Transfer of *Escherichia coli* TISTR527 from Surface to Fresh-Cut Cantaloupe

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Abstract

The objective of this research is to prove the 5- second rule and to determine the cross-contamination of *Escherichia coli* TISTR527 from wood and plastic cutting boards to fresh-cut cantaloupe (FCC). FCC were dropped onto the each surface and left on each surface in intervals of 5, 30 and 300 seconds. They were measured the amount of *E. coli* transferred to FCC. The results showed that surface types and contact times have highly effects on *E. coli* transfer. Plastic had the higher percent transfer of *E. coli* to FCC than wood. Transfer rate of *E. coli* observed since 5 second and increased with increasing the contact times. When FCC were dropped on floor in an *in situ* simulation room from a height of about 60 cm, the results showed that total viable count (approximately 9.35×10^2 CFU/cm^2) can be transferred to FCC at 5 s. This research demonstrated that the risk of illness resulting from deciding to consume food dropped on the floor or materials contaminated with pathogens.

Keywords**:** *Fresh-cut cantaloupe, Contact time, Cross-contamination, Cutting board*

1. Introduction

Cantaloupe's name come form Cantalopo, near Vatican city of an Italian town. Cantaloupes are the family in Cucurbitaceae, which is the same gourd family and other plants to raise above climber, such as watermelon and honeydew melons, includes pumpkins, squash and cucumbers. Fresh-pulp of cantaloupe has a juicy which is the great water content about 90 percent. The cantaloupe pulp is various nutrient values and antioxidant such as vitamin A in from beta-carotene, vitamin C and potassium is well mineral, but is poor calories. Antioxidants can be help protect cell damage that directs to cancer and other health symptoms. The U.S. Food and Drug Administration is specify designate on nutrition facts for one cubed (160 g) of cantaloupe pulp; there are 14 g of total carbohydrate (5 percentage daily values; %DV), 1 g of dietary fiber (6 %DV), 26 mg of sodium (1 %DV) and 13 g of sugars (Szalay, 2018). Furthermore, there are many nutrients for about 1 cup (177 g); 15.9 g of calcium, 21.2 g of magnesium and 26.6 g of phosphorus (Olsen, 2020). Cantaloupe is a popular fruit which can be consumed in its raw form without undergoing processing, and also sold in open-air market. But if they are not handled properly, it can also become a source of food- borne pathogens and hazardous to health particularly when eaten raw. Fresh- cut cantaloupes (FCC) are more susceptible to microbial attack due to injuries of plant tissues during processing. Microbial contamination can adversely affect the quality of FCC and the safety for consumer. Contamination of FCC can occur at several points during processing., i.e. peeling, cutting, washing and packing. The U.S. Food and Drug Administration (FDA) reported that the processing of fresh produce without proper sanitation procedures in the processing environment increases the potential for contamination by pathogens. 11 of 151 cantaloupes were contaminated with *Shigella* (3) and *Salmonella* (8) (FAO/WHO, 2011). In a survey of pathogens in FCC from street foods and supermarkets in Sakaeo province, Thailand, 26 of 30 FCC samples (86.67%) were found to be contaminated by fecal coliform bacteria, and out of these 3 (10%) was *E. coli* (Saelim & Lueangprasert, 2016). *E. coli* and other coliform bacteria are important food borne pathogens and are known to be the indicator of unfavorable hygienic conditions and fecal contamination in foods (Ekici & Dümen, 2019). Specifically, on factor is connected with cross-contamination from surface to food. FDA reported that precutting and mixing process (14 % outbreak) would have allowed the oppportunity for transfer from contaminated rind to inner surface or contamination from infected food handlers and the preparation environment.

Although, there is a belief that if food dropped on the floor for 5 seconds or less, it is not contaminated and safe called "The five second rule". Previous studies on five second rule shown that longer

contact time (<1, 5, 30 and 300 s) did certainly increase the transfer of *Enterobacter aerogenes* from surface (stainless steel, tile, wood and carpet) to food (bread, butter, candy and watermelon) (Miranda & Schaffner, 2016). Transfer of microorganism is dependent on the type and texture of food, type and roughness of surface, type and concentration of microorganism and residence and food contact time (Lipschutz, Kagan, Steed, Kagan, & Lipschutz, 2016; Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2013; Dawson, Han, Cox, Black, & Simmons, 2007).

2. Objectives

1. To prove the 5-second rule.
2. To determine the cross-contamination of *Escherichia coli* TISTR527 from wood and plastic cutting boards and floor in an *in situ* simulation room to FCC.

3. Materials and Methods

3.1 Bacterial strains and preparation of culture

Escherichia coli TISTR527 was purchased from the TSITR Culture Collection, Thailand Institute of Scientific and Technology Research. Bacteria was grown in Brain heart infusion broth (BHI broth, Hi Media Laboratory, Mumbai, India) at 37 °C for 18-24 h and maintained at -20 °C in medium containing 25% glycerol. Before use in experiments, *E. coli* TISTR527 was activated twice in BHI broth at 37 °C for 18-24 h. Cells were harvested by centrifugation at 4,000 ×g for 10 min at 4 ºC. The pellet cells were washed twice with 0.1% peptone water, re-suspended and immediately adjusted the turbidity to match a 1.0 McFarland standard (equivalent to $7.2 \pm 1.1 \times 10^7$ CFU/ml).

3.2 Fresh-cut fruit preparation

Cantaloupe cv. Sun Lady (*Cucumis melo* L. cv. Sun Lady) were purchased from supermarket at Sa Kaeo province, Thailand. Whole fruits were washed under running tap water for 3 min and then sanitized by immersion in 5% antiseptic solution (Dettol) for 5 min. Whole cantaloupes were drained and air dried in biosafety cabinet. Cantaloupes were cut into cubic pieces of approximately 1.0 cm^3 using sterile knife. FCC were placed in to polypropylene (PP) bags and maintained at 5 ± 2 °C and 84 ± 2% relative humidity (RH) until use (not loger than 1 h).

3.3 Preparation of cutting boards

Plastic and wooden cutting boards were purchased from local retail stores. Before each experiment, cutting boards were put into 5% antiseptic solution (Dettol) for 5 min and then washed by sterile distilled water. Cutting-board surfaces were cleaned again with 70% ethanol and air-died for 30 min under ultraviolet light in biosafety cabinet.

3.4 Transfer of E. coli TISTR527 from cutting boards to fresh-cut cantaloupes

Experimental designs of transfer were evaluated with dropping food on various surfaces (plastic and wooden cutting boards) modified by Miranda and Schaffner (2016). Ten milliliter of 1 McFarland standard *E. coli* suspension were spread onto each surface of the 15 x 15 cm squares using a glass spreader. Each surface was air-dried in biosafety cabinet. After drying, viable bacterial counts onto each surface were determined by swab test (CFU2). Moreover, FCC were dropped onto each surface from height of 12.5 cm and plated to rest for different times (0, 5, 30 and 300 s). Transfer of *E. coli* TISTR527 to FCC was evaluated by determining the number of *E. coli* TISTR527 per square centimeter of FCC (CFU1). Briefly, FCC were plated into sterile PP bags and suspended in 0.1% peptone water. The mixture was hand-massaged and homogenized for 2-3 min. The homogenates were ten-fold serially diluted with the same diluent. One microliters of appropriate dilution were placed in sterile petri dishes. Plate Count Agar (PCA, Hi Media Laboratory, Mumbai, India) was poured over the samples in the same plates and then swirled to mix its contents. The plates were incubated at 37 ºC for 24-48 h. The numbers of *E. coli* TISTR527 colonies were counted, percent transfer calculated as follow (1):

$$\%\,Transfer = \text{CFU1}\,/\text{CFU2}\,\times 100 \quad (1)$$

When CFU1 = The number of *E. coli* from FCC, CFU/cm^2
CFU2 = The number of *E. coli* from surfaces, CFU/cm^2

3.5 In situ transfer of microorganisms from floor to fresh-cut cantaloupes

The floors used as surfaces for these studies were at Food Processing Laboratory at Burapha University, Sakaeo Campus, Thailand. FCC were dropped onto each surface from height of 60 cm and plated to rest for different times (0, 5, 30 and 300 s). Swab test was used to estimate the number of microbiology in floor surface. To evaluate the amount of microbiological transfer during residence time and food contact time from floor to FCC, microbiological analyses of Total Viable Count (TVC), Yeasts and Molds, *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and coliform bacteria were carried out according to Bacteriological Analytical Manual (BAM) (Food and Drug Administration, 2020).

4. Results and Discussion

4.1 E. coli transfer from cutting boards to fresh-cut cantaloupes

Transfer of *E. coli* TISTR527 from cutting boards (plastic and wooden) to FCC is shown in Table 1 and Fig. 1. There are significant different in the number of *E. coli* TISTR527 transferred from cutting boards to FCC. The number of *E. coli* TISTR527 on FCC increases with increasing the contact times. The wood cutting board transferred about 2.07×10^2 CFU/cm^2 at the 5 s contact time. The mean *E. coli* TISTR527 counts of FCC at 30 and 300 s contact time were shown as 1.82×10^3 and 3.28×10^3 CFU/cm^2, respectively. In the same way, levels of *E. coli* TISTR527 populations transferred from plastic cutting board to FCC were approximately 5.26×10^3 CFU/cm^2 at the 5 s contact time and had increased to about 1.21×10^4 and 2.42×10^4 CFU/cm^2 at 30 and 300 s contact time, respectively.

Table 1 The number of *E. coli* TISTR527 on fresh-cut cantaloupes at different contact times and surfaces.

Contact times (seconds)	*E. coli* (CFU/cm^2)			
	wood	**	plastics	**
Control*	4.07×10^3		2.68×10^4	
0	0	c	0	d
5	2.07×10^2	c	5.26×10^3	c
30	1.82×10^3	b	1.21×10^4	b
300	3.28×10^3	a	2.42×10^4	a

* The number of initial *E. coli* TISTR527 onto the surfaces of cutting boards.
a-d Means with different letters in the same column are significantly different at $P < 0.05$.

Cross-contamination is the transfer of microorganism from foods or surfaces such as if you use a chopping board to place, peel and trim the cantaloupe and then use the same board for cutting and dicing. However, at present, there are a few published papers reported on transfer of pathogens from the surface to the food. Several research articles have been published on transfer from the food to the surface. Gkana et al. (2017) reported that the higher number of pathogens (*Listeria monocytogenes*, *S.* Typhimurium or *E. coli* O157:H7) was transferred from wood surface to beef fillets than other surfaces (stainless steel and polyethylene).

A summary of results of percent transfer from both plastic and wooden cutting boards is shown in Table 2 and Fig. 2. Type of cutting boards had a significant effect on percent transfer of *E. coli* TISTR527 to FCC at the 5 s contact time ($P = 0.013$). At the 5 s contact time, percent transfer of *E. coli* TISTR527 from plastic cutting board (19.66 ± 5.66 %transfer) was higher than from wood cutting board (5.10 ± 1.91 %transfer). Although, at 30 and 300 s contact time, there were no difference in percent transfer to FCC. There was similar trend for transfer of *E. coli* TISTR527 from plastic and wooden cutting boards to FCC. Transfer rate of *E. coli* TISTR527 strongly increased at initial contact time (30 s), and then slightly increased until at 300 s contact time.

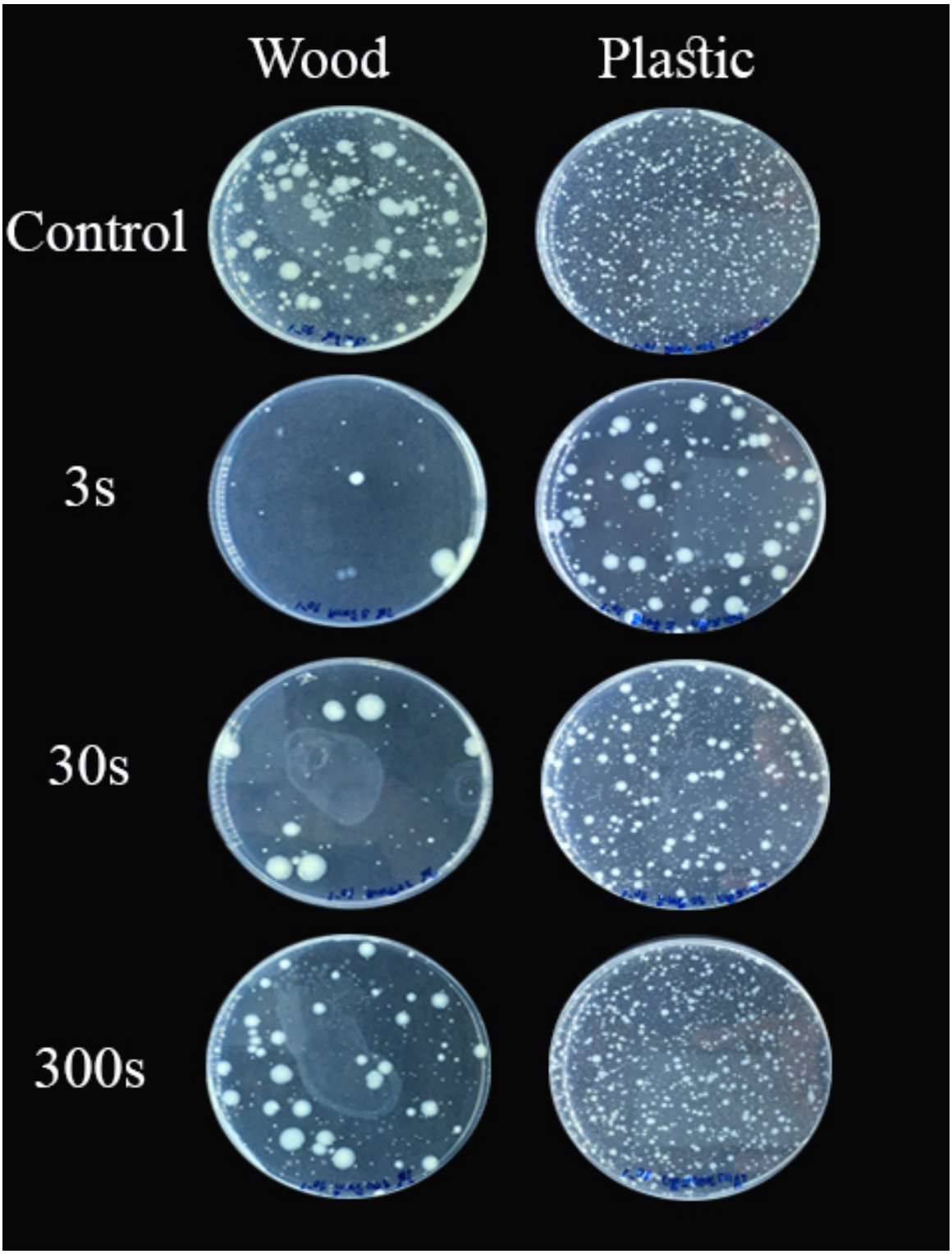

Figure 1 The numbers of *E. coli* TISTR527 colonies on Plate Count Agar (PCA).

Table 2 Transfer of *E. coli* TISTR527 from cutting boards to fresh-cut cantaloupes.

Contact times (seconds)	% Transfer		t statistic	*P* value
	wood	plastic		
0	0 [c]	0	n.d	n.d.
5	5.10 ± 1.91	19.66 ± 5.66	-4.225	0.013
30	44.89 ± 15.15	45.18 ± 8.23	-0.030	0.977
300	80.55 ± 28.80	90.58 ± 9.93	-0.570	0.616

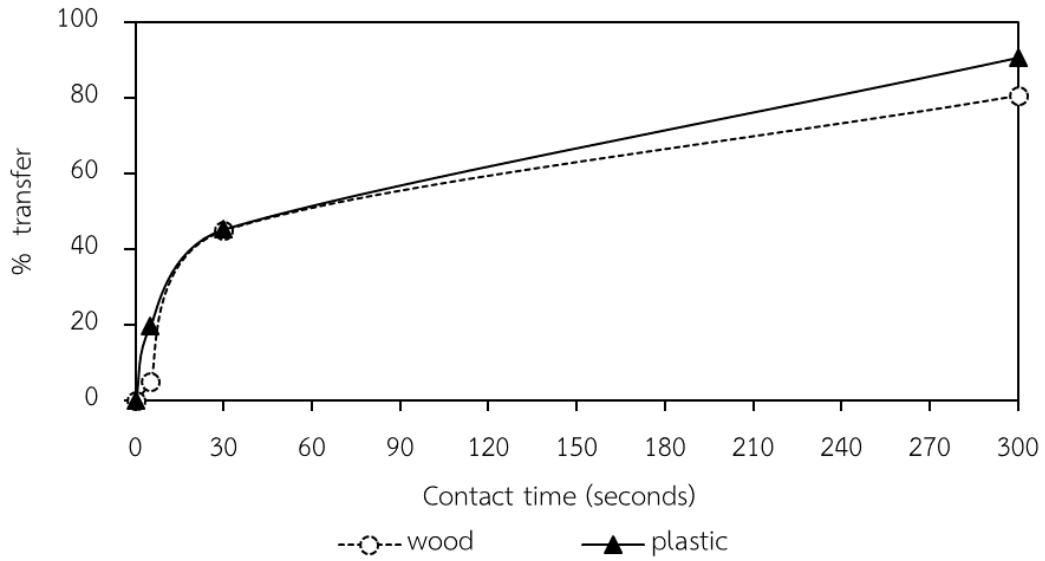

Figure 2 Transfer of *E. coli* TISTR527 from cutting boards to fresh-cut cantaloupes.

In these studies, we found that surface types and contact times were important for *E. coli* transfer to FCC. At 5s contact time, *E. coli* can instantaneously transfer to FCC, and wood surface (5.10 %transfer) had a low percent transfer of *E. coli* to FCC compared with plastic surface (19.66 %transfer). Our finding is in agreement with previous studies reported that *S.* Typhimurium transfer from surface to food was highly influenced by residence time, and was the most powerful factor in transfer of *S.* Typhimurium from wood, tile and carpet to bologna (Dawson et al., 2007). Wachtel et al., (2003) reported that 46 % of lettuce leaves, including the 25th exposed leaf, were cross-contaminated from cutting boards inoculated with *E. coli* O157:H17 at 1.25×10^2 CFU. Moore, Sheldon, and Jaykus (2003) also reported that transfers of *Salmonella* and *Campylobacter* from stainless to lettuce ranged from 23 – 66 % and 15 – 38 %, respectively.

In addition, this researches shown that contact times influence *E. coli* transfer from surfaces to FCC. Longer contact times (0, 5, 30 and 300 s) increase the transfer of *E. coli* from cutting boards to FCC. Increase in residence time from surface-to–food contact enhances the likelihood for microbial movement from surface to food. Another interesting result found that *E. coli* transfer may be observed in less than 5 second both laboratory and *in situ* simulation. Thus, the risks of illness result from deciding to consume food that has fallen on the floor.

4.2 In situ transfer of microorganisms from floor to fresh-cut cantaloupes

The results of microbial safety on floors at Food Processing Laboratory at Burapha University, Sakaeo Campus, Thailand showed that *E. coli*, *Salmonella* spp., *S. aureus* as well as yeast and molds were not detected from floors. On the contrary, TVC (6.41×10^5 CFU/cm^2) was found from floors. When FCC were dropped onto the floor, the number of TVC on FCC increases with increasing the contact times. The mean TVC of FCC at 3, 30 and 300 s contact time were shown as 9.35×10^2, 1.68×10^3 and 3.36×10^3 CFU/cm^2, respectively (Table 3).

Table 3 Microbial population on fresh-cut cantaloupe.

Samples	Microorganisms	Contact times (seconds)			
		0	**5**	**30**	**300**
Floors	TVC (CFU/cm^2)	6.41×10^5	n.d.*	n.d.	n.d.
	Yeasts & Molds (CFU/cm^2)	0	n.d.	n.d.	n.d.
	E. coli (MPN/cm^2)	0	n.d.	n.d.	n.d.
	S. aureus (CFU/cm^2)	0	n.d.	n.d.	n.d.
	Salmonella spp. (CFU/cm^2)	0	n.d.	n.d.	n.d.
Fresh-cut cantaloupes	TVC (CFU/cm^2)	0 c**	9.35×10^2 b	1.68×10^3 b	3.36×10^3 a
	Yeasts & Molds (CFU/cm^2)	0	0	0	0
	E. coli (MPN/cm^2)	0	0	0	0
	S. aureus (CFU/cm^2)	0	0	0	0
	Salmonella spp. (CFU/cm^2)	0	0	0	0

* n.d. = not detected.
** Means with different letters in the same rows are significantly different at $P < 0.05$.

5. Conclusion

Although our results exhibited that transfer of bacteria from surface to food was most affected by surface types and contact times. It should be further studied for effect of other factors to consider in bacterial transfer such as type of food, type of pathogens and initial inoculum size. Additionally, *E. coli* can instantaneously transfer to FCC when FCC comes into contact with a contaminated surface. Therefore, the sanitary quality of the processing of the produce should be concerned by applying Good Manufacturing Practices for ready-to-eat fresh-cut fruits (GMP) during preparation and selling. This will help control contamination of products and make the fruits safe for consumption.

6. Acknowledgements

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