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Antimicrobial potential of vapour phase propionic acid against *Salmonella typhimurium* contaminated on Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*)

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Abstract *Salmonella* sp. is increasingly recognized as significant cause of foodborne illness. Several decontamination procedures have been applied to reduce the number of this organism. The potential of mechanically vapourized propionic acid solution (MVP) on the reduction of *S. typhimurium* contaminated on on Cherry tomato *(Solanum lycopersicum var. cerasiforme*) was reported. *In vitro* surface inhibition at low population and high population were shown. MVP at the concentration of 70.0% demonstrated the completely inhibition within 15 min at 4 ℃. At the concentration of 70.0% the absolutely inactivated and observed within 5 min at 50 ℃. For the evaluation of antimicrobial activity of MVP over time, the results indicated that *ca.* 8.00 Log_{10} CFU/ml reduction were found within 5, 10, 15, 20, 25 and 30 min at the concentration at 5.0%, 10.0%, 30.0%, 60.0% and 70.0%, respectively. The effectiveness of MVP increased when the temperature of MPV process increased. The reduction of *S. typhimurium* contaminated on Cherry tomato using MPV was expressed. The effectiveness of MVP on the reduction of *S. typhimurium* depended on the concentration of propionic acid solution, the fumigation time and temperature. The biological and physical changes of Cherry tomato during 15 days of storage at room temperature and 4 ℃ after fumigated demonstrated that MPV at concentration of 70.0% for 5 min at 50 ℃ and 4 ℃ vapourized in 70.0% for 15 min, indicated that the completely inhibition of *S. typhimurium* contaminated Cherry tomato was accomplished. Moreover, the colour and physical appearance of fumigated Cherry tomato was not different from fresh and the control . The propionic acid in vapour phase demonstrated the antimicrobial potential against *S. typhimurium* at both after fumigation process and storage time.

Keywords: Mechanically vapourized Propionic acid, *Salmonella typhimurium*, Tomato, Antimocrobial

Introduction

The consumers have started to concern on convenience, healthiness, and freshness, which continuously resulted to increase the ready-to-eat (RTE)

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produce consumption (Tian *et al.*, 2012; Da Silva Felício *et al.*, 2015). While the demand of those has increased, it can be found that the incidence of food poisoning based on the contamination of foodborne pathogens, such as *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* has also increased (Pagadala *et al.*, 2015). The contaminations of those are contaminated by soil, water, animals, or cross-contaminated during harvest, postharvest, processing and packaging (Koseki and Isobe, 2005).

Salmonella spp. is a one of health concern and directly causes of foodborne illnesses and mortality worldwide. The gastroenteritis cases was estimated as 93 million and 155,000 deaths were reported each year (Hoffmann and Scallan, 2017; Majowicz *et al.*, 2010; Scallan *et al.*, 2015; Thomas *et al.*, 2013). Arround 87,500 illnesses, 925 hospitalizations and 17 deaths are estimated according to the infection of *Salmonella* spp. every year (CDC, 2002; Espié *et al*., 2005; Kirk *et al.*, 2004; Sangal *et al.*, 2010; Shariat *et al.*, 2013; Sivapalasingam *et al.*, 2003; Thomas *et al*., 2015; Ward *et al*., 2002).

Cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) cultivated all over the world are commonly nutritious plant food. In many cultural recipes, they contribute the function as an important ingredient in the broad range of cooked dishes and are also eaten fresh (Wanwimolruk *et al*., 2017). The benefits of consuming of different types of fruit and vegetable are admirable and tomatoes are no different. However, in last decade, a dramatical increasing number of gastrointestinal diseases linked to consumption of fresh fruits and vegetables have been literature (Bari *et al.*, 2003). As mentioned above, a wide variety of foodborne pathogens have caused these outbreaks associated with the consumption of fresh produce as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* or others (Mukherjee *et al.*, 2004). As significance, contaminations of *Salmonella* spp. on Cherry tomatoes might be posed an increasead food safe risk and become the greatest for public health concerns (Ziuzina *et al.*, 2014).

Despite the many efforts to advance effective technologies for microbial reduction contamination, food safety is still a challenge because of food market globalization (Dijksterhuis and Samson, 2006). Many possible techniques have been made for new effective, safe, and sustainable antimicrobial agents to improve food safety. Postharvest managements, sorting, washing or sanitizing, are most important procedures in view of the fact that removed, eliminated or reduced the surface contaminants. However, washing alone does not render a product completely free of pathogens (Bari *et al*., 2003).

Raw agricultural produce is washed with water in the industry; however, since they are consumed raw, washing alone does not render a product completely free of pathogens (Bari *et al.*, 2003). Conventionally, chlorine and

choline's derivertives are the most commonly used. However, they can lead to the formation of potentially carcinogenic and teratogenic trihalomethanes and haloacetic acid (Stevens, 1982; Mcwatters *et al.,* 2002).

Many food disinfectants were alternatively selected to study their antimicrobial activity. The antimicrobial activity of organic acid has also been reported (Uyttendaele *et al.,* 2004; Akbas and Olmes, 2007; Huang and Chen, 2011; Sagong *et al.,* 2011; Wang *et al.,* 2015). Organic acids such as acetic, lactic, propionic and sorbic are progressively used as preservatives, because of the good antibacterial activity and approval as generally recognized as safe substances (GRAS) (Surekha and Reddy, 2000). Propionic acid is known to have antibacterial activity and could play a role in inhibiting pathogens. This acid has been investigated as an antimicrobial agent to extend the shelf stability (Dubal *et al.,* 2004; Odgen *et al.,* 1996). Morover, the antimicrobial of volatile compounds have become popular in research and have also been exhibited antimicrobial properties in many food product (Sholberg *et al.,* 2000; Tzortzakis, 2010; Gatto *et al*., 2011; Krusong *et al.,* 2012; Cissé *et al.,* 2013). Hence, there is a need for, and interest in, a challenge to investigate the volatile antimicrobial properties of propionic acid in food product. The beneficial application of vapour phase antimicrobial substances was reported to control postharvest diseases of fresh produce including tomatoes (Tzortzakis, 2010). Thus, this study was conducted in order to investigate the efficacy of mechanically vapourized propionic acid (MVP) on the reduction of *S. typhimurium* in both vitro study and contaminated on model fresh Tomatoes.

Materials and Methods

Chemical and microbiological media used

Tryptic Soya Agar (TSA), Tryptic Soya Broth (TSB) and Peptone were purchased from Difco (Dico, USA). Propionic acid was purchased from Ajax Finechem (Auckland, New Zealand).

Preparation of tested organism

Salmonella typhimurium ATCC 13311, used in this study was kindly provided by the Foodborne Pathogenic Bacteria Research Team, Department of Food Science and Technology, Factory of Science and Technology, Thammasat University (Rangsit Centre). The culture was kept in -18°C. Activation process was prepared. Period of expose, *S. typhimurium* ATCC 13311 was sub-cultured twice in TSB at 37°C for 18 h before use as inocula.

Cherry tomatoes sample preparation

Fresh Cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) were purchased from the wholesale fresh market in PathumThani, Thailand. Visibly damaged and wilted portions were discarded. Uniform fruits were sorted in terms of size and maturity. The samples were washed with tap water to reduce the soil and debris before being drained and left in a biological safety cabinet class II (AstecMicroflow, Bioquell, UK), followed by packing in polyethylene (PE) plastic bags and storing at 12 ± 2 ℃. Fruit were subjected to treatments on the day of preparation. At the period of experiment, prepared totamoes were washed with sterile distilled water and followed by sanitization in 100 ppm chlorinated distilled water for 5 min. Residuals of chlorine were removed by soaking again in 5g/L sodium thiosulfate solution for 5 min. The fruits were dry in laminar flow cabinet for 15 min under UV lamp.

The surface test by agar overlay method

The susceptibility of *S. typhimurium* ATCC 13311 to MVP was determined *in vitro* using modified agar overlay method. Briefly, 0.1 mL at *ca*. 7.00 Log_{10} CFU/mL of *S. typhimurium*ATCC 13311 suspension was spread on TSA to obtain high level of inoculums, 0.1 mL of *ca*. 4.00 Log_{10} CFU/mL was used as low level inoculumns. Contaminated surface was aseptically placed in fumigation chamber (Fig. 1). Flask containing propionic acid solution at the concentration of 5.0%, 10.0%, 30.0%, 60.0% and 70.0% (v/v) was individually placed and directly connected with air pump. The other was connected to pure distilled water. The MVP fumigation process was conducted at the interval time as 2, 4, 6, 8 and 10 min. All plates were incubated at 37°C for 24 h. The reduction ratio was calculated. The impact of temperature on the MVP was determined at 4°C and 50°C. The rate of vapour production was calculated.

The antimicrobial activity over time (Time killing analysis)

For time killing analysis, the susceptibility of *S. typhimurium* ATCC 13311 to MVP was investigated *in vitro*. *S. typhimurium* ATCC 13311 at *ca*. 8.00 Log_{10} CFU/mL was prepared in 50 mL TSB. Fumigation tube connected with vapour generator was aseptically placed into microbial suspension. MVP was generated according to the previous experiment. Remaining population of *S. typhimurium* ATCC 13311 was withdrawn at the interval time as 5, 10, 15, and 30 min, serial dilutions were completed. Spread plate technique was used on TSA. All plates were incubated at 37°C for 24 h, and the population of

organism was calculated as Log_{10} CFU/mL. As described above, the impact of temperature on the antimicrobial potential of MVA was also determined at 4°C and 50°C.

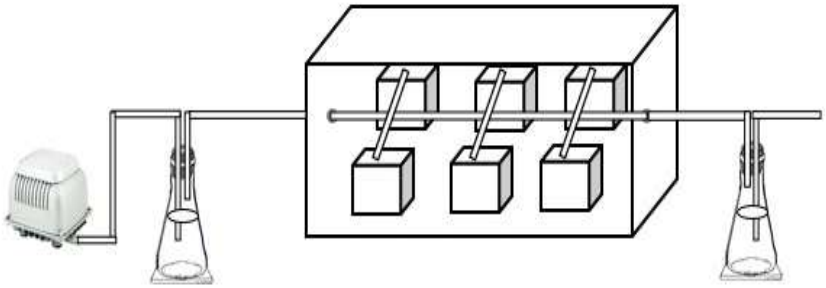

Figure 1. Schematic illustration represented the MVP fumigation chamber

MVP effects on fresh Cherry tomatoes inoculated with S. typhimurium

Stock culture of *Salmonella typhimurium* ATCC 13311 was sub-cultured twice in TSB at 37°C for 18 h. Cells were collected by centrifugation at 4000*g* for 15 min at 4°C. Cell pellets were washed twice and resuspended with 10 mL sterile 0.1% Peptone solution. The inocula were adjusted to the final concentration of cell at *approx*. 7.00-8.00 Log_{10} CFU/mL. 0.1 mL of prepared *S. typhimurium* ATCC 13311 was individually contaminated on Cherry tomatoes to obtain 6.00 Log_{10} CFU/g. The *S. typhimurium* contaminated fruits were then exposed to MVP in the fumigation chamber. Seven durations of vapour exposure were 0, 5, 10, 15, 20, 25 and 30 min at 65±2% RH. Remaining populations of *S. typhimurium* ATCC 13311 was then examined by spread plate technique. All plates were incubated at 37°C for 24 h, and the population of organism was calculated as Log_{10} CFU/g. The impact of temperature on the antimicrobial potential of MVP was also determined at 4°C and 50°C.

Statistical analysis

All experiments were performed in according to a Completely Randomized Design (CRD). The data for measurements were expressed as the average value (n=3).

Results

The Surface test by Agar overlay method

Antimicrobial potential of MVP against *S. typhimurium* ATCC contaminated on agar–surface of TSA (0.4 CFU/cm^2 for low level and 4.0 CFU/cm^2 for high of inoculums) are presented in Figure 2. The results demonstrated the microbial inhibitory potential of MVP against *S. typhimurium* ATCC 13311 at all concentration of propionic acid solution. At the low concentration of propionic solution of 5.0% (v/v), the complete destructive was not detected within 30 min. Increasing concentration of propionic acid solution of MVP process that increased in efficiency of this process. At the concentration of propionic acid as 10.0% (v/v), the complete inactivation was occurred within 20 min of fumigation time at 50°C for low level inoculum and 25 min for high inoculum at the same temperature. As presented in Figure 2, the increased concentration of propionic acid of MVP process resulted to the increased of antimicrobial properties. However, it could be noticed that at 4°C and 50°C, the inactivation effects were higher than at 29±2°C. Consideration about the impact of level of contamination, it could be indicated that the inoculum affected the antimicrobial of propionic acid in vapour phase. It was used to determine the effectiveness of the MVP in addition to the concentration of propionic acid solution and temperature of fumigation process. It was confirmed that when the low inoculum was contaminated, the accessment of vapourized propionic acid into cell was easier than in the case of high inoculum size.

The conservation ability of MVP process on the amount of acid was used and related to the increasing or decreasing acidity, the weight of the remaining solution during the fumigation process and the acidity level was evaluated. According to result, the rising rate of pH and increasing weight loss of acetic acid solution under mechanically vaporized process were the main factors affecting the antimicrobial properties as shown in Figure 3. These increasing were presented when propionic acid was exposed to high temperature. Hence, pH of tested solution and weight loss of propionic acid increased along with the rising of temperature, the antimicrobial properties of MAP also increased.

The antimicrobial activity over time (Time killing analysis)

The time killing curve, represented the antimicrobial potential of MVA against *S. typhimurium* ATCC 13311 suspension varied with the concentration, fumigation time and temperature were demonstrated (Fig.4).

At room temperature, the MVP at all concentration had no ability to provide the lethal effect within 30 min. However, AVP at 4 or 50 C demonstrated the inhibitory efficiency. The population of *S. typhimurium* ATCC 13311 was reduced to undetectable level within 30 min when the concentration of 70.0% propionic acid in MVP was used at 4 C. At 50 C, the antimicrobial activity was higher than at the low temperature.

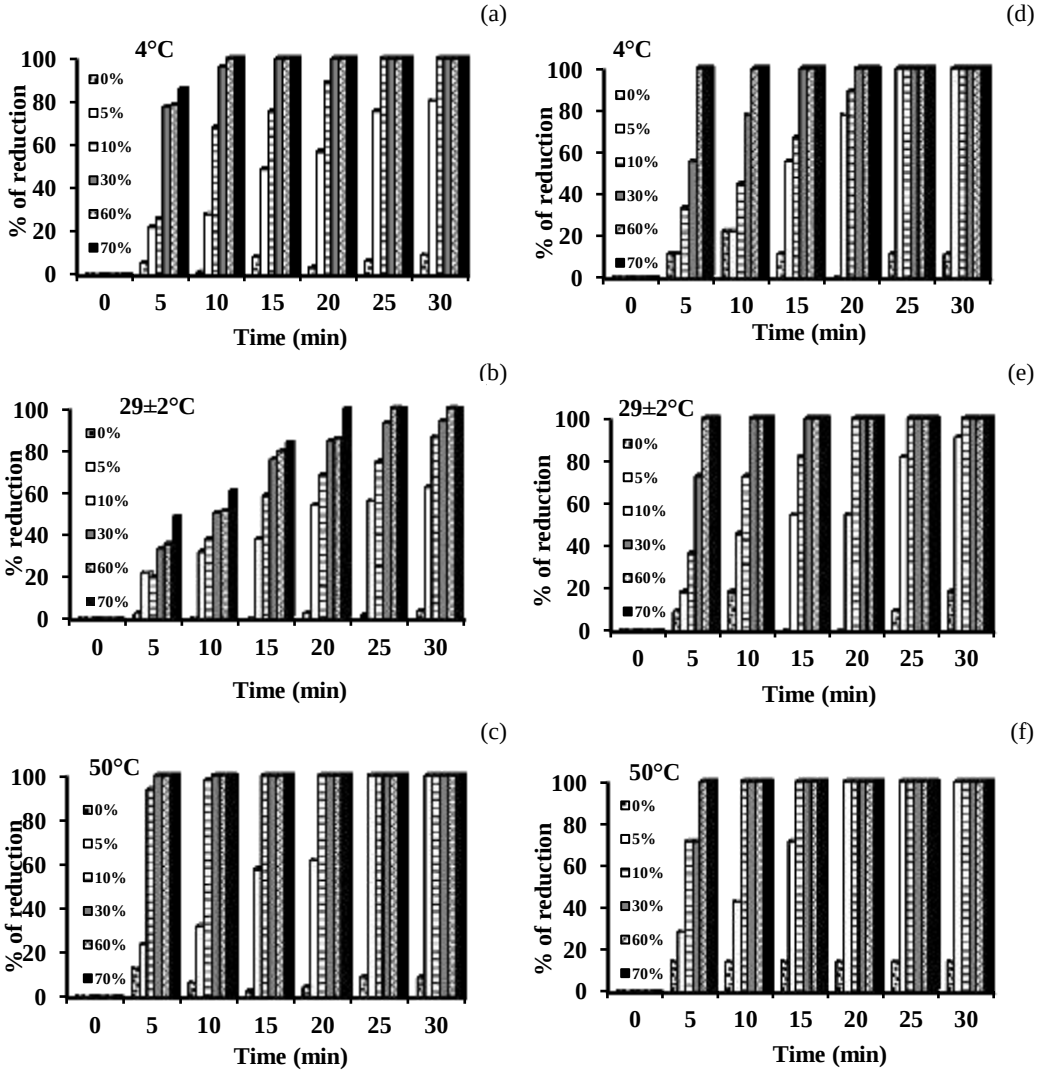

Figure 2. Reduction (%) of *Salmonella typhimurium* ATCC 13311 according to surface test during the fumigation with MVP and different concentration of propionic acid solution ((a), (b) and (c): high level iniculum; (d), (e) and (f): low level of inoculums)

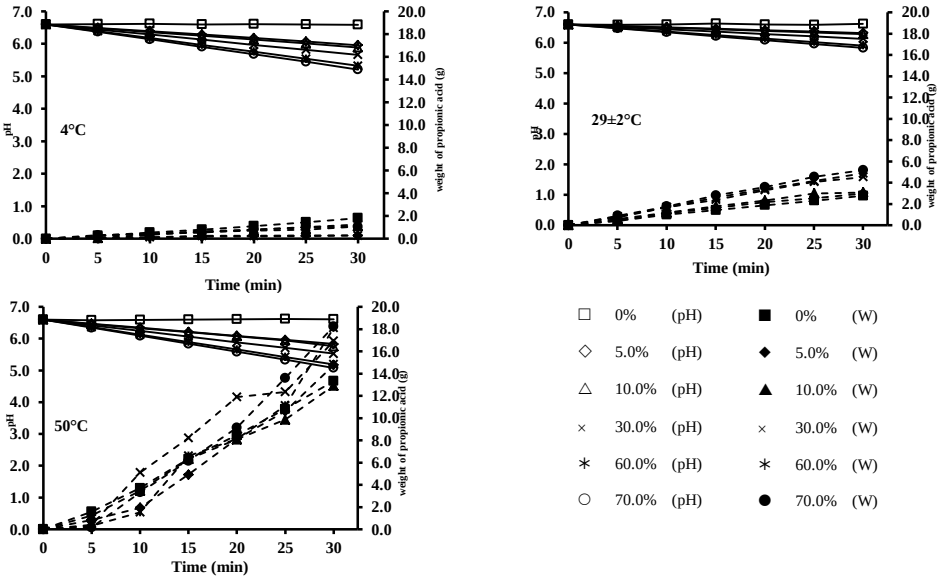

Figure 3. Weight change (g) of propionic acid solution (solid line) and pH of distilled water (dot line) during fumigation with propionic acid acid at different concentration

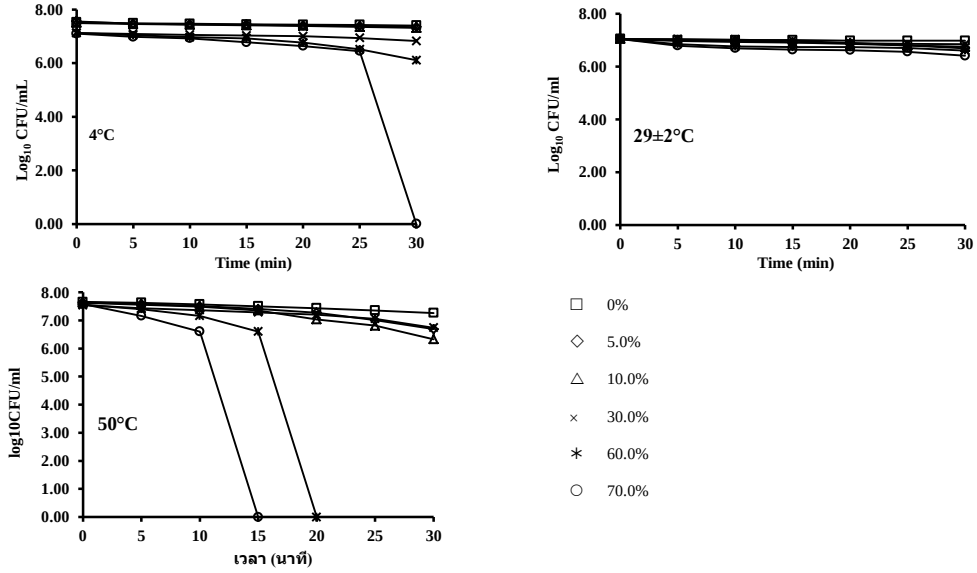

Figure 4. The population of *Salmonella typhimurium* ATCC 13311 during contact with MVP at different concentration

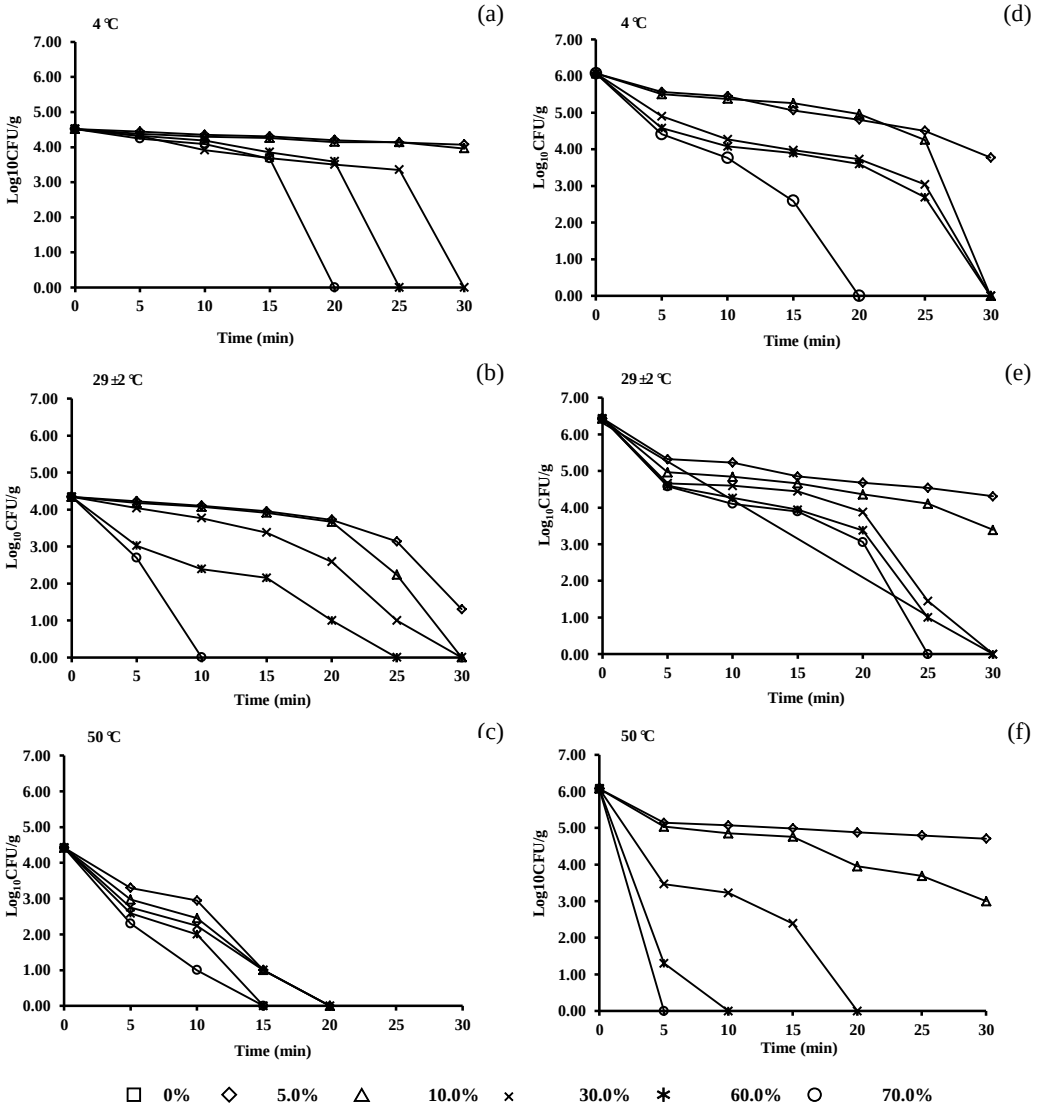

Figure 5. Total bacteria count ((a), (b), (c)) and *Salmonella typhimurium* ATCC 13311 ((d), (e), (f)) on Cherry tomatoes during contact with MVP at different concentration of propionic acid

The rapidity of bactericidal effect or the duration of a bacteriostatic effect was determined by time kill analysis (survivor curve plot) whereby the number of viable cells remaining in cell suspension after the present of antimicrobial substances is plotted against time. The time killing analysis is equal the inhibition curve, known as the 'killing curve' in clinical research.

In generally, the investigation to determine the antimicrobial action of the MVP against *S. typhimurium* ATCC 13311 at different concentration indicated that the population of organism was decreased by increasing temperature. Moreover, it could be designated that the MVP process at room temperature (29±2°C) presented less antimicrobial activity than 4 or 50°C. In addition, prolong the fumigation time the lower the necessary concentration of the MVP. Due to the fact that in the intended field of application low or high temperature and long fumigation times are expected a reduction of the concentration of the MVA might be possible. The lower the concentration the less the possible negative effect on the food, especially influence on the taste or smell could be reduced.

MVP effects on fresh Cherry tomatoes inoculated with S. typhimurium *ATCC 13311*

The initial amount of normal flora contamination determined as total bacteria count and *S. typhimurium* ATCC 13311 on Cherry tomato at ca. 6.00 Log_{10} CFU/g were exposed to MVP at the different concentration of propionic acid solution at 5.0, 10.0, 30.0, 60.0 and 70.0% along with different fumigation. The influence of operating temperature was also examined. MVP process at all temperature at more than 30.0% of propionic solution illustrated the lethal phenomena within 30 min. As the same characteristic, MVP process at 4 and 50°C demonstrated the higher antimicrobial potential than at room temperature (29±2°C). At 4°C of fumigation process, the complete elimination was detected within 30 min of 10.0, 30.0 and 60.0% of propionic solution used in MVP. The same phenomenon was detedted within 20 min. when 70.0% of propionic acid was applied.

In case of 50°C fumigation temperature, and the concentration of propionic acid at 30.0%, the lethal effect was detected within 20 min. The increasing of concentration decreased the time for complete destruction.

Discussion

The reductions of *S. typhimurium* ATCC 13311 stated in this experiment are in agreement with those reported by previous authors about using organic acids (Uyttendaele *et al.*, 2004; Akbas and Olmes, 2007; Huang and Chen, 2011; Sagong *et al.*, 2011; Wang *et al.*, 2015, Dickson and Anderson, 1992). Bhide *et al*. (2001) described the reduction of mesophiles by 4.80 log units after spraying sheep meat with 1.0% propionic acid. Odgen *et al.* 1996 found that

propionic acid at concentration of 1.0% presented the elimination effect against *Pseudomonas* sp. in pork meat after 13 days of storage.

As mentioned in the previous research of Bell and Kyriakides (2002), the inhibition potential of weak acid was acted upon the pKa and pH. The bacteriostatic and bactericidal of organic acid and the other derivatives have been attributed to the lower pH below that need for optimum growth (Yeesibsan and Krusong, 2009). Results of *in vitro* susceptibility of *S. typhimurium* ATCC 13311 on MVP using agar overlay method demonstrated the progressive inhibition of *S. typhimurium* was related to the concentration of proprionic acid solution in the MVP process. The inhibition properties of MVP in present study were similar to the previous reports. The study reported by Yeesibsan and Krusong (2009) indicated that corn vinegar at the concentration of acid of 1.0% demonstrated the bactericidal effect against *S.* Enteritidis. Several researchers suggested that organic acid presented the effective inhibitors to foodborne pathogens, especially short-chain organic acids (Devidson and Juneja, 1990) or weak acid (Buchanan *et al.*, 2004; Sengun, 2004). The protonated or un-dissociated form demonstrating the diffusion activity into cytoplasm of cell is the key factor on the antimicrobial activity of those. It is the main mechanism of acid that cause the death of bacteria (Brul and Croote, 1999; Bjornsdottir *et al*., 2006). The mechanism involved the inhibitory of enzyme, interference of nutrient transport, membrane damage or overall impact on the metabolic activity of cell (Blackburn and McClure, 2002). Propionic acid can be name as the most effective candidate among weak acids. This has been used as antimicrobial agents against foodborne pathogens.

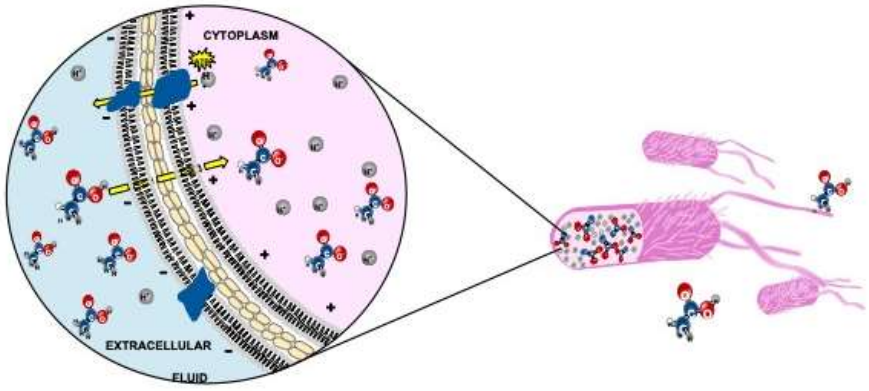

Figure 6. Schematic illustration of the possible mechanism of MVP against *Salmonella typhimurium*

Several researchers reported the ability of organic acid in vapour-phase on the decontamination of bacteria contaminated on fresh produce using fumigation process (Goñi *et al.,* 2009; López *et al.,* 2005; Lenka *et al.,* 2009). It seem that MVP prior decontaminates surfaceborne microorganism and thus sterilizes vegetable surfaces (Sholberg and Gaunce, 1996). In this research, the results demonstrated that MVP presented the inhibitory effect on both *S. typhimurium* ATCC 13311 *in vitro* and in model food as Cherry tomato. The schematic illustration of possibile mechanism of propionic acid in vapour phase was presented as in Figure 6. The mechanically vapourized process allowed the propionic acid in the form of dissociated in the solution molecule to become the un-dissociated molecule in vapour-phase. For this reason, the MVP presented more inhibitory properties against *S. typhimurium* ATCC 13311. Another advantage is, the gas phase can diffuse much more than liquid phase so the rate of cell membrane penetration become more rapid compared to liquid phase. According to the fumigation process, the downward trend of fumigation time was observed when the temperature was rising. Weak acid such as lactic acid, sorbic acid, benzoic acid and others, always demonstrate the microbial inhibition properties (Bell and de Lacy, 1987; Arroyo-López *et al.,* 2008; Stratford *et al*., 2009; Wang *et al*., 2015).

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