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ORIGINAL ARTICLE

Development and evaluation of a new device to effectively detach micro-organisms from food samples

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Abstract

Aims: To develop a new instrument of great versatility for recovering micro-organisms from all types of food samples and to compare the effects with existing sample preparation methods.
Methods and Results: To detach micro-organisms from large-size unbroken food samples such as apples, carrots, potatoes and tomatoes without preprocessing, the Spindle apparatus was newly developed. The Spindle was used to effectively detach micro-organisms from large-size samples. In a comparative study involving 51 food samples, treatment with the Spindle and Stomacher showed that recovery of total aerobic micro-organisms (naturally occurring mesophilic microflora) and foodborne pathogens (from samples inoculated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*) for both methods was highly correlated (R^2 = 0·98). Furthermore, diluents treated by the Spindle contained much less food debris than those treated by stomaching.
Conclusions: These results indicate that Spindle is a novel, effective alternative method for detaching micro-organisms from food samples including four kinds of large-size samples without the need for preprocessing.
Significance and Impact of Study: The Spindle might be used to widely detaching micro-organisms from all types of food samples for microbiological assay.

Introduction

Foodborne outbreaks from contaminated foods have been increasingly recognized in many parts of the world (Sivapalasingam *et al.* 2004; Jablasone *et al.* 2005; Lynch *et al.* 2009). Several control methods have been investigated to reduce levels of pathogens on foods. To properly evaluate potential control methods, accurate, consistent enumeration of foodborne micro-organisms is absolutely essential. This necessitates the thorough detachment and suspension of micro-organisms from food samples, which is a very basic task and the most important aspect. For accurate results, appropriate sample preparation methods according to the sample characteristics of each food are required. Several traditional sample preparation methods such as using a Stomacher (Sharpe and Jackson 1972), pulsifier (Fung *et al.* 1997), blender (Moret and Conte 1996), swab (Scherer *et al.* 2009) or hand massaging (Lee *et al.* 2006) have been employed to recover foodborne pathogens from samples. The Stomacher is the most widely used tool for homogenizing food samples in laboratories (Wu *et al.* 2003). However, stomaching produces a substantial amount of debris in suspensions, which can interfere with further analysis such as plating on Petrifilm, ATP bioluminescence and DNA assays (Kang *et al.* 2001). The pulsifier can overcome some problems inherent with stomaching (Fung *et al.* 1997), but the machine cannot process large-size samples such as apples, carrots, potatoes and tomatoes without preprocessing. Thus, large-size samples are chopped into small pieces for sampling by an apparatus or are massaged by hand (Lu and Toivonen 2000; Fleischman *et al.* 2001). The hand massaging method is labour intensive and is tremendously limited in terms of reproducibility. Therefore, it is necessary to

develop a new instrument that cannot only recover foodborne pathogens efficiently but also prevent quality deterioration of whole food samples.

The objectives of this study were to test the efficacy of a developed Spindle apparatus for recovering numbers of foodborne pathogens including *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* from the surfaces of food samples and to compare the effects with existing sample preparation methods.

Materials and methods

Bacterial strains

Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *Salm.* Typhimurium (ATCC 19586, ATCC 43174, DT 104) and *L. monocytogenes* (ATCC 7644, ATCC 19114 and ATCC 19115) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea) for this study and were used for all experiments. Stock cultures were prepared by incubating culture transfers in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) for 24 h at 37°C, then combining 0·7 ml of culture with 0·3 ml of 50% glycerol and storing at −80°C in a cryogenic vial. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37°C for 24 h and stored at 4°C.

Culture preparation

Each strain of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* was cultured in 5 ml of TSB at 37°C for 24 h, harvested by centrifugation at 4000 ***g*** for 20 min at 4°C and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately 10^8–10^9 CFU ml^{-1}. The mixed culture cocktails were prepared by blending together equal volumes of each test strain.

Sample preparation and inoculation

Food samples were obtained from a local grocery store located in Gwanak-gu, Seoul, Korea. All samples were transported to the laboratory and processed within 1 day. The foods were classified into two groups. One group was designated general foods (<200 g) for total bacterial determination by stomaching: 25 g samples aseptically weighed of beef, ground beef, broccoli, celery, lettuce, mushrooms, pork, salmon, shrimp, spinach and tomatoes. The other group was designated large-size food (*c.* 200 g) for surface bacterial determination by hand massage: whole apples, carrots, potatoes and tomatoes. For inoculation, prepared culture cocktails of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were diluted in 2 l of BPW to a concentration of 10^5–10^6 CFU ml^{-1}. Each large-size or 25 g aseptically weighed general food sample was immersed in 2 l of suspension containing *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* for 20 min at room temperature (20°C) and then dried in a laminar flow biosafety hood for 2 h with the fan running. Twenty-five grams of each inoculated general food sample was placed into two separate sterile plastic bags; 225 ml of BPW was added to each bag to make a 1:9 (10-fold) diluted sample. Each large-size food sample was placed into two separate sterile plastic bags; 300 ml of BPW was added to each bag and immediately processed as described in the next section.

Comparison of treatments by Spindle, Stomacher and hand massage

The Spindle apparatus developed in our laboratory is a novel method for physical recovery of micro-organisms from produce surfaces. One set of general samples and large-size samples were processed in sterile sample bags with diluents on the Spindle for 2 min. The Spindle apparatus consisted of very simple components: an electric motor, speed controller, time controller, sample bag container and a sample holder (Fig. 1). A sterile sample bag containing samples and diluents was placed in the sample bag container and temporarily sealed by the sample holder. The treatment time and speed (7000 ***g***) were selected before turning on the Spindle. Activating the motor caused the sample bag container, attached to the motor by a proprietary linkage (patent pending), to vibrate very rapidly and vigorously on a considerably larger scale. The sample bag container edges were placed around the sample bag and held in place with a rubber band. This ensured good transmission of impulse waves to the sample. The numerous microbes from the food samples were effectively detached and suspended in the sample diluents by the resulting robust turbulence. Another set of general samples was treated in sterile sample bags containing diluents with a Stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. The stomacher was activated by two paddles operating side by side, alternately pounding the sample bag and compressing its contents against the door. The large-size samples in sterile sample bags containing diluents were shaken and massaged by hand for 2 min. Hand massaging was performed by the same researcher to ensure consistent results.

Bacterial enumeration

After processing, the homogenate/diluent was 10-fold serially diluted in BPW, and 0·1 ml of sample was

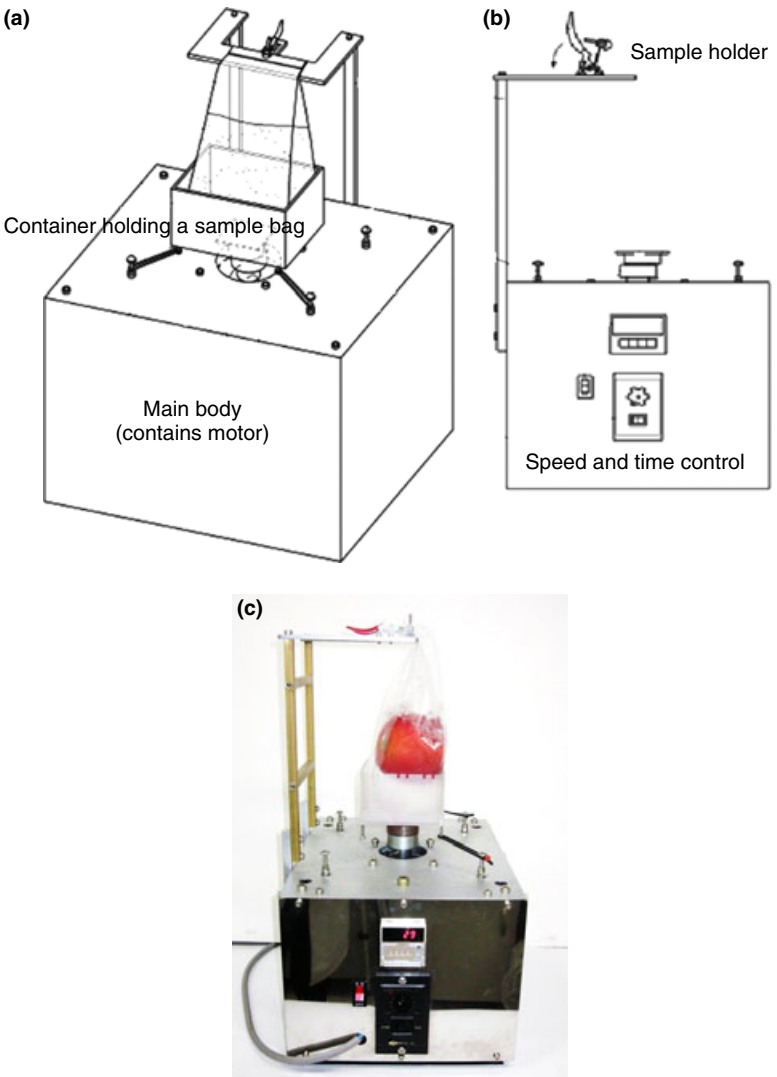

Figure 1 Front (a), side (b) view schematic diagrams and side image (c) of Spindle.

spread-plated onto appropriate media. TSA, and the selective media Sorbitol MacConkey agar (SMAC, Difco), xylose lysine desoxycholate agar (XLD, Difco) and Oxford agar base with 'Bacto™ Oxford antimicrobic supplement' (MOX, Difco) were used as appropriate media for the enumeration of total aerobic micro-organisms, *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes*, respectively (*15, 20*). The plates were incubated at 37°C or 24–48 h (*16*), and colonies were counted and calculated as log CFU g^{-1} of food samples.

Statistical analysis

All experiments were performed in triplicate. Total aerobic counts data were analysed with ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan's multiple range test to determine whether there were significant differences ($P < 0{\cdot}05$) in mean values of micro-organism populations. The number of foodborne pathogens data was analysed by the comparison method used by other research publications (Fung *et al.* 1997 and Kang *et al.* 2001). Correlation coefficients and linear regression trend lines were calculated and plotted using Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

Results

Liquid suspensions from samples treated with the Spindle apparatus were significantly clearer than from samples treated by the stomaching (Fig. 2). For example, suspensions of strawberries treated by the spindle for 2 min (Fig. 2a) were very clean and contained much less debris. On the other hand, strawberries that underwent stomaching for 2 min (Fig. 2b) were heavily broken into small debris.

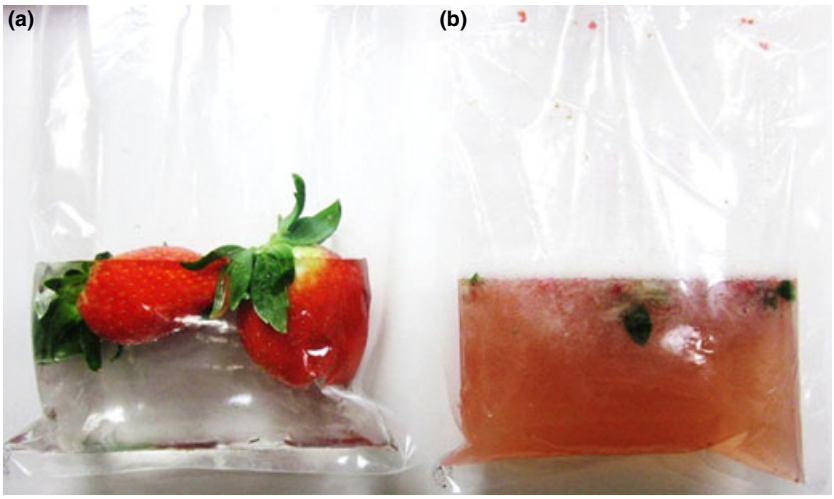

Figure 2 Comparison of strawberries treated by Spindle (a) and Stomacher (b) for 2 min.

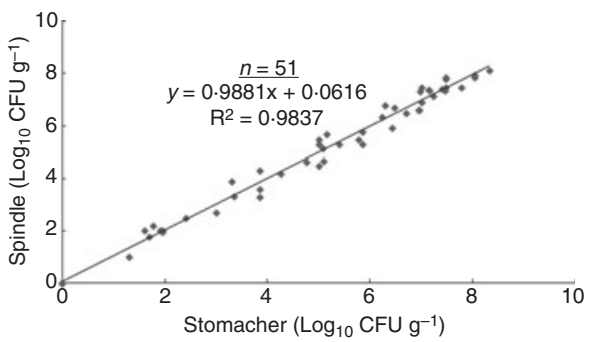

Figure 3 Comparative efficacy of Spindle and Stomacher for detaching total aerobic micro-organisms from 51 food samples.

Figure 3 shows the regression line for total aerobic micro-organisms from 51 food samples (apples, beef, ground beef, broccoli, carrots, celery, lettuce, mushrooms, pork, potatoes, salmon, shrimp, spinach and tomatoes) treated by the Spindle at 4850 ***g*** for 2 min or Stomacher for 2 min. The regression line of the logarithmically transformed data did not differ significantly from linearity. The total aerobic plate counts obtained by Spindle treatment were statistically equal to those by recovered by stomaching (R^2 = 0·9837).

The comparisons of counts for 11 kinds of general food samples that yielded virtually clear suspensions by Spindle treatment and turbid suspensions by stomaching are shown in Table 1. All samples were treated for 2 min. In the case of detaching foodborne pathogens from the general food samples, similar patterns of detaching ability were found between the Spindle and Stomacher. The average ratios for Spindle-to-Stomacher recovery of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were 1·02, 1·05 and 1·03 log CFU g^{-1}, respectively. The average differences between the samples were 0·11, 0·27 and 0·11 log CFU g^{-1}, respectively, for *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes,* indicating minimal differences between the two methods. The highest ratios appeared for ground beef, celery and pork.

Figure 4 shows the comparison of Spindle and hand massage methods for detaching total aerobic micro-organisms from each large-size food sample of apples, carrots, potatoes and tomatoes. There was no significant difference between the recovery counts of samples subjected to the Spindle and hand massage methods. Standard deviations of each treated sample by the hand massaging method were higher than those for the Spindle. The rate of recovery was lowest for whole apples.

The numbers of foodborne pathogens retrieved from large-size food samples were treated by Spindle or hand massaging for 2 min and were compared (Table 2). When the foodborne pathogen counts of large-size food samples were compared between Spindle and hand massage methods, very close counts were indicated. The average ratios between colony counts for the Spindle and hand massage methods for *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were 1·02, 1·05 and 1·03 log CFU g^{-1}, respectively. The average differences in *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* counts were 0·08, 0·15 and 0·14 log CFU g^{-1}, respectively. These minimal differences between the two methods demonstrate a very high correlation.

Discussion

Food surfaces are easily contaminated by a variety of micro-organisms (Chen *et al.* 2001), and pathogenic

Table 1 Comparison of colony counts for 11 food samples that yielded virtually clear suspensions by Spindle and turbid suspensions by Stomacher treatment

No.	Sample	*Escherichia coli* O157:H7 Log_{10} CFU g^{-1}				*Salmonella* Typhimurium Log_{10} CFU g^{-1}				*Listeria monocytogenes* Log_{10} CFU g^{-1}			
		Sp	St	Sp − St	(Sp/St)ratio	Sp	St	Sp − St	(Sp/St)ratio	Sp	St	Sp − St	(Sp/St)ratio
1	Beef	6·99	6·70	0·29	1·04	6·65	6·54	0·11	1·02	5·30	6·36	−1·06	0·83
2	Ground beef	6·51	6·85	−0·35	0·95	6·18	6·08	0·10	1·02	6·38	6·48	−0·10	0·99
3	Broccoli	6·58	6·18	0·40	1·07	6·36	5·28	1·08	1·21	6·30	6·45	−0·15	0·98
4	Celery	5·67	5·72	−0·04	0·99	5·54	5·20	0·34	1·07	5·61	5·68	−0·07	0·99
5	Lettuce	5·95	5·78	0·18	1·03	6·00	5·48	0·52	1·10	5·85	5·00	0·85	1·17
6	Mushrooms	6·34	6·85	−0·51	0·93	6·41	7·07	−0·66	0·91	5·48	6·65	−1·18	0·82
7	Pork	7·00	6·77	0·23	1·03	7·06	7·10	−0·04	0·99	6·49	6·38	0·11	1·02
8	Salmon	6·95	6·80	0·15	1·02	7·04	6·93	0·11	1·02	6·59	6·00	0·59	1·10
9	Shrimp	7·10	6·64	0·46	1·07	6·71	6·56	0·15	1·02	6·49	5·48	1·01	1·19
10	Spinach	6·04	5·48	0·56	1·10	6·26	5·30	0·95	1·18	6·04	5·00	1·04	1·21
11	Tomatoes	7·18	7·35	−0·17	0·98	7·12	6·83	0·30	1·04	6·71	6·52	0·19	1·03
	Average			0·11	1·02			0·27	1·05			0·11	1·03

Sp, Spindle; St, Stomacher.

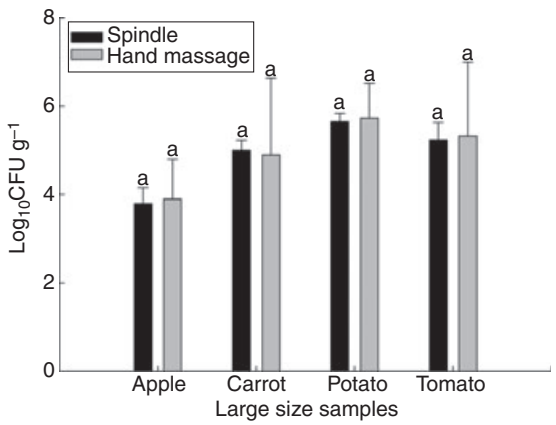

Figure 4 Comparison of Spindle and hand massage methods for recovering total aerobic micro-organisms from large-size samples of apples, carrots, potatoes and tomatoes. There are no significant differences ($P > 0·05$) among bars that share the same letter. (■) Spindle, (▤) Hand massage.

micro-organisms can also become attached to these surfaces. Therefore, many researchers have given much attention to investigating pathogens borne on foods (Hammoudia *et al.* 2009). In performing studies, sampling is a very basic task and is critically important. Stomachers (Sharpe and Jackson 1972), pulsifiers (Kang *et al.* 2001), blenders (Wilkes *et al.* 2000), ultrasound (Puleo *et al.* 1967), vortex stirring (Sharpe and Kilsby 1970), surface scraping (Williams 1967), water spraying (Clark 1965), vacuum probes (Petersen and Bond 1969), hand massaging (Lee *et al.* 2006) and electrophoresis (Tanikawa *et al.* 1966) have been used for sample preparation methods.

To the best of our knowledge, an apparatus for sampling large sizes of whole foods has not yet been developed. Therefore, in this study, we designed the Spindle for the sampling of whole-type (large size) and general-type samples. The operating principles are as follows and are illustrated schematically in Fig. 1. Briefly, an electric motor is controlled by a speed controller and connected with a plastic container by means of a mechanical linkage (patent pending). The container in which any kind of plastic film bag containing samples with BPW can be positioned is driven with a whirlpool motion produced by the motor, giving a rotational speed up to a maximum of 7000 ***g***. Because using the equipment for an extended time at the highest frequency will damage the motor, we tested the efficacy of the equipment for 2 min (the same as the Stomacher processing time). However, when using the Spindle for 1 min, the levels of detachment of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* from food surfaces were similar to 2 min of Stomacher treatment (data not shown).

The Spindle appears to have many advantages as a sample preparation method for any bacteriology laboratory dealing with various kinds of food samples, particularly in regard to the elimination of labour for hand massaging large-size samples. While existing apparatuses cannot handle large-size whole food samples, this instrument ensures skilful treatment of such samples. For example, an apple fruit could not be placed in an existing apparatus such as a Stomacher or pulsifier because the machines have a narrow entrance compared with the size of the fruit. Thus, hand massaging or wiping with a swab was almost the only practicable methods for handling large-size food samples. However, in the case of whole

Table 2 Comparison of the number of foodborne pathogens recovered from whole food samples treated by Spindle and hand massage methods for 2 min

		Escherichia coli O157:H7				*Salmonella* Typhimurium				*Listeria monocytogenes*			
		Log_{10} CFU g^{-1}				Log_{10} CFU g^{-1}				Log_{10} CFU g^{-1}			
No.	Sample	Sp	H	Sp − H	(Sp/H)ratio	Sp	H	Sp − H	(Sp/H)ratio	Sp	H	Sp − H	(Sp/H)ratio
1	Apples	4·30	4·32	−0·02	1·00	4·28	4·26	0·02	1·01	3·00	3·00	0·00	1·00
2	Carrots	6·18	6·20	−0·03	1·00	6·32	6·34	−0·02	1·00	3·90	3·60	0·30	1·08
3	Potatoes	3·00	3·00	0·00	1·00	4·38	4·08	0·30	1·07	3·30	3·00	0·30	1·10
4	Tomatoes	5·01	4·65	0·36	1·08	5·22	4·97	0·25	1·05	3·00	3·00	0·00	1·00
	Average			0·08	1·02			0·14	1·03			0·15	1·05

Sp, Spindle; H, hand massage.

food samples subjected to hand massaging, uniformity is highly operator dependent and thus lacks reproducibility. Moreover, hand massage methods are laborious and time consuming. On the other hand, the Spindle, having a fixed rotator, is far less subject to error than hand massaging that lacks an absolute standard. The margin of error from the spindle was lower than for rubbing and shaking by hand (Fig. 4). Moreover, using a Spindle is economical in regard to eliminating human labour.

Except for foods that were already comminuted, suspensions produced by the Spindle were definitely clearer than corresponding stomached suspensions that contained considerable debris and bubbles. Also, there was a high correlation between recovery of foodborne pathogens from samples processed by Spindle and Stomacher methods. The superior quality of microbial suspensions generated by Spindle treatment has implications for general detection analysis as well as for techniques such as PCR and ATP bioluminescence, which are adversely affected by tissue extracts. This Spindle can easily prepare both general and large-size food samples for more sophisticated microbial experiments.

In conclusion, this study indicates that the novel Spindle apparatus developed by the authors was highly efficacious in recovering foodborne pathogens from all types of foods tested. There were no significant differences in the numbers of recovered cells among produce treated using Spindle, Stomacher or hand massage methods. At the same time, the Spindle provided much clearer suspensions than the Stomacher and allowed for much greater reproducibility of bacterial recovery compared with the suspensions prepared by hand massaging. Thus, the spindle is a novel instrument of great versatility for detaching micro-organisms from all types of food samples.

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