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ORIGINAL ARTICLE

Evaluation of micro-organism-detaching efficacy from meat samples by spindle or stomacher treatment and quality analysis of suspensions

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Keywords
detachment, foodborne pathogen, meat, real-time PCR, sample preparation, Spindle, stomacher.

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Abstract

Aims: We investigated and compared the efficacy of a new apparatus for detaching micro-organisms from meat samples.
Methods and Results: The efficacy of Spindle and stomacher in detaching micro-organisms from meat samples was evaluated. Also, evaluation of appropriateness of suspensions generated by both methods for carrying out molecular biological analysis was implemented. A nearly identical correlation and high R^2 were obtained between Spindle and stomacher in Aerobic Plate Count (APC), and no significant differences were observed in detachment of three major foodborne pathogens. The suspension generated by the Spindle showed lower turbidity and total protein concentration. Also, significantly different threshold cycles were observed in Real-time PCR analysis using suspensions generated by both methods.
Conclusions: The Spindle shows nearly identical efficacy with stomacher treatment in detaching micro-organisms from meat samples. Furthermore, the high quality of suspensions generated by the Spindle, in terms of turbidity and total protein assay, allows for a lower threshold cycle than stomached suspension in Real-time PCR.
Significance and Impact of the Study: The Spindle could be an alternative method for detaching micro-organisms, yielding a higher quality of suspensions which may be better suited for further molecular microbiological analysis.

Introduction

Meat products are usually consumed after cooking, but meats purchased in their raw state or precooked are not free from illness-causing pathogens. Raw meat can be contaminated with various bacteria, including pathogens, throughout the process from slaughtering to consumption, and especially slaughtering offers high potential for contamination of meat. Generally, the interior of carcasses is recognized as sterile, but numerous bacteria attached to the surface of carcasses from hair or hide dust and faecal material may contaminate the interior of meat (Selgas *et al.* 1993).

From 2011 to 2014, outbreaks of *Salmonella* traced to contaminated chicken meat resulted in 143 infected persons, 31% of them requiring hospitalization. Eighty-eight persons who consumed ground beef were infected with *Salmonella* and 19 persons were hospitalized in a multistate outbreak in the United States. In 2014, there were 12 cases of *Escherichia coli* O157:H7 infection traced back to contaminated ground beef and 58% of these individuals were hospitalized (CDC 2014a,b, 2015). Although many effective interventions for controlling foodborne pathogens have been developed, of great importance is developing improved methods of foodborne pathogen detection. A critical first step for microbial analysis is detaching bacteria from food samples. If target bacteria remain on food surfaces after the initial preprocessing step, subsequent analysis will be severely affected (Rodrigues-Szulc *et al.* 1996). Micro-organisms attached to

meat surfaces tend not to be easily detached because bacterial attachment depends on properties of respective bacterial species and meat surfaces (Selgas *et al.* 1993). Bacteria attached to meats have affinity for collagen and elastin of meat due to physicochemical forces, so detaching micro-organisms from meat is more difficult than from fresh vegetables (Rodrigues-Szulc *et al.* 1996).

Many micro-organism-detaching methods have been developed. Among them, the most frequently used method is 'stomaching' which homogenizes food samples in a diluent with a stomacher (Sharpe and Jackson 1972). However, stomaching has several drawbacks such as generating much debris and producing turbid suspensions. Meat samples processed by stomaching produce many small particles of debris, become macerated, and lose their original shape. Turbid diluent interferes with subsequent detection and enumeration of micro-organisms involving PCR and flow cytometry (Rodrigues-Szulc *et al.* 1996).

In order to compensate for these limitations, our laboratory developed a new 'Spindle' apparatus which effectively detaches bacteria on surfaces of food by rotation and vibration, resulting in a treated diluent that is much clearer and can easily be used in further assays. The Spindle can also treat large samples (>200 g) at once without prepreparation or any cutting process (Kim *et al.* 2012), which allows for treating whole fruits and large cuts of meat easily. The Spindle has nearly the same ability as a stomacher for detaching like numbers of bacteria from general food samples (Kim *et al.* 2012) and previously we demonstrated a high correlation of micro-organism-detaching capacity from various fresh vegetables between a stomacher and a 4-section Spindle apparatus composed of four sample containers allowing simultaneous treatment of four samples (Kim *et al.* 2015).

In this study, we compared the effectiveness of a stomacher and a 4-section Spindle for detaching surface bacteria including the foodborne pathogens *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* from beef, chicken and pork meats. Also, we specifically investigated effects of the micro-organism-detection method for further analysis involving bicinchoninic acid (BCA) protein assay and RT-PCR.

Bicinchoninic acid protein assay is a method which quantifies total protein content by means of colorimetric detection. BCA-Cu^{+} complex exhibits a strong absorbance at 562 nm and this absorbance shows an almost linear response with increasing protein concentration across a broad working range (20–2000 μg ml^{-1}) (Smith *et al.* 1985). Concentrations of protein are commonly determined by reference to standards of a general protein such as bovine serum albumin (BSA) (Wiechelman *et al.* 1988). Therefore, samples whose concentrations were unknown were determined based on a BSA standard curve in our research.

Moreover, in order to examine the actual influence detaching methods have on molecular biological analysis, we implemented Real-time PCR (RT-PCR). Through the threshold cycle (C_t) of Spindle- or stomacher-treated diluent that indicates the PCR cycle at which the fluorescent signal passes a randomly placed threshold, we compared the amplified degree of DNA from each preprocessed sample diluent (Schmittgen and Livak 2008).

Materials and methods

Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *Salm.* Typhimurium (ATCC 19585, ATCC 43971 and DT 104) and *L. monocytogenes* (ATCC 19111, ATCC 19115 and ATCC 15313) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea). Stock cultures were stored at −80°C in a solution of 0·7 ml of Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD) and 0·3 ml of sterile 50% glycerol. In order to obtain working cultures, bacteria were streaked onto Tryptic Soy Agar (TSA; Difco), incubated at 37°C for 24 h, stored at 4°C and used within 3 days.

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 and combined in a 50-ml conical centrifuge tube. The foodborne pathogens were harvested by centrifugation at 4000 ***g*** for 20 min at 4°C and the supernatant was discarded. Pelleted cells were resuspended in sterile 0·2% Bacto peptone water (PW; Bacto, Becton, Dickinson and Company, Sparks, MD) and centrifuged. Three washing steps in PW were performed and final pelleted cells were resuspended in 9 ml PW, corresponding to approximately 10^8 to 10^9 CFU ml^{-1}.

Sample inoculation

Meat samples were purchased from a local grocery store (Seoul, Korea) and all samples were stored under refrigeration (4°C). The samples were processed within 1 day. For Aerobic Plate Count (APC), 25-g samples of beef (brisket, loin, rib), chicken (breast, leg, wing) and pork (belly, loin, rib) were aseptically weighed and processed.

For inoculation, each 25-g sample was placed on a piece of sterile aluminium foil. The resuspended bacterial

cocktail was diluted 10-fold with PW and 1 ml of the cell suspension was applied to samples. A spoon inoculation method (Dorsa *et al.* 1996; Kang *et al.* 2001) was used to allow foodborne pathogens to attach to meat tissue, and the samples were stored at 4°C overnight. In order to avoid excessive aridity of the meat surface, sample trays were covered with sterile plastic bags. Twenty-five grams of each inoculated meat sample was placed into each of five sterile stomacher bags; one was for the stomaching treatment, and the others were for Spindle treatment. Two hundred and twenty-five millilitres of 0·2% PW was poured into each bag which constituted a 10-fold diluted sample.

Detaching treatment

The four-section Spindle apparatus (Kim *et al.* 2015) (using rotational–vibrational force to detach micro-organisms) and stomacher were used for the detaching and homogenizing treatment. Four sterile bags containing meat samples and 0·2% PW were placed into each Spindle container (Spindle compartment A–D; Sp A, Sp B, Sp C and Sp D). A whirlpool effect generated by vigorous movement of the containers was applied to the meat samples in stomacher bags, which facilitated detachment of mesophilic bacteria and pathogens. A stomacher (Easy Mix; AES Chemunex, Rennes, France) was also used for detaching micro-organisms by crushing and pummelling samples with two paddles. Seven types of meat samples (beef brisket, beef loin, beef rib, chicken breast, pork belly, pork loin and pork rib) were treated by stomaching. Conversely, chicken legs and chicken wings were subjected to hand massaging as a detaching treatment because these samples had bones that were not amenable to stomacher treatment. Each method was performed for 2 min.

Enumeration of bacteria

After treatment, 10-fold serial dilutions were performed by diluting 1-ml sample aliquots into 9 ml 0·2% PW blanks and 0·1 ml of appropriate dilutions were spread-plated onto TSA or selective media (APC: TSA; *E. coli* O157:H7: Sorbitol MacConkey Agar (SMAC; Difco); *Salm*. Typhimurium: Xylose Lysine Desoxycholate Agar (XLD, Oxoid); *L. monocytogenes*: Oxford Agar Base with Oxford antimicrobic supplement (OAB, Oxoid)). All media were incubated at 37°C for 24–48 h before counting. Every colony was counted on TSA for APC and appropriate colonies (*E. coli* O157:H7: cream-coloured colonies on SMAC; *Salm*. Typhimurium, *L. monocytogenes*: black colonies on XLD or OAB) were enumerated on selective media for the detached pathogen study.

Turbidity measurements

Turbidities of Spindle- or stomacher-treated samples were measured using a turbidity meter (TU-2016; Lutron Electronic, Taipei, Taiwan). Twenty-five grams of noninoculated samples were weighed aseptically and placed into sterile stomacher strainer bags with 225 ml of 0·2% PW and processed. The strainer bags were used in order to remove large particulates. Ten millilitres of treated suspensions was pipetted into a sterile container which was included in the turbidity meter kit and turbidities were measured. This determination was replicated three times.

Total protein assay of the suspensions generated by Spindle or stomacher

Total protein concentration was measured by the BCA protein assay method (Pierce BCA Protein Assay kit; Rockford, IL) in three samples including beef rib, chicken breast and pork belly. Beef rib and pork rib were selected because they showed the largest differences in sample suspension turbidities between the Spindle and stomacher treatment, and chicken breast (boneless) was used because it was the only type of chicken that could be treated by stomaching. Twenty-five microlitres of sample suspensions from Spindle and stomacher treatments were pipetted and transferred to a 96-microplate well. Two hundred microlitres of BCA working reagent (consisting of a 50 : 1 ratio of BCA reagent A (sodium carbonate, sodium bicarbonate, BCA and sodium tartrate in 0·1 mol l^{-1} sodium hydroxide) and B (4% cupric sulphate)) was added to wells containing sample aliquots and the microplate was incubated at 37°C for 30 min. After incubation, the plate was cooled to room temperature and the absorbance of each sample was measured at 562 nm with a spectrophotometer (SpectraMax M2[e]; Molecular Devices, Sunnyvale, CA).

Real-time PCR analysis of pathogens from sample suspensions

Three types of inoculated meat samples (beef loin, chicken breast and pork belly) were placed in stomacher bags containing 225 ml of modified-TSB (mTSB) for a pre-enrichment procedure. The sample bags were stomached or Spindle-treated for 2 min and incubated at 37°C for 16 h. After 16 h, the sample bags were stomached or Spindle-treated again and 1-ml aliquots were withdrawn and transferred to 9-ml blanks of 37°C mTSB for another enrichment step at 37°C for 4 h. The enrichment broth was used for DNA isolation and plated onto each selective medium (SMAC, XLD, and OAB) with appropriate 10-fold serial dilutions in order to enumerate

each pathogen. The initial concentration of enriched inoculum was approximately 10^7 to 10^8 CFU ml^{-1}. One millilitre of pathogen-enriched suspensions were transferred to sterile micro centrifuge test tubes and centrifuged at 12 000 ***g*** for 1 min. The supernatant was discarded and 500 μl of tertiary DW (TDW) was added, followed by centrifuging at 12 000 ***g*** for 1 min again. This washing step was performed three times in succession. The final pelleted cells were resuspended with 150 μl of TDW and 95°C heat treatment was applied to the suspensions for 5 min to extract DNA. After the heating step, the tubes were centrifuged at 12 000 ***g*** for 5 min and the supernatants were withdrawn for PCR templates. Real-time PCR reaction mixture (PowerChek Real-time PCR Kit; Kogenebiotech, Seoul, Korea) composition consisted of 4 μl of primer/probe mixture of each pathogen, 10 μl of 2× Real-time PCR Master mix, 1 μl of sterile TDW, and 5 μl of template DNA (total mixture volume; 20 μl). Real-time PCR thermocycler (Exicycler 96 Real-Time Quantitative Thermal Block; Bioneer, Daejeon, Korea) conditions specified in the manufacturer's instructions was programmed for 50°C for 2 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. Also, absorbances at 260 nm and 280 nm of the template DNAs were measured in order to determine DNA purity.

Statistical analysis

All experiments were performed in triplicate. Correlation coefficients and linear regression trend lines for APC for 50 meat samples were calculated and plotted using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA). Foodborne pathogen data were analysed by ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC) and Duncan's multiple range test to determine whether there were significant differences ($P < 0{\cdot}05$) in mean values of pathogen populations of meat samples treated by the two homogenization methods. Foodborne pathogen populations of meat samples were analysed by the comparison method used by other research publications (Fung *et al.* 1998; Kang *et al.* 2001; Kim *et al.* 2012, 2015).

Results

Clarity of treated sample bags

Compared to stomacher treatment, sample bags treated by the 4-section Spindle contained much clearer suspensions because rotational force resulted in much milder sample destruction. On the other hand, samples stomached for 2 min were homogenized by crushing and pummelling which yielded much debris (data not shown).

APC of meat sample

The regression lines of APC from 50 samples of nine different types of beef (brisket, loin, rib), chicken (breast, leg, wing) and pork (belly, loin, rib) treated by the Spindle apparatus or stomacher/hand massaging for 2 min are presented in Fig. 1. The logarithmic data showed linearity and high correlation coefficients indicating that the APC of samples treated by the Spindle had detaching efficacy quite equivalent to those treated by the stomacher. The R^2 values of each Spindle section and stomacher were 0·9808 (Spindle section (Sp) A), 0·9812 (Sp B), 0·9837 (Sp C) and 0·9838 (Sp D).

Foodborne pathogen detachment from meat samples

Nine types of meat samples inoculated with *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* were processed by the Spindle and stomacher, and populations of the foodborne pathogens are shown in Tables 1–3. Foodborne pathogen populations recovered by both treatments ranged from 4 to 6 log CFU g^{-1}. All Spindle sections yielded colony counts very similar to that of stomaching treatment and no significant differences were observed ($P > 0{\cdot}05$). Also, numbers of foodborne pathogens detached from hand-massaged samples (chicken leg and wing) were not significantly different from Spindle treatment ($P > 0{\cdot}05$).

Turbidity measurement of sample suspensions

Table 4 shows turbidities of the nine meat sample diluents. Turbidity of meat suspensions generated by Spindle treatment ranged approximately from 20 to 200 Nephelometric Turbidity Units (NTU), while turbidity of stomached or hand-massaged suspensions ranged from 30 to 1500 NTU. In beef rib and pork rib samples, the turbidity of stomached suspensions was about 400–600 times greater than that of Spindle-treated suspensions. All stomacher-treated samples (beef brisket, loin, rib; chicken breast; pork belly, loin, rib) recorded at least four times larger values in turbidity than Spindle-treated samples. Chicken legs and wings treated by hand massaging showed 35 and 96 NTU, respectively, which were about twofold greater than Spindle suspensions of these same samples.

Total protein concentration measured by BCA protein assay

Total protein concentrations are presented in Table 5. Protein concentrations from Spindle treatment ranged from 300 to 600 μg ml^{-1}, while concentrations of over

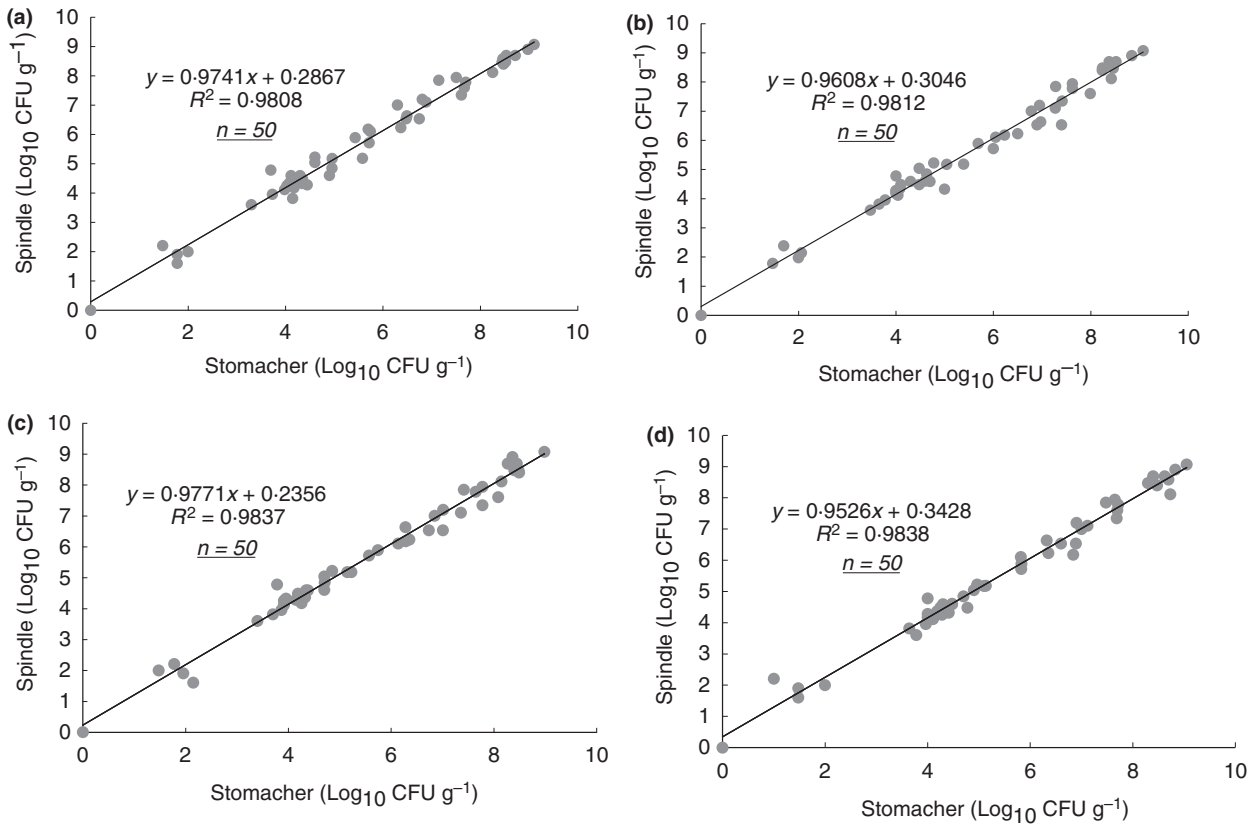

Figure 1 Comparison of Spindle and stomacher efficacy for detaching mesophilic micro-organisms from 50 meat samples. a–d indicate correlation coefficients between Spindle sections a–d with the stomacher respectively.

Table 1 Comparison of Spindle and stomaching methods for recovering *Escherichia coli* O157:H7 from nine types of meat

	E. coli O157:H7 (Log_{10} CFU g^{-1})				
Sample	Sp A*	Sp B	Sp C	Sp D	St/H†
Beef					
Brisket beef	6·21 ± 0·23 A‡	6·35 ± 0·12 A	6·21 ± 0·47 A	6·18 ± 0·29 A	6·29 ± 0·26 A
Loin	5·90 ± 0·68 A	6·15 ± 0·41 A	5·96 ± 0·74 A	6·18 ± 0·44 A	5·96 ± 0·36 A
Rib	5·20 ± 0·10 A	5·08 ± 0·01 A	5·19 ± 0·17 A	5·32 ± 0·02 A	5·41 ± 0·21 A
Chicken					
Breast	6·16 ± 0·08 A	6·30 ± 0·27 A	5·96 ± 0·03 A	6·14 ± 0·21 A	5·94 ± 0·32 A
Leg	5·64 ± 0·23 A	5·77 ± 0·20 A	5·70 ± 0·25 A	5·59 ± 0·16 A	5·85 ± 0·14 A
Wing	5·16 ± 0·28 A	5·20 ± 0·32 A	5·16 ± 0·12 A	5·22 ± 0·39 A	5·11 ± 0·12 A
Pork					
Belly	5·91 ± 0·10 A	6·07 ± 0·30 A	6·26 ± 0·32 A	5·93 ± 0·33 A	6·05 ± 0·28 A
Loin	5·97 ± 0·48 A	6·20 ± 0·52 A	6·14 ± 0·36 A	5·93 ± 0·37 A	6·18 ± 0·49 A
Rib	5·94 ± 0·14 A	5·60 ± 0·36 A	5·79 ± 0·17 A	5·98 ± 0·12 A	5·95 ± 0·15 A

*Spindle.

†Stomacher/Hand massaging.

‡Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

5000 μg ml^{-1} were measured in stomacher-treated samples. The stomaching process yielded approximately 10–20 times greater protein concentration than Spindle treatment because stomaching is a vigorous and rigorous process which induces excessive sample destruction. Especially, chicken breast showed the largest difference of

Table 2 Comparison of Spindle and stomaching methods for recovering *Salmonella* Typhimurium from nine types of meat

	Salm. Typhimurium (Log_{10} CFU g^{-1})				
Sample	Sp A*	Sp B	Sp C	Sp D	St/H†
Beef					
Brisket beef	6·24 ± 0·14 A‡	6·30 ± 0·33 A	6·10 ± 0·28 A	6·15 ± 0·27 A	6·26 ± 0·31 A
Loin	6·51 ± 0·28 A	6·36 ± 0·30 A	6·58 ± 0·52 A	6·52 ± 0·21 A	6·51 ± 0·05 A
Rib	6·54 ± 0·07 A	6·49 ± 0·15 A	6·46 ± 0·08 A	6·51 ± 0·21 A	6·58 ± 0·16 A
Chicken					
Breast	6·36 ± 0·02 A	6·44 ± 0·23 A	6·45 ± 0·24 A	6·44 ± 0·22 A	6·33 ± 0·12 A
Leg	6·09 ± 0·09 A	6·09 ± 0·13 A	6·13 ± 0·10 A	6·01 ± 0·27 A	6·09 ± 0·08 A
Wing	6·06 ± 0·13 A	5·92 ± 0·25 A	6·06 ± 0·27 A	6·01 ± 0·22 A	6·04 ± 0·22 A
Pork					
Belly	6·42 ± 0·11 A	6·39 ± 0·06 A	6·35 ± 0·26 A	6·39 ± 0·19 A	6·47 ± 0·08 A
Loin	6·43 ± 0·30 A	6·48 ± 0·25 A	6·48 ± 0·21 A	6·51 ± 0·11 A	6·58 ± 0·31 A
Rib	6·45 ± 0·05 A	6·28 ± 0·18 A	6·25 ± 0·10 A	6·44 ± 0·21 A	6·49 ± 0·07 A

*Spindle.
†Stomacher/Hand massaging.
‡Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

Table 3 Comparison of Spindle and stomaching methods for recovering *Listeria monocytogenes* from nine types of meat

	L. monocytogenes (Log_{10} CFU g^{-1})				
Sample	Sp A*	Sp B	Sp C	Sp D	St/H†
Beef					
Brisket beef	5·73 ± 0·18 A‡	5·59 ± 0·37 A	5·75 ± 0·40A	5·60 ± 0·42 A	5·76 ± 0·41 A
Loin	4·23 ± 0·40 A	4·34 ± 0·35 A	4·55 ±0·45 A	4·48 ± 0·51 A	4·59 ± 0·23 A
Rib	4·79 ± 0·16 A	4·88 ± 0·07 A	4·85 ± 0·13 A	4·93 ± 0·08 A	4·89 ± 0·17 A
Chicken					
Breast	4·65 ± 0·22 A	4·74 ± 0·13 A	4·45 ± 0 28 A	4·62 ± 0·15 A	4·51 ± 0·28 A
Leg	4·30 ± 0·33 A	4·50 ± 0·19 A	4·52 ± 0·27 A	4·44 ± 0·23 A	4·44 ± 0·42 A
Wing	4·60 ± 0·17 A	4·31 ± 0·30 A	4·62 ± 0·20 A	4·57 ± 0·20 A	4·55 ± 0·17 A
Pork					
Belly	5·09 ± 0·63 A	5·23 ± 0·59 A	5·18 ± 0·52 A	5·20 ± 0·51 A	5·20 ± 0·43 A
Loin	5·51 ± 0·57 A	5·41 ± 0·62 A	5·40 ± 0·65 A	5·60 ± 0·63 A	5·36 ± 0·67 A
Rib	5·39 ± 0·14 A	5·45 ± 0·25 A	5·44 ± 0·12 A	5·40 ± 0·34 A	5·46 ± 0·22 A

*Spindle.
†Stomacher/Hand massaging.
‡Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

protein concentration among the detaching and homogenizing methods.

Real-time PCR analysis of sample suspensions

Threshold cycles (C_t) of the three pathogens in three kinds of meat samples (beef loin, chicken breast and pork belly) were measured through real-time PCR and the results are presented in Table 6. The C_t value of each pathogen in the three samples after Spindle treatment (Sp-C_t) ranged approximately from 18 to 19 in case of *E. coli* O157:H7, 17–21 in *Salm*. Typhimurium and 25–29 in *L. monocytogenes*. The C_t values after stomaching treatment (St-C_t) were 21–22 in *E. coli* O157:H7, 20–24 in *Salm*. Typhimurium and 30–33 in *L. monocytogenes*. Numerically, St-C_t values were 3–5 units greater than those of Sp-C_t, and there were significant differences between St-C_t and Sp-C_t ($P < 0·05$). Table 7 shows a ratio of template DNA absorbance at 260 nm and 280 nm which commonly refers to DNA purity. All three samples after Spindle or stomaching processing had a ratio of approximately 1·46 and there were no significant

Table 4 Comparison of turbidities in sample solutions after Spindle or stomacher/hand-massaging treatments for 2 min

	Turbidity (NTU*)	
Sample	Sp†	St/H‡
Beef		
Brisket	29·72 ± 0·28 A§	462 ± 21·83 B
Loin	44·33 ± 1·02 A	1225 ± 71·39 B
Rib	21·38 ± 0·98 A	1243 ± 29·82 B
Chicken		
Breast	186 ± 6·08 A	869 ± 14·05 B
Leg	18·73 ± 0·09 A	34·92 ± 0·76 B
Wing	52·80 ± 3·31 A	96 ± 4·36 B
Pork		
Belly	41·54 ± 4·68 A	1012 ± 71·25 B
Loin	92·33 ± 2·89 A	886 ± 22·61 B
Rib	31·89 ± 1·17 A	1445 ± 8·08 B

*Nephelometric Turbidity Unit.
†Spindle.
‡Stomacher/Hand massaging.
§Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

Table 5 Total protein concentration of suspensions treated by Spindle or stomacher measured by the bicinchoninic acid assay (BCA) method

	Total protein concentration (μg ml^{-1})	
Sample	Spindle	Stomacher
Beef		
Rib	395 ± 63·22 A*	5154 ± 36·68 B
Chicken		
Breast	328 ± 26·23 A	5663 ± 326·01 B
Pork		
Belly	631 ± 94·98 A	5288 ± 47·50 B

*Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

differences in absorbance of DNA between Spindle and stomacher ($P > 0·05$).

Discussion

Detaching micro-organisms from meat is more difficult than from fresh vegetables due to its surface characteristics (Rodrigues-Szulc *et al.* 1996). However, detachment is an essential step for microbial assay and analysis; performing tests with samples having undergone inadequate micro-organism detachment is meaningless. Efficacy of the Spindle as an alternative to stomaching of fresh produce was validated by previous studies (Kim *et al.* 2012, 2015). Therefore, we evaluated its detachment capacity for meat samples and especially focused on the real applicability of Spindle-treated microbial solution in the present investigation.

Regarding the APC of 25 g meat samples, correlations between each section of the Spindle (A–D) and stomacher were very high and their ratios were nearly equal to one. Also, the results of Spindle processing were not significantly different from samples including bones (chicken leg, chicken wing) which were treated by hand massaging. These findings demonstrate that micro-organism-detaching efficacy of the Spindle was similar to that of stomaching and hand massaging in various types of beef, chicken and pork. The results of recovery of the foodborne pathogens *E. coli* O157:H7, *Salm*. Typhimurium and *L. monocytogenes* showed the same tendency. Log values of recovered pathogens were not significantly different between two methods. The critical strength of the Spindle apparatus which was proved in this research is that the Spindle can detach micro-organisms located on the surfaces of various shapes and types of meat, even those containing hard or sharp parts such as bones. If these bony samples were processed with a stomacher, the polyethylene bag would tear and loss of sample homogenate would occur.

The Spindle produced a much clearer diluent than stomacher treatment of vegetable samples (Kim *et al.* 2012, 2015). We proved that meat samples showed the same tendency as vegetables through the turbidity measurement test, validating the status of Spindle over stomacher treatment, but meat homogenates following stomacher treatment were far more turbid than those of vegetables, because proteinaceous meat tissues are more prone to disintegration. Therefore, these diluents contain not only detached micro-organisms but also useless and impeditive meat particles; thus, further assays such as PCR or bioluminescence using meat sample diluents would be inappropriate and produce inaccurate results (Fung *et al.* 1995).

As for BCA protein assay, diluents of stomacher-treated samples exhibited much higher protein content than those of Spindle-treated samples for all types of meat. It can be explained that stomacher-treated sample diluents contain proteins originated not only from the surface but also from inner parts of meat, because stomaching extensively frays and disintegrates meat samples, while Spindle-treated sample diluents only contain meat particles dislodged from the surface due to vibration of the Spindle.

We also implemented Real-time PCR (RT-PCR) which is a strong tool for quantifying gene expression in order to understand the real applicability of Spindle-treated solution to molecular biological analysis. The quantitative

Table 6 Threshold cycle values in Real-time PCR of sample DNAs isolated from meat sample suspensions treated by Spindle or stomacher

Sample		Organism	Threshold cycle (C_t) value	
			Spindle	Stomacher
Beef	Loin	*Escherichia coli* O157:H7	19·71 ± 0·15 A*	22·76 ± 0·46 B
		Salmonella Typhimurium	21·66 ± 0·99 A	24·16 ± 0·07 B
		Listeria monocytogenes	28·62 ± 0·16 A	31·74 ± 0·10 B
Chicken	Breast	*E. coli* O157:H7	18·89 ± 0·04 A	21·60 ± 0·13 B
		S. Typhimurium	17·85 ± 0·22 A	20·81 ± 0·17 B
		L. monocytogenes	29·04 ± 0·09 A	33·15 ± 0·14 B
Pork	Belly	*E. coli* O157:H7	19·35 ± 0·15 A	21·14 ± 0·15 B
		S. Typhimurium	19·49 ± 0·13 A	21·09 ± 1·10 A
		L. monocytogenes	25·09 ± 0·10 A	30·36 ± 0·31 B

*Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

Table 7 The absorbance ratio of sample DNA isolated from Spindle- or stomacher-treated meat sample suspensions

Sample	The absorbance ratio at 260 and 280 nm	
	Spindle	Stomacher
Beef		
Loin	1·46 ± 0·02 A*	1·45 ± 0·03 A
Chicken		
Breast	1·46 ± 0·02 A	1·48 ± 0·01 A
Pork		
Belly	1·45 ± 0·01 A	1·45 ± 0·01 A

*Data represent means ± standard deviations of three measurements after treatment. Values within rows followed by same uppercase letters are not statistically different ($P > 0·05$).

endpoint for RT-PCR is the threshold cycle (C_t) and C_t refers to the PCR cycle at which the fluorescent signal passes a randomly placed threshold. Through the C_t value, we can know that the PCR is in the exponential phase of amplification. The numerical C_t value is inversely related to the amplicon amount in the reaction (Schmittgen and Livak 2008). Sp-C_t values were lower than St-C_t values for all types of meat and their various cuts. Differences in C_t values between the two treatments were about 3–5 units which indicate that bacteria detached by the Spindle need fewer RT-PCR cycles to reach a meaningful range of amplification; this result in a more rapid reaction than for bacteria detached by stomaching. Therefore, Spindle treatment is a very effective preprocessing method to shorten the analysis time. Samples amplified with high reaction efficiencies provide lower C_t values compared to samples that are contaminated with a PCR-suppressing complex of obfuscating proteins or other compounds. As a result, stomached samples amplify with lower reaction efficiencies (Meijerink *et al.* 2001). We formulated a hypothesis that higher St-C_t values may be caused due to (i) low amplification itself resulting from meat tissue debris, or (ii) a screening effect caused by meat debris and impurities causing low luminescence of RT-PCR even if amplification of DNA samples treated by the Spindle and stomacher were adequate and almost the same. Thus, we implemented more experiments to ascertain the reason. Purity of extracted DNA was confirmed by spectrophotometry and calculated as the 260/280 OD ratio which is the traditionally used standard (Jorgez *et al.* 2006). The ratio of absorbance at 260 nm and 280 nm was almost the same between Spindle- and Stomacher-treated samples. And from our experiments implemented in this research, there was identical cell recovery between Spindle and stomacher treatments in terms of foodborne pathogens as well as APC (Tables 1–3 and Fig. 1). Of the two preprocessing methods, the DNA isolation step was processed simultaneously, so that no differences in efficiency of DNA isolation would be yielded. Furthermore, the DNA samples were amplified at the same time through RT-PCR, so that amplification efficiency of the two methods would be identical. Therefore, we could conclude that delayed C_t values of stomacher treatments were caused by attenuated fluorescence expression due to greater levels of impurities rather than to differences in DNA amplification of the two treatments in performing RT-PCR. This result indicates that Spindle treatment which generates clearer and less debris-containing suspensions has a superior advantage in RT-PCR analysis.

In conclusion, the 4-section Spindle apparatus separated nearly the same amount of bacteria as the stomacher from samples which is the basic function of a conventional stomacher. In particular, we demonstrated that the Spindle was an even more effective tool for further molecular biology-based analysis by generating much clearer, less protein-dissociated and debris-less suspensions, because it helped enable the gathering of more accurate results.

Therefore, the Spindle could be considered a superior device compared to the typically used stomacher.

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Conflict of Interest

No conflict of interest declared.

References

CDC (2014a) Reports of Selected *E. coli* Outbreak Investigations. Available at http://www.cdc.gov/ecoli/outbreaks.html (last accessed on October 2015).

CDC (2014b) Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 Infections Linked to Ground Beef (Final Update). Available at http://www.cdc.gov/ecoli/2014/O157H7-05-14/index.html (last accessed on October 2015).

CDC (2015) Reports of Selected *Salmonella* Outbreak Investigations. Available at http://www.cdc.gov/salmonella/outbreaks.html (last accessed on October 2015).

Dorsa, W.J., Cutter, C.N., Siragusa, G.R. and Koohmaraie, M. (1996) Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. *J Food Prot* **59**, 127–135.

Fung, D.Y.C., Phebus, R.K., Kang, D.H. and Kastner, C.L. (1995) Effect of alcohol-flaming on meat cutting knives. *J Rapid Methods Autom Microbiol* **3**, 237–243.

Fung, D.Y.C., Sharpe, A.N., Hart, B.C. and Liu, Y. (1998) The Pulsifier: a new instrument for preparing food suspensions for microbiological analysis. *J Rapid Methods Autom Microbiol* **6**, 43–49.

Jorgez, C.J., Dang, D.D., Simpson, J.L., Lewis, D.E. and Bischoff, F.Z. (2006) Quantity versus quality: optimal methods for cell-free DNA isolation from plasma of pregnant women. *Genet Med* **8**, 615–619.

Kang, D.H., Dougherty, R.H. and Fung, D.Y.C. (2001) Comparison of pulsifier and stomacher to detach microorganisms from lean meat tissues. *J Rapid Methods Autom Microbiol* **9**, 27–32.

Kim, Y.H., Lee, S.Y., Sagong, H.G., Heu, S., Ryu, S. and Kang, D.H. (2012) Development and evaluation of a new device to effectively detach micro-organisms from food samples. *Lett Appl Microbiol* **55**, 256–262.

Kim, S.J., Kim, D.K. and Kang, D.H. (2015) Comparison of a 4-section Spindle and Stomacher for efficacy of detaching microorganisms from fresh vegetables. *J Food Prot* **78**, 1380–1386.

Meijerink, J., Mandigers, C., van de Locht, L., Tönnissen, E., Goodsaid, F. and Raemaekers, J. (2001) A novel method to compensate for different amplification efficiencies between patient DNA samples in quantitative real-time PCR. *J Mol Diagn* **3**, 55–61.

Rodrigues-Szulc, U.M., Ventoura, G., Mackey, B.M. and Payne, M.J. (1996) Rapid physicochemical detachment, separation and concentration of bacteria from beef surfaces. *J Appl Bacteriol* **80**, 673–681.

Schmittgen, T.D. and Livak, K.J. (2008) Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* **3**, 1101–1108.

Selgas, D., Marin, M.L., Pin, C. and Casas, C. (1993) Attachment of bacteria to meat surfaces: a review. *Meat Sci* **34**, 265–273.

Sharpe, A.N. and Jackson, A.K. (1972) Stomaching: a new concept in bacteriological sample preparation. *Appl Environ Microbiol* **24**, 175–178.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M. *et al.* (1985) Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**, 76–85.

Wiechelman, K.J., Braun, R.D. and Fitzpatrick, J.D. (1988) Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation. *Anal Biochem* **175**, 231–237.