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Reduction of microbial counts during kitchen scale washing and sanitization of salad vegetables

James Noah Ssemanda [a, b], Han Joosten [a], Mark Cyubahiro Bagabe [c], Marcel H. Zwietering [a], Martine W. Reij [a, *]

[a] Laboratory of Food Microbiology, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[b] Rwanda Standards Board, KK 15 Rd, 49, P.O. Box: 7099, Kigali-Kicukiro, Rwanda
[c] Rwanda Agriculture Board, KG 175 St, P. O. Box 5016, Kigali-Remera, Rwanda

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Abbreviations:
L
Litres
FSEs
Food service establishments
NaDCC
Sodium dichloroisocyanurate
OWA
Organic washing aid
SDBS
Sodium dodecylbenzenesulfonate
AA
Acetic acid (2%)
$KMnO_4$
Potassium permanganate
SP
Sanitizing powder
NaClO
Sodium hypochlorite

ABSTRACT

Washing with or without sanitizers is one of the important steps designated to reduce or eliminate microbial hazards in fresh vegetables but the settings, conditions and effectiveness of this step remain contentious. In this study, we investigated kitchen scale salad preparation practices in a field study in Rwandan food service establishments (FSEs) and conducted laboratory trials to identify treatments that can improve reduction of microbial counts during washing and sanitization. In the field study, vegetable samples ($n = 112$) were taken from 56 FSEs before and after washing with or without sanitizer(s) to determine reduction of counts of *Enterobacteriaceae*, *Listeria* spp., and coagulase positive (CP)-staphylococci coupled with observation of the salad preparation practices from start to end. Based on the results obtained during the field study, 8 sanitizers were evaluated in the laboratory to optimize the efficacy of washing of leafy vegetables (corn salad, *Valerianella locusta*). Findings in the field study revealed that about 61% of the visited FSEs used sanitizers during washing of fresh vegetables, in particular, potassium permanganate ($KMnO_4$) in 39% of FSEs, sanitizing powder (a mixture of polyphosphate, sodium hydrogen carbonate and active chlorine), 13%; sodium hypochlorite (NaClO), 7%; and sodium dichloroisocyanurate (NaDCC) in 2%. Average inactivation ranged from 1.0 log ($KMnO_4$) to 3.1 log (NaDCC). In the laboratory study, average inactivation observed with *Listeria* spp., *Escherichia coli* and Aerobic plate count (APC) ranged from 0.7 log (water alone) to 3.0 log (NaDCC). Out of the 8 sanitizers that were evaluated, 5 sanitizers (NaDCC [90 ppm], NaClO [200 ppm], lemon juice [98%], acetic acid [2%] and sanitizing powder [4 g/L]) resulted in significantly higher inactivation compared to water alone. A contact time of 5 min and salad-sanitizer ratio of 1: 20 were considered optimal for kitchen based washing of the studied leafy vegetables with NaDCC and NaClO sanitizers.

1. Introduction

A diet rich in vegetables has been associated with health benefits like reduced risk to cancers and cardiovascular diseases (He, Nowson, Lucas, & MacGregor, 2007, pp. 717–728; Liu, 2004). Consumption of fresh vegetables is increasing year by year (FAO STAT, 2015) and reports indicate that a large portion of these

* Corresponding author.
E-mail addresses: james.ssemanda@wur.nl, james.ssemanda@rsb.gov.rw (J.N. Ssemanda), han.joosten@wur.nl (H. Joosten), mark.bagabe@rab.gov.rw (M.C. Bagabe), marcel.zwietering@wur.nl (M.H. Zwietering), martine.reij@wur.nl (M.W. Reij).

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vegetables are consumed raw (Cook, 2011). At the same time, the number of reported foodborne illnesses linked to fresh vegetables has been increasing. In the United States between 1998 and 2008, produce (fresh vegetables and nuts) accounted for 46% of foodborne illness (Painter et al., 2013) while a high number of outbreaks has also been reported in the European Union (Callejon et al., 2015). Etiological agents range from pathogenic bacteria (Callejon et al., 2015; Soon, Seaman, & Baines, 2013) to parasites (Bohaychuk et al., 2009) and viruses (Callejon et al., 2015; Laura et al., 2012).

Washing with water is a crucial postharvest step designated to reduce or eliminate field dirt and their associated microorganisms from fresh vegetables but this step also increases the chances for microbial hazards to spread in the entire batch (Sapers, 2009; Warriner & Namvar, 2014). Chemical sanitizers can be added to increase the efficacy *i.e.* by preventing cross contamination (Banach et al., 2017), but maximum reduction rates are typically around 3 logs (Warriner & Namvar, 2014). To date, efforts to further improve washing and sanitization of fresh vegetables are ongoing especially in countries with a developed commercial fresh cut industry (Abadias, Usall, Oliveira, Alegre, & Vinas, 2008; Davidson, Buchholz, & Ryser, 2013; Holvoet, Jacxsens, Sampers, & Uyttendaele, 2012; Kinsinger, Mayton, Luth, & Walker, 2017).

Such efforts include pilot and laboratory studies to evaluate the efficacy of different vegetable sanitizers and washing techniques. In countries with limited fresh cut industry, the washing and sanitization of fresh vegetables is mainly done in kitchens of food service establishments (FSEs) and households during salad preparation. However, it has been acknowledged that commercial washing and sanitization conditions are not suitable for food service or home use, because the users lack technical skills, knowledge, and equipment to apply treatments safely and effectively (Sapers, 2009). So far few studies (Amoah, Drechsel, Abaidoo, & Klutse, 2007; Beuchat, Harris, Ward, & Kajs, 2001; Kilonzo-Nthenge, Chen, & Godwin, 2006) have targeted kitchen based washing and sanitization of fresh vegetables, but also do not compare microbial inactivation in the field (FSEs or households) and in the laboratory.

In this study, we seek to identify sanitizers, conditions, treatments and techniques that enable targeted microbial reduction during washing and sanitization of fresh vegetables to propose guidelines for FSEs and households. Our study consisted of a field study in which practices for preparing vegetable salads were investigated with a focus on microbial inactivation and a laboratory simulation of the washing and sanitization of vegetables in FSEs based on the results from the field study to identify alternatives for improvement. The field study was conducted in Rwanda, a country where vegetable washing and sanitization is mainly done in FSEs and household level (Ssemanda et al., 2017).

2. Materials and methods

2.1. Field study

2.1.1. Study description and sampling

The field study was conducted in food service establishments (FSEs) in Rwanda from February to October 2015. We interacted face to face with managers and food handlers in FSEs during salad vegetable preparations, observed the unit operations (especially washing and sanitization steps) and took samples of vegetables for microbiological analysis. Efficacy of washing and sanitization of fresh vegetables in FSEs was evaluated based on the changes in counts of indicator microorganisms; APC (aerobic plate count), *Enterobacteriaceae*, *Listeria* spp. and coagulase positive staphylococci (CP-staphylococci).

FSEs were prepared for the study in a way reported in our previous related study (Ssemanda et al., 2017). In summary, out of the 280 FSEs managers invited, 168 showed interest and we were able to cover 56 of these FSEs in this study. Each FSE provided 2 samples, one sample (1–2 kg) of whole mixed vegetables was taken before washing and another sample (0.5–1 kg) after washing treatment, before cutting. Vegetables commonly used for salad making were beet root, cabbage, carrot, celery, cucumber, garlic, green pepper, lettuce, onion, parsley and tomato. Using sterile hand gloves, the 2 samples were placed and closed in sterile plastic zip bags and thereafter, all samples were stored in cooling boxes with ice packs and transported for 1–3 h to the laboratory and analyzed immediately.

2.1.2. Microbiological analysis

The 1–2 kg whole vegetable samples from FSEs as described in section 2.1.1 of different types were sliced/cut into small pieces (Mukherjee, Speh, & Diez-Gonzalez, 2007) on a sterile stainless steel tray using sterilized knives and gloves for each sample and thereafter mixed. Then 25 g of analytical unit of these samples were thereafter stomached (Model 400 Circulator, Seward, UK) in 225 mL of maximum recovery diluent (MRD) for 1 min. Thereafter, tenfold serial dilutions were prepared using MRD for the enumeration of *Enterobacteriaceae*, APC and CP-staphylococci and buffered peptone for *Listeria* spp. The culture media and consumables used were from Oxoid (Oxoid Ltd., Basingstoke, UK). The enumeration was conducted according to ISO methods *i.e.*; *Enterobacteriaceae*, ISO 21528-2, 2004; APC, ISO 4833-1, 2013; CP-staphylococci, ISO 6888-2, 1999; and *Listeria* spp., ISO 11290-2: 1998/Amd 1, 2004. For quality control of the media and positive controls of the experiments in the field study, the following strains from the Belgian Coordinated Collection of Microorganisms were used: *i.e. E. coli* (LMG 8063) for *Enterobacteriaceae*, *Listeria monocytogenes* (LMG 16783) for *Listeria* spp. and *Staphylococcus aureus* (LMG 8224) for CP-staphylococci.

2.2. Laboratory study

2.2.1. Preparing vegetables for the laboratory study

Corn salad (*Valerianella locusta*) was selected in this study because leafy vegetables are known for their high microbial attachment (Berger et al., 2009) and because they are eaten raw, easy to handle (require no cutting during washing) and available year round. For every experimental set up, prewashed, ready to eat corn salads in unit plastic packages of 75 g were purchased from local supermarkets in Wageningen. At the start of every experiment, samples of corn salad were taken for microbial analysis before artificial contamination (inoculation) to examine the counts of *Listeria* spp., *Escherichia coli*, and APC originally present.

2.2.2. Preparing strains and inoculum

Unless stated otherwise, the strains of nonpathogenic *E. coli* and *Listeria* spp. used in this study were from the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures. *E. coli* strains were DSM 498, DSM 1756 and O2K (from Laboratory of Food Microbiology, Wageningen University). *Listeria* spp. strains were *L. seeligeri* (DSM 20751), *L. welshimeri* (DSM 20650), *L. innocua* (DSM 20649). Methodology for preparing of the inoculum was based on a previous studies (López-Gálvez, Gil, Truchado, Selma, & Allende, 2010; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999) with slight modifications. The stock culture of each bacterial strain from cryovials (−80 °C) were streaked separately on brain heart infusion (BHI) agar (Oxoid Ltd., Basingstoke, UK) and incubated at 30 °C for 24 h. Thereafter, a colony from each strain was inoculated in 100 mL BHI broth and incubated at 30 °C for 24 h with agitation at 160 rpm. The cell cultures (10 mL each) were transferred into sterile tubes and concentrated by centrifugation at 11000×*g* for