast species	Mould species
		Mean	Range			
Cantaloupe pieces	5	6.34	3.18–6.69	100	*Pichia* spp. *Rhodotorula* spp.	
Citrus chunks	3	9.53	<2.0–9.72	67	*Rhodotorula* spp.	
Honeydew pieces	5	5.64	3.41–6.0	100	*Candida sake* *Candida lambica* *Rhodotorula* spp. *Pichia* spp.	
Pineapple chunks	5	7.34	3.50–7.72	100	*Debaryomyces polymorphus* *Candida pulcherrima* *Pichia* spp.	*Penicillium* spp.[a]
Cut strawberries	3	4.36	3.23–4.73	100	*Candida sake* *Rhodotorula* spp.	*Cladosporium* spp.[b]
Watermelon chunks	6	6.26	3.08–6.96	100	Candida *pulcherrima* *Cryptococcus uniguttulatus*	
Cantaloupe/honeydew	3	4.53	3.73–4.85	100	*Pichia* spp. *Zygosaccharomyces* spp.	
Mixed fruit pieces	8	6.34	3.41–7.11	100	*Rhodotorula* spp. *Debaryomyces polymorphus* *Candida pulcherrima*	*Cladosporium* spp.[c]

MY: mould and yeast.
cfu: colony forming units.
[a] *Penicillium* was found in 60% of the samples at levels between <2.0 and 3.78 $\log_{10}$ cfu/g.
[b] *Cladosporium* spp. (2.18–3.50 $\log_{10}$ cfu/g) were isolated from 100% of the samples.
[c] *Cladosporium* spp. (<2.0–2.84 $\log_{10}$ cfu/g) were found in 38% of the sample.

Table 2
Mould and yeast contamination of fruit juices

Product	Number of samples	MY $\log_{10}$ cfu/ml		Frequency (% contaminated samples)	Yeast species	Mould species
		Mean	Range			
Apple cider	5	2.0	<1.0–2.56	40	*Rhodotorula rubra*	
Apple juice	3	<1.0	—	0		
Carrot juice	6	4.99	<1.0–5.76	33	*Candida* sake	
Grape juice	5	<1.0	—	0		
Grapefruit juice	5	3.76	<1.0–4.36	40	*Candida lambica* *Kloeckera apis*	*Fusarium* spp.[a] *Geotrichum* spp[b] *Penicillium* spp.[a]
Orange juice	35	5.28	<1.0–6.83	23	*Candida lambica*	
Soy milk	6	<1.0	—	0		

MY: mould and yeast; cfu: colony forming units.
[a] *Fusarium* (<1.0–2.30 $\log_{10}$ cfu/ml) and *Penicillium* spp. (<1.0–2.40 $\log_{10}$ cfu/ml) were isolated from 20% of the samples.
[b] *Geotrichum* spp. (<1.0–2.95 $\log_{10}$ cfu/ml) were found in 40% of the samples.

mixed fruit salads (Table 1). The presence of *Cladosporium* in strawberry salads is very interesting since the major spoiling mould of this commodity is *Botrytis cinerea* which was not isolated from any of the samples analysed in this study. It is possible that *Cladosporium* spp. are always present on strawberries but they are overgrown by the fast growing *B. cinerea* when the latter is present. Pitt and Hocking (1999) also reported the spoilage of over-ripe or damaged strawberries by *Cladosporium*.

The results of fruit juice testing are summarized in Table 2. Overall, 22% of all samples were contaminated with fungi. The highest mean fungal contamination of 5.28 $\log_{10}$ cfu/ml was observed in orange juice whereas the lowest (<1.0 $\log_{10}$ cfu/ml) was found in apple and grape juice, and in soy milk (Table 2). The predominant organisms found in the various types of juice at levels ranging between <1.0 and 6.83 $\log_{10}$ cfu/ml were yeasts. The frequencies of yeast contamination within each type of juice were 40% for apple cider and grapefruit juice, 33% for carrot juice and 23% for orange juice (Table 2). Hatcher et al. (2000) and Parish and Higgins (1989) had also reported that yeasts were the major spoilers of fruit juice. Yeast numbers increased during refrigeration of the reserve samples, which indicated that these organisms could grow at temperatures around 4 °C and continue spoiling the products. Fermentation, gas production, increased turbidity of the products and swelling of the containers were also obvious in many cases (Tournas, 2002, unpublished data). Grinbaum et al. (1994) reported similar manifestations in fruit drinks spoiled by yeasts.

Yeast species commonly isolated from fruit juices *were R. rubra, C. lambica, C. sake and Kloeckera apis*, with *C. lambica* being the most frequently encountered organism in these products (Table 2). Other investigators have also reported the yeast spoilage of fruit juices. Mendoza et al. (1982) stated that *Rhodotorula, Pichia, Candida* and *Saccharomyces* were frequently isolated from pasteurized fruit juices. Beech and Davenport (1970) reported the isolation of three new *Candida* species (*C. anglica, C. cidri* and *C. pomicola*) from apple cider. *Candida* and *Saccharomyces* spp. have often been reported as spoilage-causing organisms in citrus juices (Parish and Higgins, 1989; Grawmlich et al., 1986; Hays, 1951; Teller and Parish, 1992).

Small numbers of *Penicillium* and *Fusarium* spp. were isolated from 20% whereas *Geotrichum* spp. was present in 40% of the grapefruit juice samples tested. All other products contained no moulds (Table 2). The fact that these organisms were present in very low numbers indicated that they were random contaminants not able to grow in the refrigerated juice. Mendoza et al. (1982) also reported the isolation of *Penicillium* and *Cladosporium* from pasteurized fruit juices.

In summary, fruit salads were contaminated with high levels (often higher than $6.0\,\log_{10}$ cfu/g) of yeasts. Since the normal yeast flora on fresh, undamaged fruits are generally low (less than $3.0\,\log_{10}$ cfu/g), this indicates that yeasts were probably added during preparation and/or could grow on the various salads during marketing. High yeast growth on these commodities reduces the organoleptic quality due to off-odors and discolorations of the products. Additionally, some yeast species and spoilage by-products may have an adverse health effect especially toward individuals with weakened immune systems. Adherence to GMPs during preparation and marketing would result in products with low yeast levels, thus of better quality. Mould growth was limited in fruit salads possibly due to faster growth of yeasts, which were able to take over before the filamentous fungi had a chance to germinate and establish colony development. Twenty-two per cent of the pasteurized fruit juice samples tested contained live fungi due to either inadequate pasteurization or due to post-pasteurization contamination during cooling, bulk storage and bottling. Yeasts were the most common organisms found in fruit juices. Some of the yeasts isolated from these products were capable of growing under refrigeration, completely spoiling the product before its expiration date. Therefore, juice processors and packers should take care to eliminate yeasts from juices and pack these products under strict aseptic conditions in order to avoid losses due to yeast spoilage, which results in products of poor or unacceptable quality.

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