Sensory Attributes and Safety Aspects of Germinated Small-Seeded Soybeans and Mungbeans

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ABSTRACT

Sprouts of two small-seeded soybean strains were characterized as more intense in nutty aroma and flavor and less intense in bitter, grassy and beany flavor notes than mungbean sprouts or market samples of soybean sprouts. Sprouts did not differ in sweetness. The sprouts of the two test soybeans were moderately tender and crisp but less tender than mungbean sprouts. Yeasts and fungi were found on market samples of mungbean and soybean sprouts. Fungi were also present on sprouts of one test soybean. No aflatoxins (B1, G1, G2, B2a) were identified by thin layer or high performance liquid chromatography, and no other toxins were indicated by chicken embryo bioassay.

Utilization of soybeans in the germinated form has been limited in the United States, but small-seeded legume and cereal grain sprouts are increasing in popularity. Germination modifies the flavor and nutritive value and improves the digestibility of the seeds. The flatus-causing factors, stachyose and raffinose, and the antinutrients, phytic acid and trypsin inhibitors, are reduced by germination of soybeans (1).

Little information has been published on the sensory attributes of soybean sprouts. Adjei-Twum (2) found that 3-d-old soybean sprouts were most acceptable, whereas 6-d-old sprouts were least acceptable. Further, there was no significant difference in acceptability or flavor between 5-d-old mungbean and 3-d-old soybean sprouts. Adjei-Twum (2) suggested 3-d-old soybean sprouts be used when the priority is on flavor and 6-d-old soybean sprouts when the emphasis is on digestibility. Kylen and McCreary (8) reported there was no difference in degree of acceptability between mungbean and soybean sprouts germinated for 3 d.

Small-seeded soybeans are being introduced in the United States. In the sprouted form, these may be more competitive with mungbeans than the currently available large-seeded soybeans. Sprouted mungbeans have long been used as a salad ingredient and are becoming increasingly important as a component for entrees and vegetable dishes. Therefore, this project was designed to evaluate the sensory properties of two germinated small-seeded soybean strains and mungbeans. Since conditions of germination and marketing are favorable for fungal growth, there is a potential for mycotoxin formation. Therefore, the safety of these sprouts as food was also assessed.

MATERIALS AND METHODS

Two small-seeded soybean strains, SP 75051 and Jaerejong 320-7, were provided by Dr. Harry Minor, Department of Agronomy, University of Missouri-Columbia. Mungbean seeds were purchased from a local market catering to an oriental clientele (Park Oriental Store, Columbia, MO). Damaged seeds and foreign material were separated, and the seeds were stored in a tightly sealed container at room temperature until needed for sprouting (max. 6 months). For sensory evaluation, market samples of soybean and mungbean sprouts were included along with the two test soybeans and mungbeans germinated under controlled conditions.

Germination procedure

Preliminary investigation of several pretreatments and sprouting procedures revealed that surface treatment with 1.6% sodium hypochlorite for 2 min was an effective method for controlling mold growth without inhibiting germination of soybeans. Following the surface treatment, the seeds were rinsed and soaked in tap water for 8 h. For germination, the seeds were placed in a beaker covered with cheesecloth. The beaker was tilted over a container of water so that the cheesecloth acted as a wick. Three days of germination (28°C) resulted in optimum sprout length without root formation. The germination process was the same for soybeans and mungbeans except that surface-treatment was unnecessary for mungbeans.

Microbiological studies

Plate counts of aerobes, coliforms, yeasts and fungi were made in duplicate (11). Five replications were completed. For all microbial analyses, samples were blended (2 min) in Butterfield’s buffered phosphate diluent, and serial dilutions were made in this same sterile buffer. Plate count agar (Difco) was used for aerobic plate counts, and plates were incubated at 37°C for 48 h. Coliforms were determined with violet red bile agar (Difco) overlayed with the same medium. Incubation was the same as for aerobic plate counts. Pseudomonas red colonies surrounded by a reddish zone of precipitated bile were counted as coliforms. Yeasts and fungi were enumerated with potato dextrose agar (Difco) acidified with sterile 10% tartaric acid. Plates were incubated at 25°C for 5 to 7 d.

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Determination of aflatoxin B1 in seeds and sprouts

Extraction procedure. For each sample, 2 g of powdered seeds or sprouts were blended 4 min at low speed in a laboratory blender with 10 ml of acetonitrile. The suspensions were vacuum-filtered (Whatman No. 1 filter paper), and the filtrates were dried at 50°C with a stream of filtered air. Dried filtrates were dissolved in 3 ml of 30% methanol (vol/vol) and placed on a prewashed C18-reverse phase Sep-Pak cartridge (Waters Associates, Milford, MA). The cartridges were eluted with 0.5-ml portions of water to remove polar contaminants, and then with 2.5-ml portions of 40% (vol/vol) 2-propanol to remove aflatoxin. Methanol (1 ml) was added, and the mixture was passed through a 0.5-μm pore-size filter (Type FH, Millipore Corp., Bedford, MA).

Thin layer chromatography (TLC). The extracts (100 μl) were spotted on 0.25-mm thick, unactivated silica gel plates (Analtech, Newark, DE) along with 50 Tig samples of purified aflatoxin B1. The chromatograms were developed with chloroform:acetone:water, 88:12:1.5 (vol/vol), and aflatoxin B1 was detected as a blue fluorescent spot under long wavelength UV light. Those samples containing blue fluorescing components with Rf values similar to aflatoxin B1 were analyzed by high performance liquid chromatography (HPLC).

Determination of aflatoxin B1 with high performance liquid chromatography (HPLC). The plant extracts were fractionated by HPLC by isocratic elution from a C18-reverse phase μ-Bondapak column (10-μm particle size; Waters Associates, Milford, MA) with 35% (vol/vol) acetonitrile in water (flow rate 1.2 ml/min). Aflatoxin B1 quantitation was based on the height of the peak obtained with Waters model 440 absorbance detector operated at 365 nm. Using the extraction procedure described above the practical detection limit of the system was 10 ng of aflatoxin B1 per g in the original sample.

As a control, purified aflatoxin B1 (Applied Science, State College, PA) was added to 50 samples of powdered soybean seeds at levels of 1000 or 100 ng/g. These samples were extracted and fractionated as described above, and aflatoxin B1 recoveries were measured by HPLC. The procedure gave a 95 ± 5% recovery of added aflatoxin.

Chicken embryo bioassay

For the chicken embryo bioassay (3), sprouts were dried (60°C, 24 h), ground, and a composite of the five replicate lots was made. Fertile chicken eggs were obtained from the Poultry Science Department of the University of Missouri-Columbia. The eggs were incubated at 37 to 38°C, 65% relative humidity, and manually turned every 8 h. After 7 d, the eggs were candled. The eggs with healthy embryos were surface-treated with iodine, and a hole (0.5 mm) was made with a sterile needle in the area of the air cell. Fifteen eggs were selected at random from each of the following treatments: (a) oil extract, (b) water extract, (c) sterile distilled water control, (d) sterile oil control and (e) uninoculated egg control. The procedures for preparation of oil and water extracts were those outlined by Zamora (4). The extract (0.1 ml) was injected into the eggs, and the holes were sealed with melted wax. The inoculated eggs were left undisturbed in a vertical position (air cell up) for approximately 1 h to allow the extract to disperse. Inoculated eggs were reincubated at 37 to 38°C for 4 d (4), after which the condition of the embryo was examined.

Sensory evaluation

Two types of sensory tests were done. The first was an analytical test designed to determine the intensity of the sensory attributes associated with bean sprouts. The second test was a modified consumer study using a hedonic rating to provide information pertaining to how well the sprouts were liked.

Facilities. Sensory tests were conducted in an air-conditioned facility equipped with eight individual booths. Each booth was illuminated with a red incandescent light to minimize the influence of color on the samples.

Analytical panel composition and orientation. The panel consisted of eight University students with previous experience in sensory evaluation. One week before the collection of data, four consecutive orientation sessions were conducted. For the first and second sessions, tasting and discussion of the characteristics of the sprouts were conducted informally. Agreement was reached among panelists on beany, grassy and nutty flavors and aromas. Also, five triangle tests were performed to determine if panelists were able to detect differences among sprouts. For these tests, the sprouts were ground sufficiently to eliminate differences in appearance. The five triangles represented various, but not all possible, combinations of samples. In addition to practice with sprouts, panelists were requested to identify and differentiate among various intensities of the primary tastes in aqueous solutions.

Two days before the collection of sensory data, two practice sessions simulating actual panels were conducted. The score sheet provided a 5-point scale with 1 denoting not perceived, and 5, indicating very intense for the attributes of aroma (beany, nutty), flavor (sweet, bitter, nutty, grassy, beany) and texture (crisp, tender).

Preparation and serving of the samples for the analytical panel. For each judge, approximately 5 g of sprouts were placed in a 2-oz plastic cup, bearing a 3-digit random code. Cups were immediately covered, and the samples were served one at a time to each panelist. The order of serving for each treatment was randomized. A glass of tap water at room temperature was provided for each judge to cleanse their palate before tasting each sample. Five replications were completed.

Consumer panels. Two modified consumer panels (N=50) were conducted. The first was comprised mainly of students in the College of Agriculture. The second involved students from diverse areas. Both panels were conducted in the facilities described above for the analytical panel. The sprouts were scored on a 5-point hedonic scale.

Statistical analysis

The data from sensory evaluation were subjected to analysis of variance (9). When significant differences occurred among means, Duncan's (6) new multiple range test was used to locate the differences. For the hedonic rating, the data from the two sessions (N=50) were pooled, since a t-test of the data from the two sessions showed no significant (P<0.05) difference. The pooled data (N=108) were analyzed as described above. The differences cited herein were significant at P<0.05. No statistical analyses were computed on data from microbiological assays or aflatoxin determinations.

RESULTS AND DISCUSSION

The soybean seeds were approximately 50% larger than the mungbean seeds but were substantially smaller than most soybeans which average 12 to 18 g per 100 soybeans. The mean weight of 100 seeds of Jaeraejong 320-7, SP 75051 and mungbeans were 9.1, 7.7 and 6.0 g, respectively.

Microbiological assays

The total plate, coliform, yeast and fungi counts for the five sprouts are summarized in Table 1. Soybean SP 75051 strain contained the lowest total plate count (log10 CFU/g).

| TABLE 1. Aerobic, coliform, yeasts and fungi counts in soybean and mungbean sprouts*. |
|---------------------------------|------------|--------|--------|--------|
| Type of sprout                  | Aerobic    | Coliform| Yeasts | Fungi  |
| Soybean                         |            |        |        |        |
| Jaeraejong 320-7                | 6.40       | 3.93   | none   | 0.30   |
| SP 75051                        | 5.32       | 3.11   | none   | 1.20   |
| Market sample                   | 6.43       | 3.91   | 5.00   | 1.20   |
| Mungbean                        |            |        |        |        |
| Sprouted under controlled       | 6.41       | 3.80   | none   | none   |
| conditions                      | 6.15       | 3.51   | 3.95   | 1.36   |

*Analyses in duplicate for five replications.
5.32 CFU/g) compared to the other four sprouts. Similarly, the coliform count (log_{10} 3.11 CFU/g) was lowest for the SP 75051 strain, and the highest plate count (log_{10} 3.91 CFU/g) was found in the market sample of soybean sprouts. Total plate counts for the other three sprouts, Jaeraejong 320-7, the market sample of mungbean sprouts and mungbean sprouts germinated under controlled conditions, were log_{10} 3.93, log_{10} 3.51 and log_{10} 3.80 CFU/g, respectively.

The market samples of both mungbean and soybean sprouts showed the presence of yeasts and fungi. A low level of fungi was also observed on sprouts of Jaeraejong 320-7 sprouts (Table 1).

**Determination of aflatoxin B<sub>1</sub>**

The presence of fungi on some of the sprouts (Table 1) and the fact that soybeans are known to support high levels of *Aspergillus flavus* growth and high rates of aflatoxin B<sub>1</sub> production (10) led to the search for aflatoxin B<sub>1</sub>. Since fluorescence was observed in the thin layer chromatogram of the extract from the market sample of mungbean sprouts at about the same R<sub>f</sub> value of the standard aflatoxin B<sub>1</sub>, this extract was injected into HPLC. However, there was no peak formed at the same retention time as the standard for aflatoxin B<sub>1</sub>. In addition, the samples did not contain compounds with the retention times of aflatoxin B<sub>2g</sub>, G<sub>1</sub>, G<sub>2</sub> or B<sub>2a</sub>. Therefore, it was concluded that no aflatoxin was present in any of the seeds or sprouts tested.

**Chicken embryo study**

Although TLC and HPLC indicated that no aflatoxin B<sub>1</sub> was present in any of the extracts sprouts included in this study, the absence of other toxic materials was assured by the chicken embryo bioassay. Since the mortality rate among treatments was similar, it can be postulated that there were no detectable toxic substances present in the soybean and mungbean sprouts used in this study. Non-surviving embryos for all treatments ranged from 0 to 2.

Sensitivity of chicken embryos to aflatoxin B<sub>1</sub> and G<sub>1</sub> and to extracts of aflatoxin-producing mold cultures was reported by Verret et al. (12) and confirmed by Dey (5), Wang (13) and Kazanas (7). Kazanas (7) pointed out that chicken embryos were not only susceptible to aflatoxin but also to other mycotoxins such as zearalenone, ochratoxin A and ‘zearalenone-like’ substances found in fermented sorghum meal.

**Sensory attributes of sprouts**

The two test soybean strains sprouted under controlled conditions were characterized by significantly more intense nutty aroma and flavor notes than the other sprouts. Also, bitter, grassy and beany flavor notes were significantly less apparent in the sprouts of the two soybean strains than in mungbean and the market samples of soybean. Sweetness did not differ among the five types of sprouts. The soybean sprouts were moderately tender and crisp, but less tender than mungbean sprouts (Table 2).

**Hedonic ratings for sprouts.** The mean hedonic scores for Jaeraejong 320-7 (3.5) and for SP 75051 (3.5) soybean sprouts were significantly higher than those for the other three samples. The hedonic scores also indicated that there was no significant difference among mungbean sprouts (2.9, 2.8) and the market samples of soybean sprouts (2.6). On a 5-point scale, the two test soybean strains were in the category of ‘like’, whereas the market samples and the control mungbean were in the ‘neither like nor dislike’ category (Table 3).

**General discussion**

The germinated small-seeded soybean strains tested in this study have a greater potential for successful competition with mungbean sprouts than large-seeded soybeans. The predominance of a nutty flavor and aroma, and the lesser degree of beany, grassy or bitter flavors should contribute to acceptance of the small-seeded soybean sprouts.

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### TABLE 1. Mean sensory scores<sup>a</sup> for sprouts of soybean, mungbean, and Jaeraejong 320-7

<table>
<thead>
<tr>
<th>Type of sprout</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beany</td>
<td>Sweet</td>
<td>Crisp</td>
</tr>
<tr>
<td></td>
<td>Nutty</td>
<td>Bitter</td>
<td>Tender</td>
</tr>
</tbody>
</table>

* aN = 40. Where letters following means differ within a column, means differ significantly (P<0.05) from each other. Range for scoring: 1, not perceived, to 5, very evident for aroma and flavor attributes; and 1, not crisp, to 5, very crisp, and 1, tough, to 5, tender, for texture.

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### TABLE 2. Mean sensory scores<sup>a</sup> for aroma, flavor and texture attributes of soybean and mungbean sprouts.

<table>
<thead>
<tr>
<th>Type of sprout</th>
<th>Conditions of sprouting</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td>Bitter</td>
<td>Tender</td>
</tr>
</tbody>
</table>

* aN = 108. Where letters following means differ, means differ significantly (P<0.05) from each other. Range for scoring: 1, not perceived, to 5, very evident for aroma and flavor attributes; and 1, not crisp, to 5, very crisp, and 1, tough, to 5, tender, for texture.
outs. They would probably be better liked than the soybean sprouts currently available in the market and would likely be highly competitive with mungbean sprouts which are traditionally used in some parts of the United States. As indicated in a previous publication (1), the product would be more nutritious in many parameters than the mungbean and soybean sprouts presently available. Based on data presented herein, there are no restrictions from the standpoint of safety in using these small-seeded soybean sprouts as food.

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REFERENCES