Journal of Food Technology 11 (4-6): 75-81, 2013
ISSN: 1684-8462

Efficacy of Commercial Sanitizers on the Native Microflora of Mung Bean Sprouts (*Vigna radiata*) and its Microbiological Analysis

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Abstract: The effect of five commercial sanitizers (labeled as A, B, C, D and E) available at local markets in reducing the native microflora of mung bean sprouts was evaluated. The microflora populating the sprouts was enumerated using the Plate Count Agar (PCA) for total bacterial count, Potato Dextrose Agar (PDA) for yeast and mould and *E. coli*/Coliform Agar (ECA) for *E. coli* and coliforms. All the sanitizer solutions at minimum concentration recommended by the manufacturer did not show significant differences in reducing the native microflora on the sprouts. Identification of microorganisms on the sprouts was done by using API 20E kits for Enterobacteriaceae and other non-fastidious Gram-negative bacteria and API Candida kits for yeasts. The Gram negative bacteria found on the sprouts were identified as *Rahnella aquatilis* and *Mannheimia haemolytica* (*Pasteurella haemolytica*). As for yeast, *Trichosporon mucoides* was the species found on the sprout.

Key words: Sanitizers, native microflora, mung bean sprouts, microbiological analysis, Gram negative bacteria, yeast

INTRODUCTION

In many parts of the world, mung bean, alfalfa, clover, broccoli and lentil sprouts have gained popularity and are widely consumed as cooked vegetables or as raw salad due to their high nutritional value (Toumas, 2005). A great variety of sprouts are easily available in the Malaysian markets but the most popular are the mung bean sprouts. Traditionally, they are often served lightly cooked or raw in appetizers.

As consumption of fresh products increases, concerns about microbial contamination or the safety of the food products also increase (Sharma and Demirci, 2003). Recently, several studies have been conducted to determine the microbiological quality of the sprouts (Vishwanathan and Kaur, 2001; Robertson *et al*., 2002; Abadias *et al*., 2008). It is well known that although sprouting improves the nutritional value and enhances the antioxidant content in sprouts (Marton *et al*., 2010) the warm and humid conditions also provide a conducive environment for microbial proliferation. The sprouts may have a high microbial count, possibly including pathogenic microorganisms (Molinos *et al*., 2009). These high microbial counts are the main reason for the short shelf-life of sprouts (Zhang *et al*., 2011) and may be a potential hazard to the consumer's health (Waje *et al*., 2009).

The incidence or outbreak of food-borne infections due to eating sprouts or other fresh produce have prompted many studies on decontamination methods such as the use of chemical disinfectants (hypochlorite, chlorine, bromine, iodine, acids), physical treatment (ozone, irradiation, UV) and bio-control agents (Parish *et al*., 2003). However, to date there is no single treatment available that can effectively decontaminate fresh produce (fruits and vegetables) and yield edible raw products that are safe for consumption (Yuk *et al*., 2007; Gandhi and Matthews, 2003).

A number of commercial sanitizers to clean fruits and vegetables prior to cooking or consumption are commercially available in the market. In Malaysia, varieties of sanitizers in liquid or powder form are sold at the supermarkets, pharmacies and through direct selling companies. This study was undertaken to evaluate the efficacy of various sanitizing agents in reducing the native microflora found on mung bean sprouts and to identify the microorganisms harbored by the sprouts.

MATERIALS AND METHODS

Samples: Fresh mung bean sprouts were purchased from a local grocery store and used within 24 h.

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Table 1: Sanitizers used appearance, ingredients, recommended concentrations in use

Sanitizer	Appearance	Listed ingredients	Recommended concentration	Foamy
A	White powder	Water soluble chitosan and apple acid	-0.05 g into 500 mL water	Yes
B	Clear liquid	Plant sources: Corn and palm oil	-0.5 mL into 500 mL water	Yes
C	Diluted clear liquid	Vegetable derived non-ionic surfactant, parfum, botanical extracts (lemon, cucumber, orange, ginger and cassia), potassium sorbate and citric acid	-0.92 mL into 500 mL water	Yes
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	Yes
E	Clear viscous liquid	Coco-glucoside, sodium myreth sulfate, cocamidopropyl betaine, flavor, methyl paraben and propyl paraben	-0.13 mL into 500 mL water	Yes

Sanitizers: 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 shows the specific characteristics of each washing solution. All the sanitizers used are in liquid form except for sanitizer A, a white or off-white powder.

Treatment of the mung bean sprouts: The 10 g mung bean sprouts in sterile stomacher bag (Gosselin, France) were immersed in 100 mL of treatment solution or sterile deionised Water (W) shaken at 170 rpm using a platform shaker (Innova™ 2000, New Brunswick Scientific) for 10 min at room temperature. The solutions were then decanted and the sprouts were rinsed with 100 mL sterile deionised water for 30 sec.

Microbial analysis: The 90 mL of sterile peptone water (0.1%) (Difco, USA) was added to 10 g of untreated sprouts or rinsed sprouts in the sterile stomacher bag and homogenized in a stomacher (Stomacher 400, Colworth) for 60 sec. The homogenized sprouts were serially diluted with 9 mL of 0.1% sterile peptone water. The 0.1 mL of each dilution was plated onto Plate Count Agar (PCA) (Oxoid, UK) for total bacterial count, Potato Dextrose Agar (PDA) (Oxoid, UK) for yeast and mould nd *E. coli*/Coliform Agar (ECA) (Oxoid, UK) for *E. coli* and coliforms. PCA and PDA were incubated at 32-35°C for 48 and 72 h, respectively while ECA was incubated for 24 h at 37°C. Each microbial count was the mean of triplicate determinations and was expressed as log CFU/g. Results for microbial reduction were determined by comparing treatment results with control using sterile deionised water (Water).

Isolation and identification of microorganisms by API kit: Colonies on PCA and ECA plate from the untreated sprouts were sub-cultured to Nutrient Agar (NA) and from the PDA plate to Sabourad Agar (SBA) for 18-24 h to get a single well isolated colony before continuing with the API kits. Identification of the microbes on sprouts was determined using API 20E kits for Enterobacteriaceae and other non-fastidious Gram-negative bacteria and API Candida kits for yeasts (BioMerieux Vitek, Hazelwood, MO, USA). The kits were used according to the manufacturer instructions. Both kits were incubated at 36±2°C for 18-24 h. The APIweb™ Software containing all the API databases for automated interpretation of API strip results was used for identification of the microorganisms.

Statistical analysis: Different batches of sprouts from the same source were used for each experiment and all the experiments were repeated three times independently. Triplicate samples were taken for the microbial analysis for each independent repetition. Data was analyzed by SPSS (SPSS Inc., Version 15.0, Chicago Illinois, USA) using one way Analysis of Variance (ANOVA) and Tukey post hoc test with a level of significance at $p<0.05$.

RESULTS AND DISCUSSION

Effect of sanitizers on the reduction of native microflora of mung bean sprouts: Figure 1 shows the effect of the treatment solutions on the reduction of total bacterial count. No significant differences ($p>0.05$) were found when sprouts were treated with all the sanitizer solutions compared to the sterile deionised water (Water) as control. The highest reduction was achieved with sanitizer A which reduced the total bacterial count by only 0.3 log CFU g^{-1} when compared to control.

The total surviving population of yeast and moulds on the sprouts after treatment are showed in Fig. 2. Population of yeasts and moulds for control was 7.67±0.15 log CFU g^{-1}, decreased to a non significant reduction of 0.15 and 0.11 log CFUg^{-1} when treated with sanitizer A and B. Moreover, there were also no significant differences ($p>0.05$) found compared to the control when sprouts were treated with sanitizers C, D and E.

Figure 3 shows population of *E. coli* and coliforms on the control and treated sprouts. The population of *E. coli* and coliforms was not greatly affected by most of the washing solutions as the mean reduction was ≤0.13 log CFU g^{-1} observed for all the treatments.

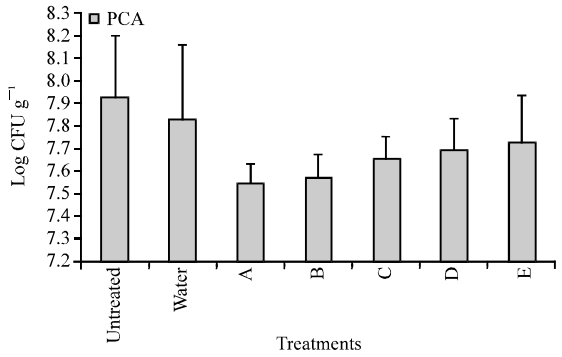

Fig. 1: Total bacterial count (mean±SD) on mung bean sprouts after treatment with different sanitizer solutions (minimum concentration)

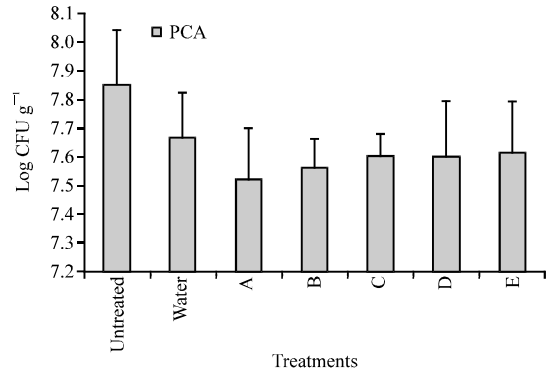

Fig. 2: Population of yeast and mould (mean±SD) on mung bean sprouts after treatment with different sanitizer solutions (minimum concentration)

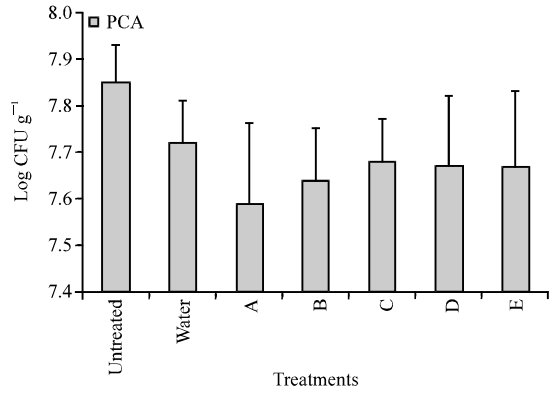

Fig. 3: Population of *E. coli* and coliforms (mean±SD) on mung bean sprouts after treatment with different sanitizer solutions (minimum concentration)

The initial *E. coli* and coliform population of 7.72±0.09 log CFU g^{-1} was reduced to 7.59±0.17 log CFU g^{-1} when treated with sanitizer A which shows the reduction of only 0.13 log CFU g^{-1}. The *E. coli* and coliform populations was also not reduced significantly ($p>0.05$) on sprouts treated with other sanitizers.

Treatment with sanitizers A and B showed significant differences ($p<0.05$) when compared to the untreated sprout for all the three types of microorganisms tested. However, washing with sterile deionised water alone did not show any significant differences ($p>0.05$) in reducing the microbial population on the sprouts compared to the untreated sprout.

Identification of microorganisms by API kit: The identification of representative colonies from the untreated mung bean sprouts using API kits demonstrated that three types of microorganisms; *Rahnella aquatilis*, *Mannheimia haemolytica* *Pasteurella haemolytica* and *Trichosporon mucoides* were isolated from the mung bean sprouts as shown in Table 2 and 3, respectively. *E. coli* was not detected or isolated from sprouts purchased from the store prior to use.

Results from this study showed that all the tested sanitizer solutions using the recommended concentration by the manufacturers did not give encouraging results as expected. All the sanitizers showed low efficacy in their ability to reduce the microbial population on the sprouts. Many studies have shown the efficacy of other treatments in reducing pathogen and microbial populations in sprouts and other vegetables (Issa-Zacharia *et al*., 2011; Mahmoud *et al*., 2010; Wu and Kim, 2007; Kim *et al*., 2006). Among the treatments found to be effective in reducing contamination in both seeds and sprouts are the application of high pressure treatment (Penas *et al*., 2008) and treatment using temperature and antimicrobial products (Penas *et al*., 2010).

Many factors that may contribute to the low efficacy of the sanitizers in reducing the microbial population on the sprouts are such as the ineffective active ingredients used in the formulation or the low concentration used for washing as recommended by the manufacturer. Increasing the concentration of sanitizer may have raised its efficacy in microbial reduction as a higher level of active ingredients will act upon the bacterial cell by in-activating or loosening its attachment on the sprouts more efficiently (Suslow, 2001). Furthermore, natural openings or cut surfaces on the plant tissue may serve as entry points as well as shelter for the microbes, in those cases where microorganisms are largely unaffected by the sanitizer solutions. Consequently, sufficient sanitizer is needed to eliminate the microorganisms attached firmly to the sprouts (Suslow, 2001).

The sanitizer effect was also influenced by the kind of vegetables used. In the earlier study, the use of the

Table 2: API 20E results for identification of microorganisms

Tests	Reactions/Enzymes	Results	
ONPG	β-galactosidase	+	-
ADH	Arginine dihydrolase	-	-
LDC	Lysine decarboxylase	-	-
ODC	Ornithine decarboxylase	-	-
CIT	Citrate utilization	-	-
H_2S	H_2S production	-	-
URE	Urease	-	-
TDA	Tryptophane deaminase	-	-
IND	Indole production	-	-
VP	Voges Proskauer	+	-
GEL	Gelatinase	-	-
Fermentation/Oxidation			
GLU	Glucose	+	-
MAN	Mannitol	+	-
INO	Inositol	-	-
SOR	Sorbitol	+	-
RHA	Rhamnose	+	-
SAC	Saccharose	+	-
MEL	Melibiose	+	-
AMY	Amygdalin	+	-
ARA	Arabinose	+	-
OX	Cytochrome-Oxidase	-	-
NO_2	NO_2 production	NT	+
N_2	Reduction to N_2 gas	NT	-
MOB	Motility	NT	-
McC	Growth on MacConkey medium	NT	-
OF-O	Glucose oxidation: exposed to the air	NT	+
OF-F	Glucose fermentation: under mineral oil	NT	-
ID %		98.9	97.9
Identified microorganism		*Rahnella aquatilis*	*Mannheimia haemolytica* (*Pasteurella haemolytica*)

NT = Not Tested; +: Positive reaction; -: Negative reaction

Table 3: API Candida result for identification of microorganism

Tests	Reactions/Enzymes	Results
Acidification		
GLU	Glucose	+
GAL	Galactose	+
SAC	Saccharose	+
TRE	Trehalose	+
RAF	Raffinose	+
β MAL	β-Maltosidase	-
α AMY	α-Amylase	+
β XYL	β-Xylosidase	-
β GUR	β-Glucuronidase	+
URE	Urease	+
β NAG	N-Acetyl-β-Glucosaminidase	+
β GAL	β-Galactosidase	+
ID %		99.7
Identified microorganism		*Trichosporon mucoides*

+: Positive reaction; -: Negative reaction

same sanitizer on mung bean seeds, showed a significant reduction of microorganisms attached on the seeds (Suraiami and Wan Nazaimoon, 2010). The different results obtained when using sprouts compared to its seeds is due to the complex physiological structure of sprouts which has enabled more microorganism cells to be trapped in the cuticle layer. These may not have been released into the peptone water when treated with sanitizers and rinsed with water and therefore would not have been counted which contributed to the poor reduction in count (Kenney and Beuchat, 2002).

The presence of biofilms on sprouts may also contribute to the inability of the sanitizers tested to reduce the microbial population. Biofilms which are found present on the different varieties of sprouts including mung bean sprouts grown in the dark (Fett, 2000; Fett and Cooke, 2005) are more resistant to the action of sanitizers than the planktonic forms (Velazquez *et al*., 2009). Other possible reasons for the low reduction in counts may be due to the less rigorous techniques or procedures used in this study when treating the sprouts.

The microorganisms found on the sprouts were *Rahnella aquatilis*, *Mannheimia haemolytica* (*Pasteurella haemolytica*) and *Trichosporon mucoides*. Basically, the microorganisms that adhere to the surface of the freshly harvested vegetables are mainly gram-negative saprophytes. Gram-positive bacteria on the other hand are mainly acquired by leafy vegetables when they come into contact with the soil (Seo *et al*., 2010).

A member of the family Enterobacteriaceae, *Rahnella aquatilis* was first isolated from freshwater in 1976 by Gavini and his colleagues at the Institute Pasteur and the species was called aquatilis because of its natural habitat is water (Tash, 2005). Nevertheless, it has also been isolated from the fruits and leaves of apples (Calvo *et al*., 2007), soil (Zdorovenko *et al*., 2004) in lager beer breweries (Hamze *et al*., 1990) and also biological specimens such as blood, surgical wounds, urine, sputum, bronchial washings and stool (Tash, 2005).

The other species found on sprouts was *Mannheimia haemolytica* which was formerly known as *Pasteurella haemolytica*. It is the new taxonomic classification as suggested by Angen *et al.* (1999). A study by Yilmaz and Kaya (2009) managed to isolate *Mannheimia haemolytica* from the UHT milk. The bacteria had changed the physical appearance of the milk so that it was thicker and had clotted. However, no published article has highlighted its presence in sprouts.

Yeast on the other hand is the most common organisms found on sprouts (Toumas, 2005; Abadias *et al*., 2008). The yeast *Trichosporon mucoides* was not only found on the mung bean sprouts as shown in this study but it was also isolated from vegetation, soil, water samples (Chaturvedi and Ren, 2004) and are occasionally found as normal flora of the skin and mouth (Nettles *et al*., 2002).

The presence of the three microbial species on sprouts was probably due to contaminated water or apparatus used during sprouting and handling. The moist and humid environment may have been conducive for their growth on the sprouts. It should be noted that there are more than three species of microorganisms attached to the sprouts but the API kits used are only able to detect Enterobactericiae and yeast and mould. Due to this reason, many microorganisms in the sample gave unacceptable, doubtful or low discrimination profiles. Unacceptable profiles may suggest that the species was not in the data index because the microorganisms were not a Gram-negative bacteria or yeast and mould. Whereas, doubtful and low discrimination profiles may occur if some of the tested characteristics did not match with the expected species in the data index (Kofli and Dayaon, 2010).

Although, the microorganisms isolated from the sprouts did not cause food-borne diseases, the etiology of the microorganisms was not fully understood because of the lack of data and studies done. Several steps should be taken to ensure that the sprout is safe enough to consume especially when eaten raw.

CONCLUSION

This study indicates that the use of water on its own and the minimum concentration of sanitizer solutions used as recommended are unable to significantly reduce the population of the native microflora on the mung bean sprouts such as *Rahnella aquatilis*, *Mannheimia haemolytica* (*Pasteurella haemolytica*) *Trichosporon mucoides* and other unidentified native microflora on the sprouts. Therefore, further investigation is needed to determine the effectiveness of these sanitizers to overcome such problems. The problems may be solved by using higher concentration or by using different methods or approaches of sanitizing such as by massaging using hands instead of by shaking. Furthermore, further studies on the DNA analysis of the isolated microorganisms need to be done for confirmation of the species.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Health Malaysia (MOH) (JPP-IMR 09-011). The researchers wish to thank the Director General of Health, Deputy Director General of Health (Research and Technical Support), the Director of IMR for permission to publish these findings and to Dr. Wan Nazaimoon Wan Mohamud for her continuous support. The assistance of Husniza Hussain with the API kits, Ismahnur Azua Ismail, Nor Diana Zubir, Isma Liyana Ismail for technical support and Dr. Harvindar Gill for editing is greatly acknowledged.

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