Microbiological Quality of Frozen "Edamame" (Vegetable Soybean)

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Journal of Food Safety

MICROBIOLOGICAL QUALITY OF FROZEN "EDAMAME" (VEGETABLE SOYBEAN)

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Accepted for Publication July 25, 2007

ABSTRACT

This study compared the microbiological quality of frozen "edamame" to other varieties of frozen beans sold in Virginia. Furthermore, the reduction of microorganisms during experimental edamame processing was investigated. Commercial frozen in-pod and shelled edamame had aerobic mesophiles at 3.4 and 3.1 log cfu/g, yeasts and molds at 2.3 and 2.1 log cfu/g, and some contained low levels of Escherichia coli *and enterotoxigenic* Bacillus *spp. Salmonellae were not found; however, 5% edamame and 4% frozen beans in general were positive for* Listeria monocytogenes. *Rinsing and shelling raw edamame caused a 1–2 log reduction of total aerobic mesophile, yeast and mold, and coliform counts. No naturally occurring yeast, mold or coliform was detected after blanching edamame at 98C for ≥30 s. Blanching for 60 s eliminated approximately 6 log cfu/g of inoculated* E. coli *and* Listeria *from in-pod edamame. Adequate processing ensures the microbial quality and safety of frozen edamame*.

PRACTICAL APPLICATIONS

Frozen "edamame" has the potential to be produced with microbial quality acceptable for direct consumption. However, current products may contain harmful bacteria such as *Escherichia coli* and *Listeria monocytogenes*; thus, thorough reheating is required for consumer safety. Strict sanitation and effective blanching practices are critical in assuring microbial quality and safety of frozen edamame. Data reported for the first time from this current study could be used to inform frozen edamame producers and consumers of the potentially associated microbial hazards and adequate means for food protection.

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Journal of Food Safety **28** (2008) 300–313.

INTRODUCTION

Vegetable soybean (*Glycine max* [L.] Merr.) is commonly called "mao dou" (translated as "hairy beans") in China or "edamame" (translated as "beans on branches") in Japan and North America. It is a large-seeded specialty soybean that can be marketed fresh or frozen (Johnson *et al.* 1999; Mebrahtu and Mohamed 2006). In East Asia, where edamame is an important vegetable, farmers harvest fresh green pods along with stems when the pods are fully filled and just before they turn yellow (Shanmugasundaram *et al.* 1991). Typically, in-pod edamame is boiled and served as a snack or as a complementary side dish; the inedible pods are removed while eating. Shelled edamame is cooked like sweet peas or lima beans, often stir fired or added to stews and soups. In the U.S.A., edamame has been introduced as a healthy ingredient for salads and is available in the frozen produce section of some groceries (Mentreddy *et al.* 2002)

Scientific information on the postharvest aspects of edamame is invaluable for production management and quality assurance. The procedures involved in industry processing and distribution of frozen edamame are documented in several publications (Benziger and Shanmugasundaram 1995; Johnson *et al.* 1999; Manuarungsan *et al.* 2005). In terms of quality attributes, Simonne *et al.* (2000) found that postharvest changes in total isoflavones and carotenoids in edamame are influenced by the processing method. Their study showed that boiling can cause a substantial increase in daidzin, genistin and genistein. Krinsky *et al.* (2006), in an effort to develop a lexicon for frozen edamame, reported that blanching shelled edamame for 60 s can significantly increase the sweet taste and can reduce the raw bean flavor associated with uncooked beans. Although research has demonstrated the influence of production and processing on the nutritional and sensory qualities of edamame, information on the microbiological quality of frozen edamame is lacking.

This study was designed to investigate the microbiological quality of frozen vegetable soybeans. We examined the microflora of frozen edamame along with other frozen beans sold at grocery stores in Virginia. Furthermore, we evaluated the reduction of natural-occurring and artificially inoculated microorganisms in the processing of freshly harvested edamame.

MATERIALS AND METHODS

Commercial Samples

Packaged in-pod and shelled edamame (frozen and not salted) each from 10 producers and four types of popular frozen beans (green beans, snap peas, green peas and lima beans; not salted) each from five producers were

purchased in duplicate at local grocery stores in central Virginia during 2006 (Table 1). Overall, 40 bags of edamame and 40 bags of other beans were evaluated to include nearly all available brands in local stores. The packages were kept frozen and microbial testing was conducted within 1 week of purchase. To open packages for sampling, one corner of each bag was spread with 70% ethanol and air-dried before cutting with flame-sterilized scissors.

Commercial Sample Analysis

For microbial enumeration, each sample (10 g) was macerated with 20 mL of peptone water (0.1%) in a laboratory blender (Masticator Silver, IUL Instruments, Barcelona, Spain) at high speed for 2 min. Appropriate dilutions of the sample were pour plated using standard method agar for aerobic mesophile counts after incubation at 35C for 48 h and for psychotropic plate counts after incubation at 7C for a week. Samples were also pour plated using acidified potato dextrose agar for yeast and mold counts after incubation for 5 days at 25C. Furthermore, the diluents were surface plated onto mannitol–egg yolk–polymyxin (MYP) agar (Difco, Sparks, MD) and incubated for 24 h at 30C for presumptive *Bacillus cereus* counts (Pao *et al.* 2005). To differentiate the presumptive *B. cereus* from culturally similar species, representative colonies (up to five isolates per positive sample) found on the MYP plates were isolated using nutrient agar and were incubated at 30C before microscopic examination for positive cell motility and Gram staining at day 1, and the absence of bipyramidal-shaped crystal formation at day 4 with basic fuchsin (Fisher Scientific, Fair Lawn, NJ) staining (Bennett and Belay 2001; Pao *et al.* 2006b). To determine the enterotoxin-producing capability of the isolated presumptive *B. cereus* strains, a loopful of each isolate was enriched with 10 mL brain heart infusion broth + 0.1% glucose for 16–18 h at 36C before a *Bacillus* diarrhea enterotoxin visual immunoassay (TECRA, Frenchs Forest, Australia) was performed (TECRA 2001).

Total coliform and *Escherichia coli* counts were determined using three-tube most probable number (MPN) evaluation with appropriate dilutions of each sample (U.S. Food and Drug Administration 1998). After incubation for 24–48 h at 35C, a loopful of culture from each positive gassing lauryl sulfate tryptose (LST) broth tube was transferred to brilliant green (BG) bile broth and EC broth containing 4-methylumbelliferyl-β-D-glucuronide (EC-MUG). After incubation for 24–48 h, BG tubes with growth and gas production (at 35C) confirmed the presence of coliforms and EC-MUG tubes with growth (at 45.5C), and fluorescence under long-wave UV light (336 nm) indicated the presence of *E. coli* (Pao *et al.* 2002). Representative isolates from the positive EC-MUG tubes were tested using biochemical kits (API 20E, Biomerieux, Durham, NC) for *E. coli* confirmation.

TABLE 1.
TYPE AND ORIGIN OF FROZEN BEANS PROCURED IN VIRGINIA

Brand	Origin	Edamame		Brand	Origin	Other beans			
		In-pod	Shelled			Snap pea	Green bean	Green pea	Lima bean
A	China	√		D	U.S.A.	√			
B	China	√		J	U.S.A.	√			
C	China	√		N	U.S.A.	√			
D	China	√*	√*	O	U.S.A. or Guatemala	√†	√		
E	China	√*	√*	P	U.S.A. or Guatemala	√†	√	√	√
F	China	√*	√*	Q	U.S.A.		√	√	√
G	China	√	√	R	U.S.A.		√		
H	China	√	√	S	U.S.A.		√		
I	China	√	√	T	U.S.A.			√	
J	U.S.A.	√	√	U	U.S.A.			√	
K	U.S.A.		√	V	U.S.A.			√	√
L	China		√*	W	U.S.A.				√
M	China		√	X	U.S.A.				√

* Organic product.
† Product of Guatemala.

Salmonella and *Listeria* detections were performed using Association of Official Analytical Chemists-approved methods for foods (TECRA 2002, 2004). For *Salmonella*, each sample (25 g) was macerated and pre-enriched in lactose broth (225 mL) at 36C for 20 h, followed by enrichment in Rappaport–Vassiliadis broth at 42C for 18 h and postenrichment in M broth at 36C for 7 h before an enzyme-linked immunoassay was performed using a *Salmonella* visual immunoassay test kit (TECRA). For *Listeria* spp., each sample (25 g) was macerated and enriched in 1:10 dilution in UVM-modified *Listeria* enrichment broth. After incubation at 30C for 24 h, 0.1 mL of the primary enrichment broth was transferred into 9.9 mL of Fraser broth for an additional enrichment at 30C for 23 h before a *Listeria* visual immunoassay (TECRA) was performed. Fraser broth that showed positive response in the immunoassay was streaked on Oxford *Listeria* agar for isolation. Identification of representative isolates was conducted using API *Listeria* kits.

Fresh Vegetable Soybeans

Vegetable soybean (*G. max* [L.] Merr.) VS03-604, a Virginia State University (VSU) advanced breeding line developed from a cross between Tomahomare and V81-1603, was harvested in October 2006 from the VSU Randolph Research Farm (Petersburg, VA) either manually (wearing sterile gloves) or by using a mechanical harvester (model BH100, Pixall, Byron, New York, NY). Immediately after harvesting, the pods were transported to the VSU Agricultural Research Station (Petersburg, VA) for experimental processing within 24 h.

Processing Evaluation on Natural-occurring Organisms

At random, in-pod beans (100 g/sample) were taken in triplicate and subjected to various combinations of the following treatments: (1) twice rinsing with gentle agitation in 250 mL autoclave-sterilized city water for 30 s at 45C to facilitate dirt removal; (2) immersion in 3 L of hot water (98 $\pm$ 2C) for 30, 60 or 120 s followed by cooling in 500 mL of cold water (4C) for 1 min; (3) shelling either by hands (wearing sterile gloves) or by using a mechanical shelling device (Mr. Pea Sheller, Lee Mfg., Dallas, TX); and (4) storing in sterile bags at −18C for 30 days before microbial analysis. The samples were tested for naturally occurring total aerobic mesophiles, yeasts and molds, coliforms, *E. coli*, *Salmonella* spp. and *Listeria* spp. using methods described earlier for commercial samples.

Inoculum Preparation

For the inoculation study, three strains of *E. coli* (*Escherichia coli* O157:H7 American Type Culture Collection [ATCC] 35150, *Escherichia coli*

O157:H7 ATCC 700728 and *Escherichia coli* ATCC 25922) or *Listeria* spp. (*Listeria monocytogenes* ATCC 7644, *Listeria monocytogenes* ATCC 19115 and *Listeria innocua* ATCC 33090) were cultured in tryptic soy broth (Difco) and were individually streaked onto tryp-soy agar (unless otherwise stated, all media were obtained from Biotrace International, Bothell, WA). After overnight incubation at 35C, colonies from the three strains of *E. coli* or *Listeria* were pooled in Butterfield's phosphate buffer and vortexed thoroughly before dilution to ~10^7 cfu/mL (confirmed by plating) for inoculation.

Processing Evaluation on Inoculated Organisms

Fresh in-pod edamame (100 g/sample) were inoculated with 10 mL of either *E. coli* or *Listeria* culture followed by gentle mixing for 1 min to allow thorough wetting of soybean surfaces (Pao *et al.* 2006a). The inoculated soybeans were air-dried for 2 h at 25C in a forced-air chamber (model 680A, Lab-Line, Dubuque, IA). For treatment groups, each sample was immersed in hot water (98 ± 2C) for 1 min. Treated samples or untreated controls were immediately macerated in 900 mL of LST for *E. coli* or UVM-modified *Listeria* enrichment broth for *Listeria* pre-enrichment before microbial detection. Then, the same protocols used for testing *E. coli* and *Listeria* spp. in commercial samples were followed except EC broth was used for the final *E. coli* enrichment. All positive EC tubes were streaked to eosin–methylene blue agar before purple colonies (with or without a green metallic sheen) were isolated and evaluated by API 20E test strips for *E. coli* confirmation (Pao *et al.* 1998).

Statistical Analysis

Log levels of microbial populations were analyzed by one-way analysis of variance or *t*-test using SigmaStat (version 3.0, SPSS Inc., Chicago, IL) software. Significance of difference was defined at $P \leq 0.05$. In processing evaluation, microbial populations were analyzed on the basis of a minimum of three replications per treatment.

RESULTS

Frozen Product Evaluation

The average aerobic mesophile counts of frozen in-pod edamame, green beans, snap peas, shelled edamame, green peas and lima beans were 3.4, 3.1, 2.9, 3.1, 3.0 and 3.2 log cfu/g, respectively (Fig. 1). The respective psychotropic plate counts were 2.3, 2.0, 2.4, 2.3, 2.3 and 2.4 log cfu/g. No significant

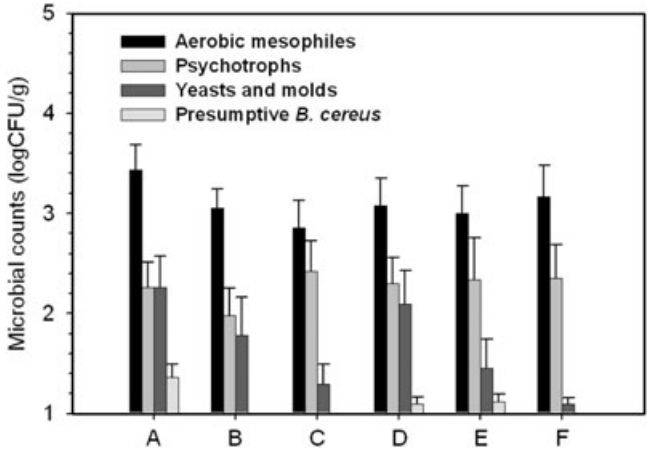

FIG. 1. MICROBIAL COUNTS OF FROZEN BEANS PERCURED IN VIRGINIA: (A) IN-POD EDAMAME, (B) GREEN BEAN, (C) SNAP PEA, (D) SHELLED EDAMAME, (E) GREEN PEA AND (F) LIMA BEAN

difference ($P > 0.05$) on counts of either aerobic mesophiles or psychotrophs was found among the six types of beans, between domestic and foreign origins of the grouped results from all beans, or between organic and conventional sources in the combined results from in-pod and shelled edamame.

The average yeast and mold counts of the in-pod edamame, green beans, snap peas, shelled edamame, green peas and lima beans were 2.3, 1.8 1.3, 2.1, 1.5 and 1.1 log cfu/g, respectively (Fig. 1). Although no significant difference was found among the types or between the origins the beans, the grouped results from organic edamame had yeast and mold counts at 1.3 log cfu/g, which was approximately 1.4 log lower ($P < 0.05$) than that of the conventional edamame. The average counts of presumptive *B. cereus* for all types of beans were all below 1.5 log cfu/g with only 13 of the 80 samples having counts above the minimum detection level (1 log cfu/g). Microscopic observations identified that the representative colonies on MYP plates were exclusively gram-positive bacilli. No isolates resembling *Bacillus anthracis* (a nonmotile bacterium) or *Bacillus thuringiensis* (bipyramidal-shaped crystal producer) were observed. Furthermore, immunoassays on the presumptive *B. cereus* isolates confirmed that *Bacillus* diarrheal toxin-producing *Bacillus* spp. were the predominate isolates found on the MYP positive samples.

The total coliform counts were -0.2 ± 0.2, -0.3 ± 0.1, 0.3 ± 0.2, -0.1 ± 0.2, -0.2 ± 0.2, and 0.6 ± 0.2 log MPN/g for in-pod edamame, green beans, snap peas, shelled edamame, green peas and lima beans, respectively. A

significant difference ($P < 0.05$) in the counts was found between green beans and lima beans. Low levels of *E. coli* were found on one lima bean (2.3 MPN/g), one shelled edamame (0.4 MPN/g) and one in-pod edamame (0.4 MPN/g) sample. The positive samples included both domestic and foreign products, but none from an organic source. Furthermore, salmonellae were not found in any tested samples.

Listeria spp. were detected in 15% of the overall samples ($n = 80$) and in 10% of the edamame ($n = 40$) samples. The positive samples included all types, origins and sources of beans, except snap peas. Most of the *Listeria* isolates were identified as *Listeria innocua*. *Listeria monocytogenes* was found in ~4% of the overall samples and in 5% of the edamame samples. The *L. monocytogenes*-positive samples included one nonorganic lima bean sample from the U.S.A. and two organic, shelled edamame samples from a company in China.

Processing Evaluation

Freshly harvested vegetable soybeans had total aerobic mesophile, yeast and mold, and coliform counts at about 6.8 log cfu, 3.5 log cfu, and 2.3 log MPN/g, respectively (Table 2). *E. coli*, *Salmonella* and *Listeria* were not found in the raw beans. No significant difference in the initial microbial load was observed between manually and mechanically harvested edamame.

For in-pod vegetable soybeans, rinsing alone did not yield any significant reduction of naturally occurring organisms. However, blanching for 30 s at 98 ± 2C caused a ≥3 log cfu/g reduction of aerobic mesophiles and rendered yeast, mold and coliform counts below detection levels. Additional microbial reduction was not observed by increasing the blanching time up to 2 min. Microorganisms that survived blanching for 60 s were further reduced by about 1 log cfu/g during storage at −18C for 1 month.

Rinsing combined with shelling caused a 1–2 log reduction of the total microbial load, yeast and mold, and total coliform counts of edamame. The addition of pod blanching (60 s at 98 ± 2C) prior to shelling helped reduce initial microbial loads to approximately 1.8 cfu/g and decreased yeast, mold and coliform counts to levels below detection. Storage at −18C for 1 month did not cause any further microbial reduction. No difference in the microbial quality of manually and mechanically shelled edamame was found at any stage of the experimental processing.

In the challenge study, raw edamame (in-pod) were inoculated with approximately 6.0 log cfu/g of either *E. coli* or *Listeria* spp. These artificially introduced organisms were detected from the inoculated samples but were not recovered after blanching these samples at 98 ± 2C for 60 s. No *E. coli* or *Listeria* organisms were detected in noninoculated samples.

TABLE 2.
EFFECT OF PROCESSING ON THE MICROBIAL QUALITY OF FRESH VEGETABLE SOYBEANS

Operations*	Aerobic mesophiles (log cfu/g)		Yeast and molds (log cfu/g)		Total coliforms (log MPN/g)	
	Manual	Mechanical	Manual	Mechanical	Manual	Mechanical
Control (harvested in-pod beans)	6.8 ± 0.3a†	6.8 ± 0.1a	3.6 ± 0.5a	3.3 ± 0.3ab	2.0 ± 0.2a	2.6 ± 0.1a
Rinsed	6.7 ± 0.2a	6.3 ± 0.1a	3.7 ± 0.5a	3.1 ± 0.4ab	1.6 ± 0.4ab	1.9 ± 0.3a
Rinsed + shelled	5.0 ± 0.1bc	5.1 ± 0.1b	1.9 ± 0.2bc	2.2 ± 0.1abc	1.4 ± 0.5ab	1.2 ± 0.3a
Rinsed + blanched (30 s)	2.4 ± 0.3cd	2.6 ± 0.1c	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b
Rinsed + blanched (60 s)	2.3 ± 0.1cd	2.5 ± 0.1c	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b
Rinsed + blanched (120 s)	1.7 ± 0.2cde	2.4 ± 0.1cd	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b
Rinsed + blanched (60 s) + frozen	1.6 ± 0.4cde	1.1 ± 0.1e	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b
Rinsed + blanched (60 s) + shelled	1.7 ± 0.1cde	1.8 ± 0.2cde	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b
Rinsed + blanched (60 s) + shelled + frozen	1.1 ± 0.1e	1.5 ± 0.1cde	<1.0 ± 0.0c	<1.0 ± 0.0c	<–0.5 ± 0.0b	<–0.5 ± 0.0b

* All operations (harvesting, rinsing, blanching and shelling) were carried out manually except that a harvester (model BH100, Pixall, Byron, New York, NY) and a shelling device (Mr. Pea Sheller, Lee Mfg., Dallas, TX) were use in the mechanical operation.

† Data represent means ± SEs of three replications. Within the same organism group, means not followed by at least one identical letter are significantly different ($P < 0.05$).

MPN, most probable number.

DISCUSSION

In general, the microbial quality of frozen edamame evaluated in this study was comparable to other, more common varieties of frozen beans, even though most of the edamame was foreign produced, whereas the majority of other beans tested in this study were manufactured domestically. Imports represent an increasingly important share of U.S. produce consumption and thus, a potential source of foodborne illness outbreaks (Calvin 2003). The finding of this study agrees with previous reports by the U.S. Department of Agriculture showing no clear evidence that imported produce is any more prone to food safety problems than are domestic products (Zepp *et al.* 1998; Calvin 2003). Data in the literature do not always support the idea that organic and conventional produce pose different risks of pathogen contamination (McMahon and Wilson 2001; Sagoo *et al.* 2001; Johannessen *et al.* 2004; Mukherjee *et al.* 2004). In this study, however, we noted that frozen organic edamame had significantly less yeast and mold contamination than that of the conventional products. A recent survey also showed that organic seeds sold through the Internet market had less microbial contamination than conventional seeds (Pao *et al.* 2005). Additional research at produce operations is needed to confirm that organic practices can help minimize microbial contamination.

Because not all consumers will follow reheating instructions before adding thawed beans directly to salads (Manani *et al.* 2006), it is desirable to produce frozen beans that are free from infectious organisms. In this study, *L. monocytogenes* was detected in 5% of the edamame samples. This result indicates that currently available frozen edamame, consistent with label instructions, requires additional heat treatment to ensure food safety. Similarly, previous investigations found *L. monocytogenes* in 12% of vegetables in Taiwan and in 2% of frozen peas in Botswana (Wong *et al.* 1990; Manani *et al.* 2006). Recently, the persistence of *Listeria* spp. in a frozen vegetable processing plant has been reported as a potential health risk (Agudao *et al.* 2002). The presence of *E. coli* in 5% of frozen edamame evaluated in this study is an indication of production-related fecal contamination (U.S. Food and Drug Administration 1998; Pao *et al.* 2002). Although *E. coli* and *L. monocytogenes* were both found in edamame, no sample had the two organisms together. This finding, along with a previous report by Manani *et al.* (2006), highlights the importance of not relying on either *E. coli* or *Listeria* spp. as the sole indicator of pathogen contamination in frozen produce.

Blanching to inactivate enzymes is a required step for commercial processing of frozen edamame (Mentreddy *et al.* 2002; Mebrahtu and Mohamed 2006). In this study, we demonstrated that a brief blanching treatment could

also reduce background microflora and could effectively eliminate potentially infectious organisms such as *E. coli* and *Listeria* spp. The isolation of *E. coli* and *Listeria* spp. in our product survey indicates that postblanching contamination and/or inadequate blanching operations exist in some commercial operations. With improved processing sanitation and microbial intervention, it is possible to produce frozen edamame that requires no additional heating for foodservice or consumer usage in cold dishes. Results of this study also show that brief blanching is effective in removing yeast, mold and coliform organisms. Thus, the presence of significant levels of yeasts, molds or coliforms in packaged products may be used as additional evidence of processing or storage deficiencies.

Although rinsing alone is not a significant microbial reduction step, it helps to remove dirt and debris from raw edamame, which is beneficial to product quality. Shelling by either manual or mechanical method is effective in reducing 1–2 log cfu/g of surface microbial contaminants in the absence of blanching. This result is consistent with previous research findings on the removal of surface microflora by manual nut shelling and mechanical fruit extraction (Pao and Davis 2001; Pao *et al.* 2006a). In some Asian countries, raw edamame is shelled at produce markets for sale directly to consumers. Although this practice does not eliminate microbial hazards, it is understood that cooking by the consumer is required to prevent foodborne illness.

In conclusion, strict sanitation and effective blanching practices are critical in assuring microbial quality and safety of frozen edamame. Producers and handlers should implement food safety programs including good agricultural practices and hazard analysis and critical control points to prevent product contamination. Frozen edamame currently sold in central Virginia has comparable microbial quality to other types of beans. Frozen vegetable soybeans have the potential to be produced with microbial quality acceptable for direct consumption. However, current products may contain harmful bacteria such as *E. coli* and *L. monocytogenes*; thus, thorough reheating is required for consumer safety.

ACKNOWLEDGMENTS

This investigation was funded by the United States Department of Agriculture and Virginia Agricultural Council. The article is a contribution of VSU, Agricultural Research Station (Journal Article Series Number 257). Technical support from Catherine Baxley is acknowledged.

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