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A Research Note

Microbiological Evaluation of Vegetable Sprouts and Seeds

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ABSTRACT

Microbiological analyses of commercial sprout seeds indicated aerobic plate counts (APC) and confirmed coliforms ranging from < 30 x 10^2 to 40 x 10^5 and from 0 to > 11 x 10^3/g, respectively. Fecal coliforms were detected in the range from 7.3 to 11 x 10^2/g; *Listeria* or *Salmonella* were not detected. Seeds, pregerminated in potable water (16-18 h, 20-22°C), contained an APC ca. 1 logarithm higher than the corresponding dry seeds. Increased levels of confirmed and fecal coliforms were also detected as a result of soaking. The APC of retail sprouts ranged from 3.6 x 10^3 to 3.7 x 10^9/g. All samples contained confirmed and fecal coliforms. Coagulase positive staphylococci were detected in ca. 24% of samples analyzed.

Alfalfa and bean sprouts have been reported to harbor relatively high levels of microorganisms. For example, Patterson and Woodburn *(4)* reported total aerobic and coliform counts of 10^8 and 10^6 cells/g, respectively. Splittstoesser et al. (*5*) reported similar findings with mung bean sprouts and indicated that growth of the bean microflora during germination and not contamination contributed to the high microbial load. The presence of pathogens in sprouts, although infrequent (*1,5*), has nevertheless contributed to at least one outbreak of food poisoning (*2*). Since sprouts are normally consumed with no or minimal heat treatment, a potential exists for additional outbreaks of food poisoning, especially as the popularity of these products increase.

The present study was conducted to assess the microbial quality of locally produced sprouts and to substantiate results of earlier reports. The microflora of sprout seeds was also evaluated in order to determine the presence of potentially hazardous microorganisms which could eventually contaminate the final product.

MATERIALS AND METHODS

Sprouts produced by a major processor were obtained from three retail outlets. The varieties sampled at each outlet consisted of: alfalfa sprouts, onion sprouts, and a sprout mixture composed of alfalfa, radish, clover, mustard and lentil. Samples were transported on ice and analyzed within 2 h; all sprouts were packaged in rigid, enclosed plastic containers. Each store was sampled three times over a 3-month period.

Samples (25-g) were blended in peptone water (0.1%, 225 ml) for 1 min at high speed. Aerobic plate counts (APC; 32°C, 24-48 h) were performed using plate count agar (Difco, Detroit, MI). Total coliforms were determined by the most probable number procedure using lauryl sulfate tryptose broth (Difco) followed by confirmation of gas positive tubes using brilliant green lactose bile broth (Difco). Incubation in both cases was at 35-37°C for 24-48 h. Fecal coliforms were determined using EC broth (Difco; 45.5°C, 24-48 h). *Staphylococcus aureus* was determined by surface spreading homogenate dilutions (10^1 and 10^2, 0.1 ml) on Baird-Parker medium (Difco; 37°C, 30 and 48 h). Typical colonies were subjected to a coagulase test.

The microbiological analysis of commercial sprout seeds was performed in cooperation with a local processor. Four seed types, consisting of two alfalfa (alfalfa-1, alfalfa-2), one onion, and a mixture composed of alfalfa, radish, clover, mustard and lentil seeds, were analyzed for total and fecal coliforms, aerobic plate counts, and *S. aureus* as previously outlined. *Salmonella* was analyzed by inoculating seed (50 g) in lactose broth (Difco, 450 ml) at 37°C for 24 h. Portions of broth (1.0-ml) were transferred to tubes containing selenite-cystine and tetrathionate brilliant green broth (Difco, 100 ml). Following incubation (37°C, 24 h), portions were streaked onto brilliant green sulfadiazine agar (Difco) and incubated (37°C, 24 h). Suspect *Salmonella* colonies were used to inoculate lysine iron agar and triple sugar iron agar slants (Difco). Following incubation (37°C, 24 h), suspect colonies giving a typical *Salmonella* profile were tested by slide agglutination with commercial O antiserum (Difco). For the presence of *Listeria*, seed samples (25 g) were added to *Listeria* enrichment broth (LEB, Difco, 225 ml) and incubated at 30°C for 24 h. A 0.1-ml portion from the primary enrichment was transferred to a secondary enrichment (LEB) and incubated at 4°C for 28 d. Following secondary enrichment, 0.1-ml portions were surface plated onto modified McBride *Listeria* agar (MMLA) and lithium chloride phenyl ethanol moxalactum agar (LPM) and incubated at 35°C for 48 h in an atmosphere containing 10% $C0_2$. The MMLA consisted of (g/L): McBride *Listeria* agar (Difco) 46.0; lithium chloride (Sigma Chemical Co., St. Louis, MO) 4.6; cycloheximide (Sigma) 0.2. The LPM agar consisted of (g/L): phenylethanol agar (Difco) 35.5; glycine anhydride (Sigma) 10; lithium chloride, 5.0; moxalactum (Sigma) 0.020. Culture plates were examined for the presence of typical blue *Listeria* colonies using Henry's oblique light system. Suspect colonies were subjected to a Gram

TABLE 1. *Microbiological profile of dry and water soaked sprout seeds and corresponding filtrates.*

Seed[a] type	Sample	APC (g or ml)	Confirmed coliform (g or ml)	Fecal coliform (g or ml)	*S. aureus*[b]
A-1	Dry	31 x 10^4 - 40 x 10^5[c]	>1,100	0	1
	Soaked	>30 x 10^4 - 12 x 10^6[d]	>11,000	0	0
	Filtrate	>30 x 10^5 - 79 x 10^6	>11,000	0	1
A-2	Dry	<30 x 10^2 - 54 x 10^2[c]	0-23	0	0
	Soaked	<30 x 10^2	0-23	0	0
	Filtrate	<30 x 10^2 - 11 x 10^3	0-23	0	0
M	Dry	33 x 10^2 - 32 x 10^3	0	0	1
	Soaked	42 x 10^2 - 15 x 10^4	0-240	0	1
	Filtrate	11 x 10^3 - 15 x 10^4	0-240	0	0
O	Dry	35 x 10^4 - 41 x 10^4	240-1,100	7.3 - 1,000	0
	Soaked	51 x 10^5 - 15 x 10^6	>11,100	21 - >1,100	0
	Filtrate	90 x 10^4 - 12 x 10^5	>11,100	21 - >1,100	0

[a]Commercial seed varieties: (A-1) alfalfa-1, (A-2) alfalfa-2, (M) mixture, (o) onion.
[b]Number of samples, out of four, yielding coagulase positive *Staphylococcus*.
[c]Range of four samples.
[d]Highest dilution analyzed.
[e]Lowest dilution analyzed.

TABLE 2. *Microbial flora associated with vegetable sprouts at the retail level.*

Retail[a] outlet	Sprout[b] type	APC (x 10^7/g)	Confirmed coliform (g)	Fecal coliform (g)	*S. aureus*[c]
1	A-1	64 - 68[d]	>11,000[e]	1,100 - >1,100	1
	M	230 - 300	>11,000	240 - 1,100	1
	O	>300 - 378	>11,000	>1,100	1
2	A-1	80-120	>11,000	>1,100	2
	M	84 - 209	>11,000	240 -->1,100	1
	O	86 - 161	>11,000	>1,100	2
3	A-1	27 - 225	>11,000	3.6 ->1,100	1
	M	52 - 300	>11,000	3.6 ->1,100	3
	O	36 - 95	>11,000	3.6 - 240	1

[a]Sprouts maintained on crushed ice in outlets 1 and 2 and on a refrigerated shelf in outlet 3.
[b]Alfalfa (A-1), mixture (M), onion (O).
[c]Number of samples, out of six, yielding coagulase positive *Staphylococcus*.
[d]Range of six samples.
[e]Highest dilution tested.

stain and catalase test. Isolates were tested for motility by stabbing a tube containing semisolid motility media (Difco) which was incubated at room temperature for 3 d.

The influence of pregermination on the microbial load of sprout seeds was evaluated by immersing seeds (130 g) in tap water (500 ml, 20-22°C) for 16-18 h. The seed water ratios reflect those used in commercial practice. Following filtration (Whatman No. 4), both the soaked seeds and the filtrates were evaluated for the presence of APCs, total and fecal coliforms, and coagulase positive *S. aureus* as previously outlined.

RESULTS AND DISCUSSION

The APC of the dry sprout seeds ranged from < 30 x 10^2 to 40 x 10^5/g (Table 1). Confirmed coliforms ranged from zero to > 11,000/g; fecal coliforms, detected only with the onion seeds, ranged from 7.3 to 1,100/g. Coagulase positive staphylococci were detected in alfalfa-1 and mixed sprout seeds; approximately 12.5% of the dry seed samples examined contained coagulase positive staphylococci. No *Listeria* or *Salmonella* was detected from any of the seed samples.

The APC of the soaked seeds was either similar to or one logarithm higher than the APC for the corresponding dry seed. In the case of the onion seeds, however, the APC was ca. 1-2 logarithms higher following soaking. Seed soaking also appeared to result in an increase in both confirmed and fecal coliforms. In the case of alfalfa-1 and onion seeds, the levels of confirmed coliforms increased by more than 10-fold, during soaking. In only one sample, mixed sprout seeds, were coagulase positive staphylococci detected. Since *Listeria* and *Salmonella* were not detected from the dry seed samples, their analyses on the corre-

sponding soaked seeds and filtrates were not performed. Analysis of the seed filtrates indicated microbial levels for APC, confirmed and fecal coliforms, to be similar to those levels of the corresponding soaked seeds. Only one filtrate sample, alfalfa-1, contained coagulase positive staphylococci.

With regard to the initial microflora of sprout seeds, Splittstoesser et al. (*5*) reported that mung beans also contained a varied APC population (10^5-10^7/g) which subsequently increased to ca. 10^8/g following 2 d of germination. Initial presumptive coliforms for many seeds, reported by these authors, ranged from ca. 10^2 to 10^5/g. The absence of *Salmonella* or *Listeria* isolates from seeds in the present study is in general agreement with previous studies which reported few pathogens (*4,5*). The increase in microbial levels associated with seed soaking undoubtedly contributes to the high microbial counts associated with the final product. Since alfalfa seeds contain sufficient nutrients (*3*) to support microbial growth, nutrient leaching could foster growth both in the soak water and on the seed surface.

Microbiological evaluation of vegetable sprouts at the retail level indicated APC levels from 10^7 to 10^9/g and confirmed coliforms from 1,100 to >11,000/g (Table 2). Fecal coliforms ranged from 0 to >1,100/g. Coagulase positive staphylococci were detected in 13 of 54 samples (ca. 24%) analyzed. Although the APC and coliform counts for the retails sprouts were similar to those previously reported by Patterson and Woodburn (*4*), the incidence of coagulase positive staphylococci appears higher than that reported by Splittstoesser et al. (*5*). The sanitary quality of the seeds used for sprouting would undoubtedly exert some influence in this area. In addition, the time-temperature regimes used for soaking and germination of seeds could allow for extensive cross-contamination.

Overall, critical control points such as heat or refrigeration are generally lacking in the processing of vegetable sprouts. The microbiology of the raw materials used viz. sprout seeds should therefore be of special concern and be evaluated as a major quality control factor.

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