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Growth inhibition of *Klebsiella quasipneumoniae* contamination on fresh-cut mango by vapor-phase vinegar and pure acetic acid

Mintita Nathong[1], Aphacha Jindaprasert[2], Wiramsri Sriphochanart[2] and Warawut Krusong[2,*]

[1]*Master student of Department of Food Safety Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

[2]*Division of Fermentation Technology, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand*

** Corresponding author; email:warawut.kr@kmitl.ac.th*
DOI:

ABSTRACT

The purposes of this study were to survey of coliform contamination on fresh-cut mango and to investigate the effect of vapor-phase (V) upland rice vinegar (URV) and pure acetic acid (PAA) on growth inhibition of coliform contaminant on fresh-cut mango. Thirty samples were taken from local markets-Ladkrabang and Minburi areas of Bangkok, Thailand for examination. The predominant contaminant in 76.7% of samples tested with the same colony characteristic on the MacConkey agar plates were found. By 16S rDNA sequence determination, it was clearly identified as *Klebsiella quasipneumoniae*. Treatment of V-URV and V-PAA on inhibition of *Kb. quasipneumoniae* were further investigated. After plating with 3 log CFU/ml (low level) and 6 log CFU/ml (high level) of *Kb. quasipneumoniae* on Trypticase Soy Agar, they were exposed to of V-URV or V-PAA in vapor exposure box (0.25x0.35x0.25 m) at room temperature (30±1 °C). Results showed that vaporization period inhibitory affected directly on the growth of *Kb. quasipneumoniae*. Complete growth inhibition of the low level after exposing with V-URV for 35 min (0.87 $mmolL^{-1}$ AA) as well as with V-PAA for 35 min (0.80 $mmolL^{-1}$ AA) were achieved. Meanwhile complete growth inhibition of the high level

after exposing with V-URV for 40 min (0.99 mmolL^{-1} AA) as well as with V-PAA for 40 min (0.91mmolL^{-1} AA) were achieved. Results demonstrated that both V-URV and V-PAA are appropriable for applying as a bio-control agent for reducing coliform contamination of fresh cut products.

Keywords: fresh-cut mango, *Klebsiella quasipneumoniae*, vapor phase, vinegar, pure acetic acid

INTRODUCTION

Klebsiella spp. is ubiquitous in nature. They probably have two common habitats, one being the environment, where they are found in surface water, sewage and soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horse or swine, *Klebsiella* is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to a serve pyogenic infection which has a high fatality rate if untreated. The vast majority of *Klebsiella* infections are associated with hospitalized and suffer from serve underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. (Podschun and Ullmann, 1998)

Normally, fresh-cut fruits are susceptible to microbial contamination in any phase of the production or distribution. The destruction of natural protective barriers, high water and nutrient contents of fruits cause to support the microbial pathogens growth. Additionally, they are neither heat treated nor contain added preservatives. As a result, They may cause consumer health's problems.

Outbreaks caused by foodborne pathogens (FBP) have been found in minimally processed produce. FBP found are *Escherichia coli* O157:H7 (Ackers et al.,1998), *Salmonella* spp. (Lin et al.,1996; Salleh et al., 2003) and *Listeria monocytogenes* (Beuchat,1996). Fresh apple products, especially juices, have been associated with outbreaks of illness caused by *E. coli* O157:H7 (Burnett and Beuchat, 2000; Dingman, 2000) and several studies have shown that *E. coli* O157:H7 can survive and grow on fresh apple tissues stored in air (Dingman, 2000; Fisher and Golden, 1998; Gunes and Hotchkiss, 2002; Janisiewicz et al., 1999). In addition, *Salmonella* spp. has been implicated in human illnesses that have been

associated with consumption of apple cider and unpasteurized orange juices (CDC, 1975, CDC, 1995, CDC, 1999; Krause et al., 2001).
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 et al., 2007). However, their risk potential arising of carcinogenic residues is increasingly being focused. Therefore, an alternative sanitizers and disinfectants with high activity are continually being sought. One of these, which is of special interest to us is vinegar (acetic acid). It is biologically and environmental friendly and also safe, yet it has excellent sanitizing properties (Meatherall et al., 2009; Sholberg et al., 2000). Many investigators have reported on the effective sanitizing properties of liquid acetic acid and vinegar 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 et al., 2006; Sengun & Karapinar, 2004; Sholberg et al., 2000). The vapor-phase of vinegar has also been shown to exhibit the antibacterial and antifungal properties in many food products such as eggs, tomatoes, apples, apricots, lettuces and strawberries (Krusong et al., 2012; Sholberg et al., 2000; Tzortzakis, 2010). The aim of this work were to investigate the extent of coliform contamination on fresh-cut mango in the marketplace, and to investigate the effect of vapor-phase upland rice vinegar and pure acetic acid on growth inhibition of the dominate *Kb. quasipneumoniae* contamination on fresh-cut mango.

MATERIAL AND METHODS

Materials

Fresh-cut mango was purchased from a local market. Upland rice vinegar (URV) containing 8±0.1% of acetic acid was provided from Laboratory of Fermentation Technology, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Pure acetic acid (PAA) was obtained from Merck KGaA (Darmstadt, Germany). The medium used for inoculum preparation and for viability assessment was Trypticase Soy Agar (TSA; Difco, France)

Microbial survey of market-fresh-cut mango

Thirty samples of fresh cut mango from local markets in the Ladkrabang and Minburi areas of Bangkok, Thailand were randomly purchased. Sample were held at 3-5 ºC after purchasing and transported to the laboratory for analysis within 2 h for total aerobic bacteria count.

Microbiology method

25 g sample of shredded material was placed with 225 ml 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). By serial dilution with sterile 0.1% PW, sub-samples of the resulting suspension were analyzed for the total coliform and the fecal coliform using the procedures described by Gauthier and Archibald (2001). 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 test, 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 identified by Single strand 16S rDNA sequencing.

In vitro susceptibility of *Klebsiella quasipneumoniae* to vapor-phase of upland rice vinegar (V-URV) and pure acetic acid (V-PAA)

Pure culture of selected *Kb. quasipneumoniae* isolates was confirmed by single strand 16S rDNA sequencing. It was maintained on Trypticase soy agar (TSA) slants at 3-5 ºC. Inoculum preparation, it was spread on TSA plates overnight at 37 ºC 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 8 log CFU/ml) by spectrophotometer (UV-1601 Shimadzu, Japan) before use.

The *in vitro* susceptibilities of *Kb. quasipneumoniae* to V-URV and V- PAA were investigated in a vapor exposure plastic box (0.25x0.30x0.25 m) (see Fig. 1) with a slide cover to prevent build up during vapor treatment (Krusong *et al.*, 2016). Two levels of *Kb. quasipneumoniae* inoculum were prepared consisting of low level (3 log CFU/ml) and high level (6 log CFU/ml). A volume of 1 ml of *Kb. quasipneumoniae* was spread on a plate of TSA (with no cover) and, then, placed on sterile steel rack in the box. The evaporated vapor phase was applied by pumping the ambient cleaned air to liquid URV (8% acetic acid content, 500 ml in volume) and PAA (8% v/v, 500 ml in volume) in a 1,000 ml sterile closed

bottle. The delivery of V- URV and V-PAA from the headspace of the bottle was pump to the box. Eight exposure periods of each vapor-phase substance for 0, 10, 20, 30, 35, 40, 50 and 60 min were used. After vapor treatment all plates were covered and incubated at 30±1 °C for 22-24 h. The number of *Kb. quasipneumoniae* was recorded and the susceptibility was expressed as the proportion (%) of reduction.

Analytical methods

The acidity (%v/v) of vinegar was determined by acid-base titration with 0.1 M NaOH using phenol- phthalein as pH indicator (Fregapane et al.,2001).

Statistical analysis

The data obtained from sample were analyzed by Complete Randomized Design (CRD) variance for vapor substances. Significant differences among means were determined by using Duncan's New Multiple Range Test (DMRT) at $p \leq 0.05$. Statistical analysis was performed using the SPSS program version 17.0 for windows.

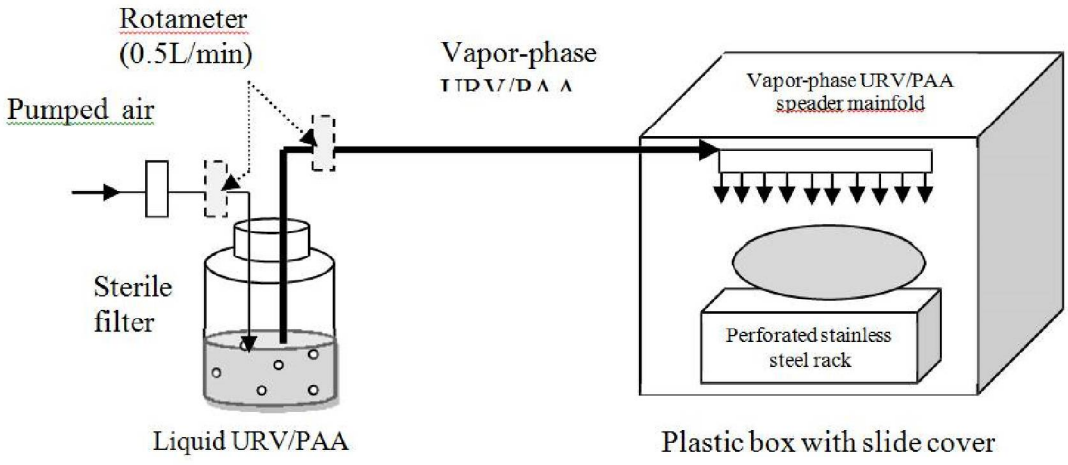

Figure 1. Schematic of the vapor exposure box (0.25x0.30x0.25 m) for exposing samples to vapor-phase upland rice vinegar (URV) and pure acetic acid (PAA) (Krusong *et al.*, 2016)

RESULTS AND DISCUSSION

The predominant contaminants in 76.7% of the 30 samples tested with the same colony characteristic on the MacConkey agar plates were found. By 16S rDNA sequence determination, it was clearly identified as *Klebsiella quasipneumoniae*.

Results *in vitro* susceptibility of *Kb. quasipneumoniae* to V-URV (Table 1) and V-PAA (Table 2) showed that vapor exposure period affected directly in inhibition of *Kb. quasipneumoniae*. Complete growth inhibition of the low level after exposing with V-URV for 35 min (0.87 mmol L^{-1} AA) as well as with V-PAA for 35 min (0.80 mmol L^{-1} AA) were achieved. Meanwhile complete growth inhibition of the high level after exposing with V-URV for 40 min (0.99 mmol L^{-1} AA) and with V-PAA for 40 min (0.91mmol L^{-1} AA) were obtained. There are many investigations report that vapour of vinegar has been shown to exhibit antibacterial and antifungal properties in many food products such as on tomatoes, grapes, sweet cherries, straw- berries, and egg (Krusong *et al*., 2012; Sholberg *et al*., 2000; Tzortzakis, 2010; Chu *et al*., 1999). Additionally, vinegar containing of 2.5 to 3.6 mol L^{-1}AA have also been reported to be used for effective inhibition of conidia of brown rot, gray mold and blue mold from germination which normally cause decay of stone fruit, strawberries and apples (Sholberg et al., 2000). These results are belonged to the effect of organic acid which many studies suggest that they are effective antimicrobial agent. The weak acids (Buchanan, *et al*., 2004; Sengun and Karapinar, 2004) or short-chain organic acids (Davidson and Juneja, 1990) especially the un-dissociated (uncharged) forms can more easily diffuse into the microbial cells and react with their compositions causing in inhibiting or destroying the cells (Bjornsdottir et al., 2006; Brul and Croote, 1999). Moreover, V-URV with 8% AA for 50 min exposure period showed the complete inhibition of *Kb. pneumonia* contamination on fresh coriander (Krusong *et al.*, 2015).

Table 1. Effect of exposure to vapor-phase upland rice vinegar on survival of two level of *Klebsiella quasipneumoniae* colonies spread on TSA at 30±2 ºC

Exposure period (min)	AA content in vapor-phase URV used (g)	LI		HI	
		Survival of *Klebsiella quasipneumoniae* (log CFU/ml)	Proportion of inhibition (%)	Survival of *Klebsiella quasipneum niae* (log CFU/ml)	Proportion of inhibition (%)
0	0	7.04±0.07	0	TNTC	0
10	0.26±0.0094	6.51±0.27	7.5	TNTC	0
20	0.491±0.0031	6.42±0.34	8.81	TNTC	0
30	0.744±0.0220	5.95±0.67	15.48	8.34±0.62	12.33
35	0.871±0.0189	ND	100	3.35±4.74	64.66
40	0.987±0.0000	N	10	ND	100
50	1.209±0.0314	N	10	ND	100
60	1.444±0.0251	ND	100	ND	100

Using 8% upland rice vinegar. *V-URV was calculated based on the weight loss of the vinegar solution used at aeration rate of 0.5 L min^{-1} during exposure period and molecular weight of vinegar (60) in the 37.5 L volume of the vapor exposure box.(Model Box: Krusong *et al.*, 2016). Abbreviation: URV, Upland rice vinegar; ND, NOT Detected; TNTC, Estimated count

Table 2. Effect of exposure to PAA vapor on survival of two level of *Klebsiella quasipneumoniae* colonies spread on TSA at 30±2 ºC

Exposure period (min)	AA content in vapor-phase PAA used (g)	LI		HI	
		Survival of *Klebsiella quasipneumo niae.* (log CFU/ml)	Proportion of inhibition (%)	Survival of *Klebsiella quasipneumo niae.* (log CFU/ml)	Proportion of inhibition (%)
0	0	6.95±0.06	0	TNTC	0
10	0.251±0.0157	6.84±0.07	1.6	TNTC	0
20	0.467±0.0063	3.00±4.24	56.8	TNTC	0
30	0.691±0.0031	2.65±3.75	61.9	TNTC	0
35	0.802±0.0031	ND	100	TNTC	0
40	0.909±0.0220	ND	100	ND	100
50	1.138±0.0314	ND	100	ND	100
60	1.344±0.0534	ND	100	ND	100

Using 8% Pure acetic acid. *V-PAA was calculated based on the weight loss of t1he vinegar solution used at aeration rate of 0.5 L min-1 during exposure period and molecular weight of vinegar (60) in the 37.5 L volume of the vapor exposure box.(Model Box: Krusong *et al.*, 2016). Abbreviation: PAA, Pure acetic acid; ND, NOT Detected; TNTC, Estimated count

CONCLUSION

Completeness of growth inhibition of the low level of *Klebsiella quasipneumoniae* after exposing with V-URV and V-PAA. It means that URV can be applied for operational practices during preparation of fresh-cut produce. It can be claimed as an applicable source of natural antimicrobial agent

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