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Bridging technique failure through low-tech improvisation: A case study of food microbiology

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Modern technology for food safety studies includes standardized protocols and equipment. However, appropriate technology needs to step in to bridge technology dys- or malfunctioning. We examined different low-tech methods for extraction of bacteria from fresh vegetables. Standard equipment including stomacher and filter bags were compared to extraction using bread stick and alternative filter material (nylon stocking, mosquito net). Comparison of microspheres' (ø: 53-63 µm; ø: 63-75 µm) passage through filter bags, nylon stockings with different densities (15 DEN, 20 DEN, 25 DEN, 40 DEN) and mosquito net showed no significant difference between filter bag and nylon stocking. A significantly higher number of both size microspheres (ø: 53-63 and ø: 63-75 µm) passed through the mosquito net than filter bag and nylon stocking. Manual extraction of romaine lettuce leaf was performed by three technicians. Viable counts of leaf associated bacteria were influenced by the technician and choice of filter material. Viable bacterial counts obtained from breadstick with filter bag manual extraction did not show any significant difference from standard method. We conclude that standard procedures can be replaced by low-tech approaches in the event of malfunctioning equipment. However, method validation is imperative.

Key words: Homogenizer, low-tech method, microbial load, romaine lettuce.

INTRODUCTION

The production of fresh vegetables has increased by 30% in the last few years (Hess and Sutcliffe, 2018; Balali et al., 2020) and consumption of fresh-cut vegetables is considered as one of the essential nutritious diets to maintain the proper functioning of the body. Various studies also documented the association between the consumption of fresh vegetables and the outbreaks of foodborne diseases especially in developing countries

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(Balali et al., 2020; Khan et al., 2016; Nguyen-the and Carlin, 1994). In developing counties, a lot of vegetables and fruits are produced locally and encounter various food quality and safety issues due to poor water quality and insect infestation thus facilitate to induce microbial contamination (Amoah et al., 2016). Bacterial foodborne illnesses pose a serious threat to public health (Manning, 2017). Study has shown that outbreaks of diseases especially dysentery, diarrhoea, and even cholera are because of the consumption of pathogenic microbes such as *Escherichia coli* through fresh-cut vegetables (Bhunia, 2018). Recently, food borne illness was outbreaks in Ghana due to the consumption of bacterial infested vegetables (Al-Mamun et al., 2018). Therefore, it is an imperative need to ensure food safety through proper investigation. More efficient sampling and detection methods of pathogens are needed to assess the potential health hazards. To investigate food safety, locally available a handy technology is an essential part of this process.

Viable bacterial count is the basis of many standard methods to monitor the safety of food items (Lambertz et al., 2012). For preparing bacterial suspension associated with viable count, stomaching is widely used homogenization technique (Kim et al., 2019; Sharp and Jackson, 1972). The high shear forces generated by stomacher are released even deep-seated bacteria. In spite of the many advances of stomacher technique, due to unavailability and/or unforeseen incidents such as power shortage/electricity failure, instrument malfunction, and confiscated equipment might limit its use and eventually force to adopt makeshift low cost alternative methods, especially in remote areas. Therefore, low cost alternatives based on manual homogenization were tested following the same mechanism as stomaching.

To prevent such bottlenecks, this study addresses improvised scientific low-tech solutions. Low technology often refer to a traditional or non-mechanical methods which is smiley be practiced by local people without any modern tools. Hand-massaging or wiping methods were applied for preparation of sample have been previously been used (Kim et al., 2015). However, these adopted methods for whole sample preparation showed poor uniformity. To the best of our knowledge, this is the first paper of its kind that deals with alternative manual methods to standard stomaching and extraction of microbes associated to vegetable and salad. This paper not only demonstrates different low-tech extraction methods and filter material, but also offers the possibility to compare with standard procedures. The main advantage of the low-tech methods is likely to be low cost and easy availability of the equipment is required for the methods running. We compared the effectiveness of low-tech procedures with standard stomacher by assessing number of microbial release from leafy vegetables. The aim of the study was to identify an appropriate low-tech extraction method and their potential shortcomings that may jeopardize the reliability when evaluating microbial food safety hazards.

MATERIALS AND METHODS

Filter material and microsphere (comparison of filtration)

Two sizes of microspheres (∅: 53-63 µm and 63-75 µm) were passed through the four sizes of nylon stockings (15, 20, 25 and 40 DEN, knee highs, Lindex, made in Italy), mosquito net bag (Mosquito PyramidNet, mesh size 177 µm, Jun Xing Knitting Commodity Co., Ltd., Jiangmen, China) and standard filter bag (mesh size 70 µm, Separator 400, Grade product Ltd., Leicestershire, England). This procedure was repeated six times. The percentage of passed microspheres was calculated.

Media preparation for bacterial colony formation

Diluted (0.1x) Tryptic Soy Agar (TSA) [Tryptic Soy Agar 4.0 g (DIFCO, USA); Bacto Agar (DIFCO, USA) 15.0 g; Aq dest 1000 ml], 0.85% NaCl [8.5 g NaCl (Sodium chloride, CAS-No: 7647-14-5, Belgium); Aq dest 1000 ml] and TRIS-buffer [1.58 g TRIS (Hydroxymethyl-aminomethane hydrochloride, CAS-No: 1185-53-1, Germany); Aq dest 1000 ml] was autoclaved at 121°C and 1 bar pressure, and cooled to 50°C before pouring 20 ml in sterile disposable petri dishes. Prepared agar plates were stored in sterile bags upside down at 4°C until use. Autoclaved 0.85% NaCl and TRIS-buffer were kept at room temperature and used within three days.

Sample preparation

Bagged and sealed romaine lettuce (*Lactuca sativa longifolia*; Romaine, class 1, from Spain) purchased from food chain outlet in Sweden (COOP, Lomma, Sweden) was used as a model leafy vegetable. The fresh lettuce leaf sample (25 g) was placed into filter bags, nylon stocking (20 DEN) or mosquito net sachet covered with a plastic bag (Grade product Ltd., Leicestershire, England), respectively. The single samples were extracted as displayed in Table 1.

Aliquots of 50 ml of TRIS-buffer solution were added in each bag and homogenized by stomacher (normal mode, duration 1 min, Smasher, AES, France) or breadstick for one minute (Figure 1). Extraction with breadsticks was performed manually by technicians. Each extraction method was performed with six replicates by three technicians (technician 1 (woman): ♀ H 160 cm × 69 kg, technician 2 (man): ♂ H 180 cm × W 84 kg, and technician 3 (woman): ♀ H 158 cm × 67 kg) and was repeated twice. The filtered liquid sample was collected in a falcon tube and immediately 10-fold diluted serially with 0.85% NaCl solution. Aliquots of 100 µl were spread on TSA and incubated at 25°C for 72 h. Plates with 30-300 colony-forming units (CFU) were counted.

Statistical analysis

Viable counts (heterotrophic bacterial plate counts, HPC) were related to g fresh weight and log-transformed before statistical analysis (log CFU+1). The statistical analysis was performed using general linear model (GLM) followed by Dunnett test ($p<0.05$) by using Minitab version 17 and mean values are displayed.

Table 1. Experimental set-up for extraction treatments.

Extraction treatment	Filtration matrix		
	Filter bag (F)	Nylon stocking sachet (S)	Mosquito net sachet (M)
Stomacher (S)	■		
Breadstick (B)	■	■	■

Figure 1. Photographs of different extraction techniques. Photographs were taken during extraction and after extraction.

RESULTS

Different types of extraction materials were used in this study and significant differences were observed. The choice of filtration matrix alternative to filter bags is imperative for extraction efficiency. As expected, less dense mesh (that is, mosquito net) allowed the passage of both microsphere sizes in contrast to denser material nylon stockings and filter bag. The percentage of passed microspheres through mosquito net was significantly higher than all nylon stockings and filter bags (Figure 2). The percentage of passed microspheres through 15 DEN nylon stocking was higher than standard filter but not significantly different. While the percentage of passed microspheres was almost similar between 20 DEN nylon stocking (94.2%) and standard filter (94.1%), a lower percentage of microspheres was found with 25 and 40 DEN nylon stocking compared to standard filter. However, no significant difference was stated.

The different low-tech extraction methods showed statistically significant differences in HPC (Figures 3 and 4). The HPC was observed significantly higher in BM and BS technique compared to BF and standard. The HPC was significantly higher in BM and BS with all technicians compared to BF and standard. However, there was no significant difference observed between BF and standard.

DISCUSSION

Rapid and efficient sampling methods are prerequisites to control probable food borne diseases. On-farm sampling requires transportation of produce to the laboratory, necessitating adequate space and rapid transit (Heredia et al., 2015). Our present study proposed an alternative technology to resolve the current challenges in food safety research arena when standard high-tech methods cannot properly be implemented due to technological constraints. Design of alternative technology should be based on the available local resources, which meet demand in resource-limited settings (Papanek, 1972) so that the less advantaged countries can derive maximum benefits out of an appropriate alternative technology (Jokhu and Kutay, 2020; Vaccari et al., 2012; Manju et al., 2016; Joshi and Seay, 2016).

Our data exhibited that nylon stockings with all density (15-40 DEN) allow comparable passage of microspheres as filter bags. Therefore, nylon stocking could be used as

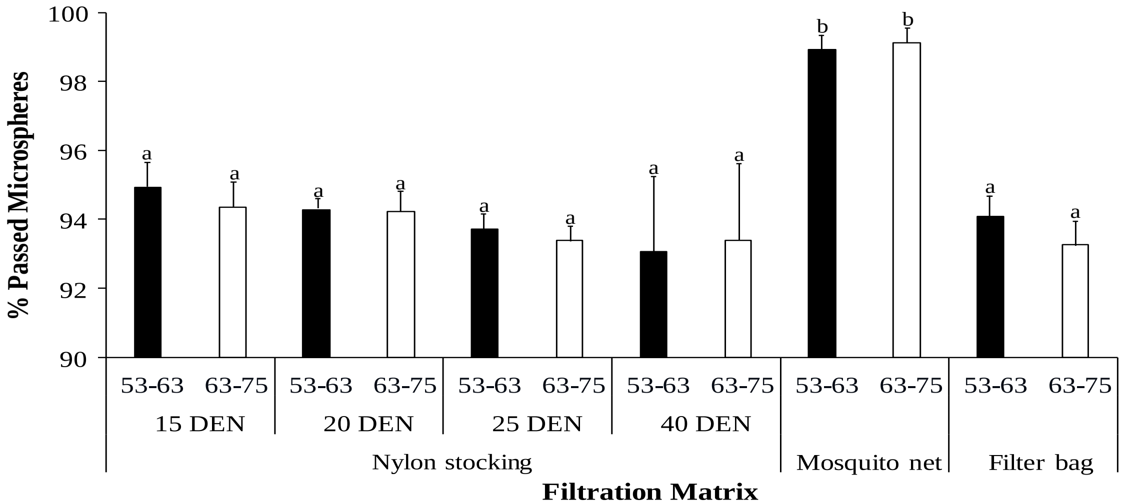

Figure 2. Comparison of filtration with different sizes of microspheres (Ø 53-63 µm and Ø 63-75 µm) through different filtration matrix (Nylon stockings: 15, 20 25 and 40 DEN; mosquito net and filter bag (control). Bars represent the standard deviation (n = 6). Values with different letters are significantly different from control. Data were analyzed by analysis of variance (ANOVA) combined with the Dunnett test (p<0.05).

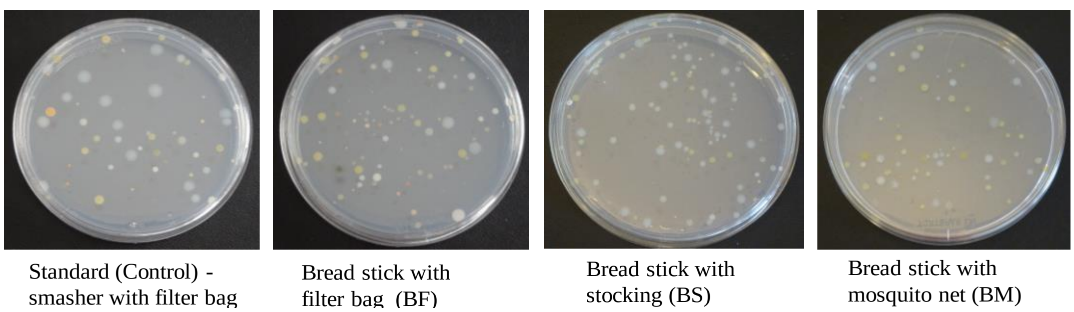

Figure 3. Photographs of microbial colonies under different extraction techniques.

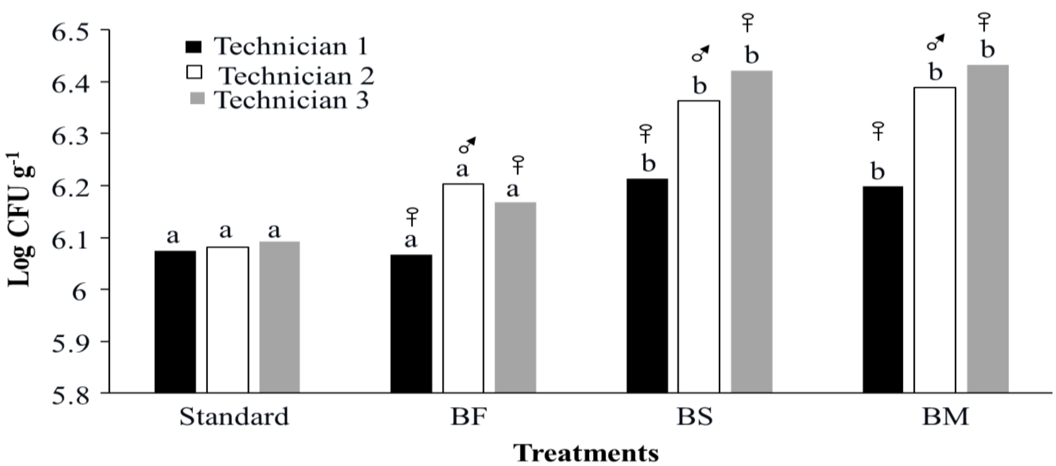

Figure 4. Number of microbial colony count in different extraction techniques (Standard (control): stomacher with filter bag, BF: breadstick with filter bag; BS: breadstick with nylon stocking and BM: breadstick with mosquito net). Values with different letters are significantly different from control. Data were analyzed by analysis of variance (ANOVA) combined with the Dunnett test (p<0.05). Man is represented by ♂ sign while woman is by ♀ sign.

an alternative to filter bags. As expected, breadstick extraction using filter bags also compares to standard stomaching with filter bags, whereas mosquito net and nylon stockings lead to overestimation of viable counts. More importantly, the presented approach demonstrates that the operator (technician) in command is a factor for result interpretation. Our data showed that, samples extracted by Technician 2 and 3 resulted in higher counts for filter bag combined breadstick extraction than Technician 1 but not significantly difference. A similar trend was found also for alternative methods using mosquito net and nylon stockings. Hence, in a low-tech situation, responsibilities within the research team may not be switched. Also, there is a need for post hoc comparison with standard procedures to determine deviations between the low tech and standard procedures to validate the results.

Our results demonstrated that the efficacy of the low-tech homogenizer methods was the same as standard stomacher dose, in some cases even higher. Relative accuracy, sensitivity and specificity of the low-tech method was evaluated and performed equivalently to the reference methods. The low-tech methods are simple and inexpensive compared to other more complicated methods. Although statistically significant differences were stated, these are of technical, but not of biological significance. Stomaching is treated as a good homogenization method for preparing a food sample for the assessment of inner-matrix bacterial contamination of fruits and vegetables (Kim et al., 2019; Rohde et al., 2015). The obtained result based on counting released of bacteria proved the efficiency and applicability of the low-tech methods in the food safety analysis in limited technological facilities areas. In this study, we established that low-tech methods for extraction of microorganisms from plant matrices performed similar to the traditional and widely used stomacher methods. The low-tech method is an efficient, and can replace standard methods in field and baseline studies, given that standardization and validation is done.

In conclusion, the low-tech method used in this study creates a new way to combat any undesired circumstances. This study provides an alternative way to assess the microbial contamination of fresh-cut vegetables using the low-tech method in limited technological areas as well as in low-income countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al-Mamun M, Chowdhury T, Biswas B, Absar N (2018). Food poisoning and intoxication: A global leading concern for human health, food safelty and preservation, pp. 307-352. Elseiver, Amsterdam, Netherlands.

Amoah ID, Abubakari A, Stenström TA, Abaidoo RC, Seidu R (2016). Contribution of wastewater irrigation to soil transmitted Heliminths infection among vegetable farmers in Kumasi, Ghana. PLoS Neglected Tropical Diseases 10(12):e0005161.

Balali GI, Yar DD, Dela VGA, Adjei-Kusi P (2020). Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. International Journal of Microbiology 2020:1-13.

Bhunia AK (2018) Foodborne microbial pathogens: Mechanisms and pathogenesis. Springer, Berlin, Germany.

Heredia N, Solis-Soto L, Venegas F, Bartz FE, Aceituno AFD, Jaykus LA, Leon JS, Garcia S (2015). Validation of novel rinse and filtration method for efficient processing of fresh produce samples for microbiological indicator enumeration. Journal of Food Protection 78:525-530.

Hess T, Sutcliffe C (2018). The exposure of a fresh fruit and vegetable supply chain to global water-related risks. Water International 43(6):746-761.

Jokhu PD, Kutay C (2020). Observations on appropriate technology application in indigenous community using system dynamics modeling. Sustainability 12:2245.

Joshi CA, Seay JR (2016). An appropriate technology based solution to convert waste plastic into fuel oil in underdeveloped regions. Journal of Sustainable Development 9 (4):133-143.

Khan I, Miskeen S, Khalil AT, Phull AR, Kim SJ, Oh DH (2016). Foodborne pathogens: *Staphylococcus aureus* and listeria monocytogenes, an unsolved problem of the food industry. Pakistan Journal of Nutrition 15(6):505-514.

Kim D, Kim S, Kang D (2015). Comparison of a four-section spindle and stomacher for efficacy of detaching microorganisms from fresh vegetables. Journal of Food Protection 78(7):1380-1386.

Kim W, Jeong K-O, Cheon H-L, Kang D-H (2019). Comparison of spindle and stomacher efficacy for detaching biofilms from stainless steel, PVC, and green leafy vegetable surface. British Food Journal 122(2):503-514.

Lambertz ST, Nilsson C, Brådenmark A, Sylvén S, Johansson A, Jansson LM, Lindblad M (2012). Prevalence and level of listeria monocytogenes in ready-to-eat foods in Sweden 2010. International Journal of Food Microbiology 160(1):24-31.

Manju S, Cassidy T, Carnie B (2016). The development and evaluation of a specialty hand knitting yarn using appropriate technology for the empowerment of women in rural India. Research Journal of Textile and Apparel 20(3):136-154.

Manning L (2017). Categorizing food-related illness: Have we got it right? Critical Reviews in Food Science and Nutrition 57(9):1938-1949.

Nguyen-the C, Carlin F (1994). The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition 34(4):371-401.

Papanek V (1972). Design for the real world. Thames and Hudson, London.

Rohde A, Hammerl JA, Appel B, Dieckmann R, Al Dahouk S (2015). FISHing for bacteria in food—A promising tool for the reliable detection of pathogenic bacteria. Food Microbiology 46(1):395-407.

Sharp AN, Jackson AK (1972). Stomaching: A new concept in bacteriological sample preparation. Applied Microbiology 24(2):175-178.

Vaccari M, Vitali F, Mazzu A (2012). Improved cook-stove as an appropriate technology for the Logone Valley (Chad–Cameroon): analysis of fuel and cost savings. Renewable Energy 47:45-54.