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The Effect of Sanitizers on the Native Microflora of Mung Bean Seeds (*Vigna radiata*)

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Abstract: The ingredients of sanitizers varies from natural products such as botanical extracts (lemon and orange extract) and cleaning agents derived from natural plant oil to chemicals such as surfactant solutions, combinations of surfactants with organic or mineral acids and alkaline washes that can remove wax, soil, harmful substances including agricultural chemicals found on fruits and vegetables. Most companies do not make any claims about the microbial reduction in fruits and vegetables by using their sanitizers. This study was therefore conducted to evaluate the effect of five commercial sanitizers available in the local market (labeled as A, B, C, D and E) in reducing microbial population found on the mung bean seeds. About 10 g of mung bean seeds in a stomacher bag were treated with the test solutions for 10 min, shaken at 170 rpm and compared with untreated seeds and sterile deionised water. Microbial populations were enumerated by using Plate Count Agar (PCA) for total bacterial counts, Potato Dextrose Agar (PDA) for yeast and mould and *E. coli*/coliform Agar for *E. coli* and coliforms. All cleansers were tested at minimum concentration as recommended by the manufacturer. Among the 5 commercial sanitizers, A, C and D were effective in significantly reducing yeast and mould to an undetectable level compared to B and E. Sanitizers A, C and D also showed comparable results in reducing the total bacterial counts compared to the other sanitizers. Only sanitizers A and B were effective in significantly reducing the *E. coli* and coliforms. All the 5 sanitizers did not inhibit the seeds from sprouting although, sanitizer D germination percentage was statistically difference from untreated seeds or seeds treated with sterile deionised water.

Key words: Sanitizers, microflora, mung bean seeds, sprouts, concentration, Malaysia

INTRODUCTION

Sprouts, germinating forms of seeds and beans are easy to produce and are nutritionally rich making them an important part of the diet. Sprouts have low fat and calories and provide substantial amounts of key nutrients such as fibre, folate and vitamin C (FDA, 1999). Multiple outbreaks linked to the consumption of raw sprouts have occurred in spite of it being a popular health food. The source of microbial contamination on sprouts is thought to be from seeds which are improperly disinfected before sprouting, rather than contamination of sprouts during or after production (Scouten and Beuchat, 2002; National Advisory Committee on Microbiological Criteria for Food, 1999). From 1973-2005, 37 outbreaks associated with seed sprouts were reported around the world. The most common seed sprouts involved in the outbreaks were mung bean, alfafa and radish (Canada Health, 2007; Taormina *et al*., 1999). In Malaysia, mung bean are the most common and popular variety of sprouts that are consumed. Mung bean sprouts can be easily found in the marketplace and can be grown at home. Although, no outbreak due to the consumption of these sprouts have been reported in Malaysia, safety precautions are still an important issue to look at. Seeds can get contaminated during cultivation from the manure used from animal feces and during growth, harvest, processing, storage or shipping (CDCP, 1997).

Most processors and consumers assumed that washing and sanitizing fresh fruits and vegetables will reduce the microbial load (Sapers, 2001). The United States Food and Drug Administration (FDA) recommend the treatment of sprouting seeds with 20,000 ppm (mg L^{-1}) of free chlorine from calcium hypochlorite or an equivalent antimicrobial treatment in order to minimize microbial contamination of the sprouts prior to consumption (FDA, 1999).

The increasing public health concern related to the microbial safety of sprouts has resulted in increased numbers of studies that analyze the efficiency of different

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methods for reducing the microbial load of the seed sprouts. Various treatments have been evaluated for improving the safety of seeds including heat and ultrasound treatment (Scouten and Beuchat, 2002), numerous chemical treatments such as chlorine or hypochlorite (Fett, 2002), radiation (Saroj *et al*., 2007) high hydrostatic pressure (Neetoo *et al*., 2008) and commercial disinfectants (Pandrangi *et al*., 2003).

A number of commercial sanitizers found in the Malaysian market are sold at the supermarket, pharmacies and through direct selling companies. Most of them are in liquid form and only a number are in powder form. Due to the lack of information on the efficiency of these sanitizing agents in reducing microbial contamination of fruits and vegetables, this study was undertaken to evaluate the effectiveness of these various sanitizing agents in reducing the native microflora on mung bean seeds.

MATERIALS AND METHODS

Samples: Mung bean seeds were purchased from a local grocery store and stored at 4°C in a sterile plastic bag until used.

Sanitizers: All the commercial sanitizers (labeled as A, B, C, D and E) used in this study were bought from local stores. The sanitizers were diluted with sterile deionised water and tested at minimum concentration as recommended by the manufacturers (Table 1).

Treatment of the mung bean seeds: A triplicate sample of 10 g mung bean seeds in sterile stomacher bag (Gosselin, France) were treated by dipping them into solution of 100 mL sterile deionised water or commercial sanitizer solution for 10 min and shaken at 170 rpm using a platform shaker (InnovaTM 2000, New Brunswick Scientific). Following this treatment, the solutions were decanted and the seeds were rinsed with 100 mL sterile deionised water for 30 sec. Both untreated seeds and sterile deionised water were used as controls. Microbial analysis was then carried out.

Microbial analysis: About 10 g of untreated seeds or rinsed seed in the sterile stomacher bag were added with 90 mL of 0.1% sterile peptone water (Difco, USA). Samples were then homogenized using a stomacher (Stomacher 400, Colworth), pummeled at medium speed for 60 sec. About 1 mL of the stomached seed slurry was then serially diluted into 9 mL of sterile peptone water (0.1%). About 0.1 mL of each dilution was then plated onto Plate Count Agar (PCA) (Oxoid, UK) for total bacterial count, Potato Dextrose Agar (PDA) (Oxoid, UK) for yeast and mould and *E. coli*/coliform Agar (Oxoid, UK) for *E. coli* and coliforms. PCA and PDA were incubated at 32-35°C for 48 h while *E. coli*/coliform Agar was incubated for 24 h at 37°C. Each microbial count was the mean of three determinations and was expressed as log CFU g^{-1}.

Evaluation of various treatments on seed germination: The effect of non-treatment and all washing treatments on mung bean seed germination was studied. About 10 g of seeds in a sterile beaker were soaked with 100 mL of sterile deionised water or commercial sanitizer solutions for 10 min with shaking at 170 rpm. The treatment solution was decanted and the seeds were then rinsed with 100 mL sterile deionised water for 30 sec. The untreated and treated seeds were placed between 2, water-saturated filter study (Whatman #1) in sterile petri dishes: 100 seeds per dish, 4 dishes per treatment to determine the percentage of seed germination. The seeds were germinated at room temperature by moistening the filter study daily with sterile deionised water.

Additional sterile deionised water was added to keep the filter study moist during germination. Germination (seeds developing hypocotyls) was checked after 72 h and a total percent germination was recorded.

Statistical analysis: All experiments were repeated twice independently using seeds from different batches in each

Table 1: Sanitizers used appearance, ingredients, recommended concentrations in use and estimated cost per volume

Sanitizers	Appearance	Listed ingredients	Recommended concentration	Estimated cost per vol^{-1} (# cents 500 mL^{-1})
A	White powder	Water soluble chitosan, Apple acid	0.05 g into 500 mL water	3
B	Clear liquid	Plant sources: Corn and palm oil	0.5 mL into 500 mL water	1
C	Diluted clear liquid	Vegetable derived non-ionic surfactant, parfum, botanical extracts (lemon, cucumber, orange,ginger, cassia), potassium sorbate, citric acid	0.92 mL into 500 mL water	11
D	Very diluted clear liquid	Non-ionic and anionic surfactants, polysorbate-20, grapefruit seed extract, lemon and orange extract	13.75 mL into 500 mL water	98
E	Clear viscous liquid	Coco-glucoside, sodium myreth sulfate, cocamidopropyl betaine, flavor, methyl paraben, propyl paraben	0.125 mL into 500 mL water	<1

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experiment. In each independent repetition, triplicate samples were taken for all microbial analysis. Data was subjected to one-way Analysis of Variance (ANOVA) and Tukey post hoc test using SPSS (SPSS Inc., version 15.0, Chicago Illinois, USA) with a level of significance at ($p<0.05$).

RESULTS AND DISCUSSION

Effect of sanitizers on the reduction of native microflora of mung bean seeds: The results of microbiological analysis of mung bean seeds after treatment with different sanitizers are shown in Fig. 1-3. The initial population of total bacterial count was 3.08±0.19 log CFU g^{-1} as shown in Fig. 1. After treatment, the population of the microbial load was reduced to 2.83±0.17, 2.05±0.12, 2.55±0.30, 2.05±0.12, 2.23±0.19 and 2.45±0.37 log CFU g^{-1} for sterile deionised water (Water), sanitizers A, B, C, D and E, respectively. Treatment with sanitizers showed significant difference compared to the untreated seeds with A, C, D and E ($p<0.001$) and B ($p = 0.002$). On the other hand, compared to washing with sterile deionised water, only sanitizers A, C and D showed comparable reduction with significant difference ($p<0.001$). Result for the population of yeast and mould in the mung bean seeds are demonstrated in Fig. 2.

Initial populations of yeast and mould were 2.65±0.07 log CFU g^{-1}. The most effective treatment was with sanitizers A, C and D. These treatments reduced the population of yeast and mould in the mung bean seeds to an undetectable level. Treatment with sanitizers A, C and D reduced the yeast and mould by >2 log CFU g^{-1} compared to the untreated seeds and seeds treated with sterile deionised water. However, washing with sanitizers B and E produced no significant difference compared to sterile deionised water.

Initial populations of *E. coli* and coliforms in the mung bean seeds were 2.25±0.23 log CFU g^{-1} (Fig. 3). Significant reduction of *E. coli* and coliforms occurred with sanitizers A ($p<0.001$) and B ($p<0.001$). In particular, the treatment reduced the *E. coli* and coliforms by >2 log CFU g^{-1} compared to the untreated seeds and to seeds treated with sterile deionised water. There was no significant difference in microbial counts from beans washed with sterile deionised water and those washed with sanitizers C, D and E.

Effect of various treatments on seed germination: The effect of sanitizers on the germination process of mung bean seeds is shown in Table 2. The percent germination of seeds treated with sanitizers ranged between 95.4 and 97.3% compared to 98.5% with sterile deionised water and

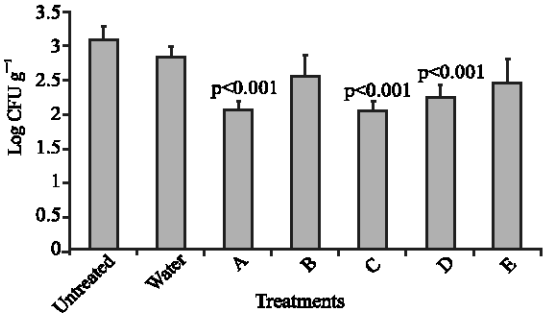

Fig. 1: Total bacterial count (mean±SD) in mung bean seeds after treatment with different sanitizer solution. Significant difference between water and commercial sanitizers at $p<0.001$

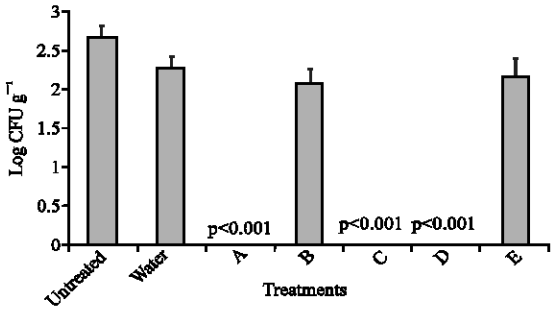

Fig. 2: Population of yeast and mould (mean±SD) in mung bean seeds after treatment with different sanitizer solution. Significant difference between water and commercial sanitizers at $p<0.001$

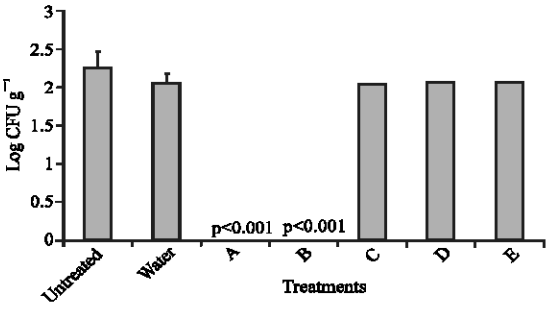

Fig. 3: Population of *E. coli* and coliforms (mean±SD) in mung bean seeds after treatment with different sanitizer solution. Significant difference between water and commercial sanitizers at $p<0.001$

98.9% to untreated seeds. Only the percent germination of seeds treated with sanitizer D (95.4%) was statistically different compared to sterile deionised water ($p<0.05$) and untreated seeds ($p = 0.015$). The results indicated that different sanitizers may be used for sanitizing sprouting seeds without adverse effects on germination and

Table 2: Percent germination of mung bean seeds following treatments with various washing solutions for 10 min at 170 rpm

Treatments	Germination (%)
Untreated	98.9±0.6
Sterile deionised water	98.5±1.7
A	96.7±2.4
B	97.3±2.6
C	95.8±2.1
D	95.4±2.2*
E	96.5±2.4

Values are the mean of four replicates. Significant difference between untreated and sterile deionised water with commercial sanitizers at $p<0.05$ are represented as (*)

demonstrated that all the sanitizers did not inhibit the seeds from sprouting. Most of the sanitizers used in this study were in liquid form except for one, sanitizer A which was in powder form. The volume of sanitizers used as recommended by the manufacturer was minimal except for sanitizer D which required a larger volume to achieve the minimum cleansing effectiveness. Estimated cost per volume^{-1} of sanitizer as shown in Table 1 indicated the most expensive sanitizer was D (98 cents/500 mL) and the cheapest was E (<1 cent/500 mL).

This study demonstrated that the application of sanitizers on mung bean seeds did reduced the microbial populations on the surface of the seeds. These results showed that sanitizers were effective in microbial decontamination compared to washing with sterile deionised water. The effectiveness of other sanitizing agents in reducing pathogen levels on seeds and sprouts has been reported in the literature (Penas *et al*., 2010; Lee *et al*., 2007; Gandhi and Matthews, 2003). Gamma or electron beam irradiation has also been an effective antimicrobial treatment for both seeds and sprouts (Waje *et al*., 2009; Saroj *et al*., 2007).

Sanitizer A which contained chitosan was the most effective compared to other sanitizers. Several studies (Campaniello *et al*., 2008; Pranoto *et al*., 2005; Devlieghere *et al*., 2004) have shown the antimicrobial properties of chitosan as a coating film for food. Chitosan exhibited various promising biological activities including antimicrobial activity, antitumor activity, hemostatic activity and acceleration of wound healing. One of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi *et al*., 1999).

The anionic and ionic surfactants found in sanitizer C and D are common ingredients found in detergents used for cleansing purposes. Chemical and biosurfactants are potentially toxic to specific microbes and may be exploited as antimicrobial agents against plant, animal and human microbial pathogens (Colores *et al*., 2000; Boyette *et al*., 2002; Cameotra and Makkar, 2004). The Food and Drug Administration has proposed that sanitizing treatment of fresh vegetables should reduce pathogen loads by a minimum of 5 log CFU without affecting the sensory characteristics of the treated produce (Venkitanarayanan *et al*., 2002). In this study, bacterial reduction of <5 log CFU obtained from mung bean seeds immediately after sanitizing was due to the low initial microbial population found on the mung bean seeds. As reported by Venkitanarayanan *et al*. (2002) the short time of exposure to chemicals was sufficient to reduce the bacterial count.

Compared to washing with sterile deionised water, sanitizer E was the least effective in reducing all three types of microbes examined. This could be due to the ineffectiveness of the active compounds in the formulation compared to the other sanitizers. Sanitizer A was effective against all types of microbes tested in the mung bean seed. Meanwhile, sanitizer B was only effective on *E. coli* and coliforms. Sanitizers C and D on the other hand were found to be quite effective for total bacterial count and yeast and mould but not for *E. coli* and coliforms. Sapers (2001) observed that higher efficiencies of the sanitizers could be related to the higher oxidizing capacity or higher surfactant activity which allows better contact between attached bacteria and the active compound. While all sanitizers were able to remove dirt particles, chemicals, fertilizers and pesticides, one of the sanitizer can also remove fats and stains. The manufacturers of 2 sanitizers, A and D claimed that they were effective in removing bacteria from the surface of fruits and vegetables. The results indicate that most of the sanitizers showed promising effects in the reduction of microbial load of the mung bean seeds. This will be advantageous to the consumers using the sanitizers for cleansing purpose.

CONCLUSION

This study indicates that the sanitizers tested can be used to improve microbial safety of mung bean seeds. In particular, treatment with sanitizer significantly reduced the specific microbial populations compared to washing with sterile deionised water without affecting seed viability and germination. This information will be useful to the Malaysian consumers when selecting a suitable sanitizer for their domestic purposes. However, for practical application by the food industry, the efficacy of these sanitizers needs to be further investigated in a bigger scale setting.

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