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Liquid and vapor-phase vinegar reduces *Klebsiella pneumoniae* on fresh coriander

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ABSTRACT

Samples of fresh coriander were sourced from several markets in the Ladkrabang and Minburi areas of Bangkok, Thailand. These were examined in the laboratory for fecal coliform contamination. The bacterium *Klebsiella pneumoniae* was the predominant contaminant in 48.5–84.0% of the 30 samples tested. Inhibition of surface contaminations of *K. pneumoniae* by vinegar was measured *in vitro* for either liquid- or vapor-phase exposures. For liquid-phase exposure, levels above 2.4% (v/v) acetic acid (AA) completely inhibited *K. pneumoniae*. After 50 min of vapor-phase exposure, 8% AA completely inhibited *K. pneumoniae* spread on Mueller Hilton agar (4.10 ± 0.04 log CFU/ml, 30 ± 2 °C, 80 ± 2% RH). Reduction of *K. pneumoniae* contamination on fresh coriander leaves using vinegar vapor was also examined. The effectiveness of inhibition was related to the level of *K. pneumoniae* inoculation, with vinegar vapor being shown to be an effective control agent for surface contaminations on fresh coriander so reducing the potential health risks to consumers.

1. Introduction

Coriander can be used in the kitchen as a vegetable, as a herb or as a spice. It can be consumed raw (fresh or dried) or added to food after cooking depending recipe. Due to the internal and external morphologies of coriander leaves, these can harbor specific groups of microbial contaminants (Beuchat, 2002). Many reports note occurrences of fecal coliform contamination on the leaves and other parts of these plants. Sometimes these arise from the soil in which they are grown (Kaneko et al., 1999; Zhao, Clavero, Doyle, & Beuchat, 1997). Coriander itself offers no antimicrobial activity against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* or *Salmonella Anatum* (Shan, Cai, Brooks, & Corke, 2007). On the other hand, the extracted coriander oil can be an effective antimicrobial agent against some food-borne pathogenic bacteria including *S. aureus*, *E. coli*, *L. monocytogenes*, *Pseudomonas aeruginosa* (Delaquis, Stanich, Girard, & Mazza, 2002; Singh, Kapoor, Pandey, Singh, & Singh, 2002).

Next after *E. coli*, the rod-shaped bacterium *Klebsiella pneumoniae* is widely recognized as the agent responsible for the second most common Gram-negative bloodstream infection in human populations (Meatherall, Gregson, Ross, Pitout, & Laupland, 2009; Wong, Cullimore, & Bruce, 1985). *K. pneumoniae* belongs to the same family of Enterobacteriaceae as *E. coli* and is found widely in the environment and also in the intestinal tracts of mammals. It can cause many diseases such as ventilator-associated pneumonia (Pawar et al., 2003), septicemia and various soft-tissue infections (Yang, Lauderdale, & Lo, 2004). Virtually, no reports exist on the incidence of *K. pneumoniae* on coriander, but there are reports of contamination by this bacterium on other foods such as in orange juice concentrate (Fuentes, Hazen, López-Torres, & Rechani, 1985), dried bush okra and African spider herb (Mpuchane & Gashe, 1996), on a hamburger in Houston, Texas (Sabota et al., 1998), raw and ready-to-eat foods from restaurants in Spain (Soriano, Rico, Moltó, & Mañes, 2001), fruit juices sold in Tripoli–Libya (Ghenghesh, Belhaj, El-Amin, El-Nefathi, & Zalmum, 2004) and street foods sold in Malaysia (Haryani et al., 2007). Some study has been reported that *K. pneumoniae* is the predominant fecal coliform found in frozen orange concentrates (Fuentes et al., 1985).

A large number of sanitizers and disinfectants, including hydrogen peroxide, chlorine and quaternary ammonium compounds have been used for reduction of total mesophilic bacteria and coliforms (Lee, Park, & Ha, 2007). However, health concerns are increasingly being focused on these substances due to their risk

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potential arising of carcinogenic residues. Concern is also being expressed because of their decreasing antimicrobial effectiveness. Therefore, alternative, high activity, sanitizers and disinfectants are continually being sought. One of these, which is of special interest to us is vinegar (acetic acid). It is biologically and environmentally friendly and also safe, yet it has excellent sanitizing properties (Meatherall et al., 2009; Sholberg, Haag, Hocking, & Bedford, 2000). Many investigators have reported on the effective sanitizing properties of acetic acid and vinegar in liquid form which is already used for limiting microbial contaminants on fresh produce such as in apples, tomatoes, carrots, stone fruits, lettuces and strawberries (Chang & Fang, 2007; Kilonzo-Nthenge, Chen, & Godwin, 2006; Sengun & Karapinar, 2004; Sholberg et al., 2000). The vapor of vinegar has also been shown to exhibit antibacterial and antifungal properties in many food products such as eggs, tomatoes, apples, apricots, lettuces and strawberries (Krusong, Dansai, & Itharat, 2012; Sholberg et al., 2000; Tzortzakis, 2010).

The aims of this work were: (1) to investigate the extent of *K. pneumoniae* contamination on fresh coriander purchased in the marketplace, (2) to determine if *K. pneumoniae* can be inhibited by exposure to liquid- and vapor-phase vinegar, and (3) to determine if vinegar vapor is effective as a control agent for *K. pneumoniae* inoculation on fresh coriander leaves.

2. Materials and methods

2.1. Materials

Fresh coriander (*Coriandrum sativum* L.) was purchased from a local market. Upland rice wine vinegar (URV) containing 8 ± 0.1% (v/v) of acetic acid was produced at the Laboratory of Fermentation Technology, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Filter paper discs (6 mm diameter) were used *in vitro* to expose *K. pneumoniae* to URV liquid.

2.2. Microbial survey of market-fresh coriander and leaf surface morphology

Thirty samples of fresh coriander were obtained from local markets in the Ladkrabang and Minburi areas of Bangkok, Thailand. These were purchased randomly between November 2013 and January 2014. Samples were held at 3–5 °C after purchase and transported to the laboratory for analysis within 2 h for total aerobic bacteria and for *K. pneumoniae*. Leaf surface morphology was also examined using a scanning electron microscope (SEM).

2.3. Sample preparation and microbiological method

Removal of soil and foreign matter, the samples were first washed with sterile distilled water for 5 min before drying aseptically in laminar airflow for 5 min. Any wilted leaf parts were aseptically removed using scissors and discarded. The unwilted remains were aseptically shredded and the leaves, stems and roots were combined and mixed together well. For analysis, a 25 g sample of shredded material was placed with 225 ml of sterile 0.1% peptone water (PW) in a sterile plastic bag in which it was homogenized for 2 min using a stomacher (Model BA 7021, Seward, UK). Sub-samples of the resulting suspension were serially diluted using sterile 0.1% PW and analyzed for total aerobic bacteria using Trypticase Soy agar (TSA) by a spreading technique. Prior to assessing the *K. pneumoniae* in the samples, it was necessary first to determine the total coliform and the fecal coliform levels in the serially-diluted suspensions using the procedures described by Gauthier and Archibald (2001). These were as follows: total coliform and fecal coliform were counted by the MPN (most probable number) method using lauryl tryptose broth MPN tubes and brilliant-green lactose bile broth for presumptive and confirmative tests, respectively. Typical colony isolates (Gram-negative rods) were selected randomly from MacConKey agar (Merck) plates of the completed confirmative tests. The species of each isolate was identified by the standard Rapid ID 32E biochemical reaction profile (Biomérieux). After this, counting of *Klebsiella pneuminiae* was done by spreading 0.1 ml the serially diluted suspension on MacConKey-inositol-carbenicillin as described by many investigators (Tomás, Ciurana, & Jofre, 1986).

2.4. In vitro susceptibility of K. pneumoniae to the liquid and vapor of URV

Pure cultures of selected *K. pneumoniae* isolates were confirmed by standard Rapid ID 32E (Biomérieux) and maintained on trypticase soy agar (TSA) slants at 3–5 °C. Inoculum prepared from these was spread on TSA plates overnight at 37 °C before suspending several colonies in trypticase soy broth (TSB). The suspended culture was diluted with sterile 0.1% PW until achieving McFarland 1.0 (approximately 7 log CFU/ml) by spectrophotometer (UV-1601 Shimadzu, Japan) and used as inoculum.

The susceptibility of *K. pneumoniae* to liquid-phase URV was examined *in vitro* using the agar overlay disc diffusion method with slight modification of the procedures described by Chaudhry and Tariq (2006). A 20 μl volume of inoculum was suspended in 5 ml of sloppy Mueller Hinton agar (MHA; Merck) before spreading this on a plate containing 10 ml of already-set MHA agar and allowing the whole to set for 20 min at room temperature. The sterilized disc filters were soaked for 2 min in 1 ml of diluted URV containing: 0, 1, 2, 3, 4, 5 or 8% (v/v) acetic acid (AA). The soaked disc filters were then placed aseptically on the surfaces of inoculated MHA plates. After incubation at 37 °C for 24 h, the inhibition zones around the discs were measured.

The susceptibility of *K. pneumoniae* to direct, liquid-phase contact with URV was examined *in vitro*. The test was conducted in sterilized TSB acidified with URV containing AA in the selected concentration range resulting from the *in vitro* inhibitory effect on *K. pneumoniae* by the agar overlay disc diffusion test. The lowest AA content with step-wised 0.1% (v/v) increments were prepared aseptically into sterilized TSB by calculation, based on the known AA content of URV (8%, v/v) to give a final volume of 10 ml in each tube. A volume of 1 ml of *K. pneumoniae* inoculum containing 7 log CFU/ml was then inoculated into each tube. The pH of each AA tube was also measured. An interval of 10 min contact time at 37 °C was allowed before the residual viability of *K. pneumoniae* in each acidified TSB tube was assayed by spreading over a plate of solid MHA agar followed by incubation at 37 °C for 24 h. Susceptibility to direct, liquid-phase contact with URV was expressed in terms of fractional (%) inhibition.

Susceptibility of *K. pneumoniae* to URV vapor was also investigated *in vitro* using a vapor exposure box. This comprised a plastic box (0.25 × 0.30 × 0.25 m) with a slide cover and vents to prevent pressure build up during vapor treatment. The URV vapor source incorporated a pumped air stream of ambient air, bubbled through a 1000 ml glass bottle containing URV (acetic acid content 8% v/v, at room temperature 30 ± 2 °C). The URV vapor in the headspace of the bottle was delivered to a vapor spreader manifold in the vapor exposure box. The rate of vapor production was calculated from the rate of weight loss of the URV in the bottle as 0.042 ± 0.002 g URV/min. For the test, 20 μl of inoculum was spread on a plate of 10 ml MHA agar and allowed to solidify for 20 min at room temperature. The inoculated plates (without covers) were aseptically transferred to a sterile perforated stainless steel rack. Six durations of vapor