Microbial Flora on Restaurant Beverage Lemon Slices

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Abstract

Restaurateurs often place a lemon slice on the rim of a beverage glass, or afloat in the beverage, as a flavor-enhancer or a decorative garnish. The handling of the lemons before their placement in the beverage may not follow sanitary procedures. The study reported here investigated whether beverage lemon slices contain microbial contamination that could be consumed by a restaurant patron.

Swabbed samples of the flesh and rind of lemon slices on the rims of beverage glasses were analyzed for microbial contents. Seventy-six lemons from 21 restaurants were sampled during 43 visits. Fifty-three (69.7 percent) of the lemon slices produced microbial growth. Twenty-three (30.3 percent) of the lemon slices produced no microbial growth. A total of 25 different microbial species were recovered from the samples.

Introduction

The antimicrobial properties of lemons are well documented. One study (Dabbah, Edwards, & Moats, 1970) demonstrated significant inhibition of bacterial growth in nutrient broth when lemon oil was added. Other studies report similar antimicrobial activity by lemons and lemon extract against numerous microbes, including *Candida albicans, Escherichia coli, Escherichia coli O157:H7, Helicobacter pylori, Klebsiella pneumoniae, Listeria innocua, Listeria monocytogenes, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Penicillium digitatum, Penicillium italicum, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Salmonella* spp., *Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus*, and *Streptococcus faecalis.* (Adeleye & Opiah, 2003; Belletti et al., 2004; Brock & Ketchum, 1951; Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998; Dabbah et al., 2002; Dada, Alade, Ahmad, & Yadock, 2002; Francis & O'Beime, 2002; Nogueira, Oyarzabal, & Gombas, 2003; Ohno et al., 2003; & Saleem, Afza, Anwar, Hai, & Ali, 2003). Lemon juice has even been shown to be useful as an anti-HIV agent when applied vaginally in sexually active women (Potts, Perlman, Mandara, Prata, & Campbell, 2004; Short, McCoombe, Maslin, & Crowe, 2004). Another study reported significant larvicidal activity by a fresh lemon peel extract (Salvatore, Borkosky, Willink, & Bardon, 2004). Many nonscientific Web sites proclaim the antimicrobial effects of lemons and lemon juice as a benefit in food preparation, sterilization of the rind of fruits and vegetables, sterilization of kitchen cutting boards, and as a sore-throat remedy (Boschen, n.d.; iVillage, 2002; Rall & Center for Unhindered Living, 2005; Podleski, 2006; Weiss, 2005). One site encourages restaurant patrons to squeeze lemon juice into drinking water, onto the hands, and all over the silverware in order to kill microbes (Tufarelli, n.d.).

Water containing lemon, however, was found to actually enhance the growth of *Pseudomonas aeruginos*a in one study (Ibrahim & Ogunmodede, 1991). Moreover, some lemon exporters spray the fruit with antimicrobial chemicals in order to kill *Vibrio cholerae*, *Penicillium digitatum*, *Botrytis cinerea*, and other microbes that may be contaminating the rind; this procedure indicates a lack of faith in the antimicrobial properties of lemon. (Cheah & Hunt, 1994; Cheah & Tran, 1995; de Castillo et al., 1998).

In restaurants, a lemon slice is commonly placed on the rim of a beverage glass, or afloat in the beverage, as a flavor enhancer or a decorative garnish. Although a patron might ask for this embellishment, frequently the lemon is added without the customer's request. Our study investigated whether these lemon slices contain microbial contamination that might be ingested by restaurant patrons.

Materials and Methods

Samples were collected surreptitiously, without the knowledge of the restaurant staff. Two StarPlex® brand specimen-collection swabs were used for each sample. Samples were taken as soon as the beverage was served, before a sip was taken, and before the lemon slice was touched by the patron. One swab was rubbed along the rind. The second swab was rubbed along the flesh of the fruit. A total of 76 lemons from 21 restaurants were sampled during 43 visits. Water and soda were the only beverages used in the study.

TABLE 1

Positive Culture Results*

Sample**	Site	Culture Results	Sample**	Site	Culture Results	Sample**	Site	Culture Results
1	Rind	*A. baumanii, C. guilliermondii*	19	Rind	*C. krusei*	37	Rind	*S. viridans*
	Flesh	*E. cloacae, E. sakazakii, S. epidermidis, S. viridans*		Flesh	*C. tropicalis*		Flesh	*A. baumanii*
2	Rind	No growth	20	Rind	*C. lusitaniae*	38	Rind	No growth
	Flesh	*C. lusitaniae*		Flesh	*C. lusitaniae*		Flesh	*B. subtilis*
3	Rind	No growth	21	Rind	*C. guilliermondii*	39	Rind	No growth
	Flesh	*C. lusitaniae*		Flesh	*S. epidermidis*		Flesh	*B. subtilis*
4	Rind	No growth	22	Rind	*Bacillus spp.*	40	Rind	*S. epidermidis*
	Flesh	*C. lusitaniae*		Flesh	*C. parapsilosis*		Flesh	No growth
5	Rind	*A. baumanii, S. epidermidis*	23	Rind	*C. parapsilosis*	41	Rind	No growth
	Flesh	*A. baumanii*		Flesh	*C. parapsilosis*		Flesh	*Micrococcus* spp.
6	Rind	*A. baumanii*	24	Rind	*Enterococcus* spp., *S. epidermidis*	42	Rind	*E. coli*
	Flesh	*A. baumanii, S. epidermidis, Corynebacterium* spp.		Flesh	*C. guilliermondii*		Flesh	*E. coli*
7	Rind	No growth	25	Rind	*S. epidermidis, C. parapsilosis*	43	Rind	*E. coli, P. mirabilis*
	Flesh	*B. subtilis*		Flesh	*C. parapsilosis*		Flesh	*E. coli*
8	Rind	*S. epidermidis, S. viridans*	26	Rind	No growth	44	Rind	*S. epidermidis, Bacillus* spp., *Enterococcus* spp.
	Flesh	No growth		Flesh	*S. marcescens*		Flesh	*S. epidermidis, Bacillus* spp., *Enterococcus* spp.
9	Rind	*B. subtilis*	27	Rind	*C. guilliermondii*	45	Rind	*Bacillus* spp.
	Flesh	No growth		Flesh	*C. guilliermondii*		Flesh	No growth
10	Rind	*C. parapsilosis*	28	Rind	*A. baumanii, C. parapsilosis*	46	Rind	*Bacillus* spp.
	Flesh	No growth		Flesh	No growth		Flesh	No growth
11	Rind	*C. tropicalis*	29	Rind	*C. guilliermondii*	47	Rind	*E. coli*
	Flesh	*C. krusei*		Flesh	*E. cloacae*		Flesh	*E. coli*
12	Rind	*T. glabrata*	30	Rind	*K. oxytoca*	48	Rind	*E. coli*
	Flesh	*C. tropicalis*		Flesh	*C. guilliermondii, T. asahii*		Flesh	No growth
13	Rind	*C. tropicalis, Bacillus* spp.	31	Rind	No growth	49	Rind	*Bacillus* spp.
	Flesh	*C. tropicalis*		Flesh	*B. subtilis*		Flesh	No growth
14	Rind	*C. tropicalis*	32	Rind	*B. cereus*	50	Rind	*Bacillus* spp.
	Flesh	*T. glabrata, C. krusei*		Flesh	No growth		Flesh	*Bacillus* spp.
15	Rind	*C. albicans, Bacillus* spp.	33	Rind	*P. fluorescens, P. putida*	51	Rind	*S. viridans*
	Flesh	No growth		Flesh	No growth		Flesh	*C. tropicalis*
16	Rind	*B. subtilis*	34	Rind	*C. krusei*	52	Rind	No growth
	Flesh	*B. subtilis*		Flesh	*C. guilliermondii*		Flesh	*Enterococcus* spp.
17	Rind	*C. guilliermondii*	35	Rind	*C. krusei*	53	Rind	*Enterococcus* spp.
	Flesh	*C. guilliermondii*		Flesh	*C. guilliermondii*		Flesh	No growth
18	Rind	*B. subtilis*	36	Rind	No growth			
	Flesh	*C. guilliermondii*		Flesh	*B. subtilis*			

* 53 of the 76 lemon samples produced some microbial growth on the rind, the flesh, or both.
** Shading denotes one restaurant visit.

Each swab was cultured onto a TSA-II 5 percent sheep's blood agar plate and a MacConkey II agar plate. Plates were incubated at 35ºC in a CO_2-enriched aerobic atmosphere. Since samples were taken from the surfaces of the lemon slices, anaerobe recovery was not attempted. Culture plates were examined for growth at 24 hours, reincubated, and examined again after 48 hours. Isolates were identified by Gram stain, colony characteristics, API 20C Aux® for yeast, API 20E® for Enterobacteriaceae, PYR Test Kit for *Enterococcus*, H_2O_2 for catalase, and rabbit plasma for coagulase. Isolates were not quantified.

Results

Culture results are found in Table 1, Table 2, Table 3, and Table 4. Twenty-three (30.3 percent) of the lemon slices produced no microbial growth from the rind or the flesh. A total of 25 different microorganisms were recovered, including bacteria and yeasts. Fifty-three (69.7 percent) of the lemon slices produced some microbial growth, either from the rind, the flesh, or both (Table 1). Thirteen (17.1 percent) of the lemon slices had microbes only on the rind; this number represented 24.5 percent of the lemon slices that produced microbial growth (Table 2). Eleven (14.5 percent) of the lemon slices had microbes only on the flesh; this number represented 20.8 percent of the lemon slices that produced microbial growth (Table 3). Twenty-nine (38.2 percent) of the lemon slices had microbes on both the flesh and the rind; this represented 54.7 percent of those lemon slices that produced microbial growth (Table 4). Of the 29 samples that had microbial growth on the flesh and the rind, 9 (31 percent) had exactly the same microorganism or microorganisms on both locations, whereas 20 (69 percent) had some differences in the microorganisms that were recovered from the rind and the flesh (Table 3). In 15 instances (19.7 percent), the microorganisms recovered from the rind were completely different from those that were recovered from the flesh; this situation occurred in 51.7 percent of the 29 slices that produced microbial growth from both the flesh and the rind (Table 3). Six of the lemon slices (7.9 percent) produced three or more species; this number represented 11.3 percent of the lemon slices that produced microbial growth (Table 3).

Discussion

Possible Origins of the Microbial Contaminants

It is not possible to definitively identify the origins of the microorganisms. While the Enterobacteriaceae and nonfermentative Gram-negative bacilli could have come from the fingertips of a restaurant employee via human fecal or raw-meat or poultry contamination, they might have contaminated the lemons before they even arrived at the restaurant. The Gram-positive cocci and *Corynebacterium* isolates may have been introduced onto the lemons from the skin or oral flora of anyone who handled them, before or after they arrived in the restaurant. The *Bacillus* species are ubiquitous and could have had numerous sources, including airborne spores landing on the fruit or on the knife used to cut the lemon.

There are three possible origins for the various yeasts that were isolated. Some yeasts commonly colonize lemons and other foods, and

TABLE 2

Culture Results from Samples with Growth on the Rind*

Sample**	Site	Culture Results
1	Rind	*S. epidermidis, S. viridans*
	Flesh	No growth
2	Rind	*B. subtilis*
	Flesh	No growth
3	Rind	*C. parapsilosis*
	Flesh	No growth
4	Rind	*C. albicans, Bacillus spp.*
	Flesh	No growth
5	Rind	*A. baumanii, C. parapsilosis*
	Flesh	No growth
6	Rind	*B. cereus*
	Flesh	No growth
7	Rind	*P. fluorescens, P. putida*
	Flesh	No growth
8	Rind	*S. epidermidis*
	Flesh	No growth
9	Rind	*Bacillus spp.*
	Flesh	No growth
10	Rind	*Bacillus spp.*
	Flesh	No growth
11	Rind	*E. coli*
	Flesh	No growth
12	Rind	*Bacillus spp.*
	Flesh	No growth
13	Rind	*Enterococcus spp.*
	Flesh	No growth

* 13 samples produced microbial growth only on the rind.
** Shading denotes one restaurant visit.

TABLE 3

Culture Results from Samples with Growth Only on the Flesh*

Sample**	Site	Culture Results
1	Rind	No growth
	Flesh	*C. lusitaniae*
2	Rind	No growth
	Flesh	*C. lusitaniae*
3	Rind	No growth
	Flesh	*C. lusitaniae*
4	Rind	No growth
	Flesh	*B. subtilis*
5	Rind	No growth
	Flesh	*S. marcescens*
6	Rind	No growth
	Flesh	*B. subtilis*
7	Rind	No growth
	Flesh	*B. subtilis*
8	Rind	No growth
	Flesh	*B. subtilis*
9	Rind	No growth
	Flesh	*B. subtilis*
10	Rind	No growth
	Flesh	*Micrococcus spp.*
11	Rind	No growth
	Flesh	*Enterococcus spp.*

* 11 samples produced microbial growth only on the flesh.
** Shading denotes one restaurant visit.

TABLE 4

Culture Results from Samples with Growth on the Flesh and the Rind*

Sample**	Site	Culture Results	Sample**	Site	Culture Results
1	Rind	*A. baumanii, C. guilliermondii*	16	Rind	*Enterococcus* spp., *S. epidermidis*
	Flesh	*E. cloacae, E. sakazakii, S. epidermidis, S. viridans*		Flesh	*C. guilliermondii*
2	Rind	*A. baumanii, S. epidermidis*	17	Rind	*S. epidermidis, C. parapsilosis*
	Flesh	*A. baumanii*		Flesh	*C. parapsilosis*
3	Rind	*A. baumanii*	18	Rind	*C. guilliermondii*
	Flesh	*A. baumanii, S. epidermidis, Corynebacterium spp.*		Flesh	*C. guilliermondii*
4	Rind	*C. tropicalis*	19	Rind	*C. guilliermondii*
	Flesh	*C. krusei*		Flesh	*E. cloacae*
5	Rind	*T. glabrata*	20	Rind	*K. oxytoca*
	Flesh	*C. tropicalis*		Flesh	*C. guilliermondii, T. asahii*
6	Rind	*C. tropicalis, Bacillus* spp.	21	Rind	*C. krusei*
	Flesh	*C. tropicalis*		Flesh	*C. guilliermondii*
7	Rind	*C. tropicalis*	22	Rind	*C. krusei*
	Flesh	*T. glabrata, C. krusei*		Flesh	*C. guilliermondii*
8	Rind	*B. subtilis*	23	Rind	*S. viridans*
	Flesh	*B. subtilis*		Flesh	*A. baumanii*
9	Rind	*C. guilliermondii*	24	Rind	*E. coli*
	Flesh	*C. guilliermondii*		Flesh	*E. coli*
10	Rind	*B. subtilis*	25	Rind	*E. coli, P.mirabilis*
	Flesh	*C. guilliermondii*		Flesh	*E. coli*
11	Rind	*C. krusei*	26	Rind	*S. epidermidis, Bacillus* spp., *Enterococcus* spp.
	Flesh	*C. tropicalis*		Flesh	*S. epidermidis, Bacillus* spp., *Enterococcus* spp.
12	Rind	*C. lusitaniae*	27	Rind	*E. coli*
	Flesh	*C. lusitaniae*		Flesh	*E. coli*
13	Rind	*C. guilliermondii*	28	Rind	*Bacillus* spp.
	Flesh	*S. epidermidis*		Flesh	*Bacillus* spp.
14	Rind	*Bacillus* spp.	29	Rind	*S. viridans*
	Flesh	*C. parapsilosis*		Flesh	*C. tropicalis*
15	Rind	*C. parapsilosis*			
	Flesh	*C. parapsilosis*			

* 29 samples produced microbial growth on both the flesh and the rind.
** Shading denotes one restaurant visit.

are classified by the food industry as "food spoilage yeasts" (Adegoke, Iwahashi, Komatsu, Obuchi, & Iwahashi, 2000). Some distributors add yeasts to lemons and other fruits in order to retard the growth of other, destructive fungi (Cheah et al., 1994; Cheah et al., 1995; Droby, Chalutz, & Wilson, 1991). Finally, the yeasts could have originated from oral, fecal, or vaginal secretions contaminating the fingertips of a restaurant employee or another food handler.

Diseases Caused by the Microbes Found on the Lemon Samples

The microbes found on the lemon samples in our investigation all have the potential to cause infectious diseases at various body sites, although the likelihood was not determined in this study. An extensive search of the literature yielded no reported outbreaks or illnesses attributed to lemon slices in beverages. Establishment of an infection would depend upon the number of microbes introduced; this investigation did not include a quantitative determination of the numbers of microorganisms on the lemons. Other factors that would contribute to the establishment of an infection would include whether the organisms were resistant to multiple antibiotics, the general health and age of the individual, the status of the immune system, and the integrity of the mucous membranes of the lips and mouth.

Conclusion

Although lemons have known antimicrobial properties, the results of our study indicate that a wide variety of microorganisms may survive on the flesh and the rind of a sliced lemon. Restaurant patrons should be aware that lemon slices added to beverages may include potentially pathogenic microbes. Further investigations could determine the source of these microorganisms, establish the actual threat (if any) posed by their presence on the rim of a beverage, and develop possible means for preventing the contamination of the lemons. It could also be worthwhile to study contamination on other beverage garnishes, such as olives, limes, celery, and cherries, and to investigate whether alcoholic beverages have an effect not seen with water and soda.

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