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Microbiological quality of selected organically-grownfruits and vegetables in Luzon, Philippines

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Abstract

Contamination of pathogenic microorganisms in fruits and vegetables eaten raw was linked to world-wide disease outbreaks.This study aimed to determine the microbial loads of organically-grown produce at thefarm and market level andidentify points of contamination by investigating the prevalence of coliforms, *Escherichia coli (E. coli)* and *Salmonella* spp. Effects of simple washing techniques on the microbial quality of Romaine lettuce were also investigated. Freshly harvested organically-grown cabbages have high coliform counts (525 cfu/g) and are *E.coli*-positive while strawberries are *Salmonella*-positive. In the market, all organically-grown produceare *E. coli*-negative. *E. coli* and higher loads of total coliforms were detected on conventionally-growncommodities,broccoli(150 cfu/g), cabbage (590 cfu/g), celery (985 cfu/g),and lettuce (955 cfu/g). Lettuces are the most susceptible to contamination due to its large surface area where microbes can easily cling on.The critical control point in the lettuce value chain was trimming, with scissors, crate as containerand farmer's hand being *E. coli*-positive. Washing eradicated contamination on the produce. However, re-contamination occurred as heads got in contact with *E. coli*-positive dryer. In the washing experiment, lettuce washed with chlorinated water exhibited the highest (31%) coliform reduction. Even when corrective strategies are applied, the produce is still at risk of contamination if proper handling will not be observed in the succeeding processes. Produce sanitation coupled with sanitation of equipment and facilities can create a positive impact on microbial quality of the products from the farms and markets under study.

Introduction

World-wide food-borne disease outbreaks related to consumption of raw organic fruits and vegetables has led the government and industries to exert efforts to systematically determine and control the significant checkpoints along the food safety chain (Othman, 2010; Connor, 2015). For the past decade, this trendhas been popular particularly in Southeast Asian countries, including the Philippines (Tamayo *et al.*, 2013). Most organic commodities are consumed without further processing, thus, their microbial content may pose risks on consumer's health and become a food safety problem (Falomir *et al.*, 2010; Othman, 2010). Consumption of contaminated produce enables fast transmission of bacterial, parasitic and viral infections which can cause illnesses to humans (Olaimat and Holley, 2012; Van Boxstael *et al.*, 2013). Contamination can occur during production, harvesting, postharvest handling and distribution (Johnston *et al.*, 2005; Lynch *et al.*, 2009; Mathur *et a*l., 2014).The escalating demand on organic produce in this countrycalls for vigilant implementation of food safety measures and regulations to provide people with wholesome, safe and good quality fresh organic vegetables (Porciuncula *et al.*, 2014; Vital *et al.*, 2014). Among these strategies is the implementation of the Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) (Prabhakar *et al.*, 2010; Agriculture and Agri-Food Canada, 2012).

The Food and Agriculture Organization (FAO) structured the Hazard Analysis and Critical Control Points (HACCP) System, a systematic tool or system used to identify hazards and measures to control these hazards to ensure food safety. This evaluates the identified hazards in order to establish control systems that acts preventively as compared to end-product testing. In this system, critical control points and hazards are also identified. Critical control points (CCP) are steps at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level while hazards are any biological, chemical or physical agent in,

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or condition of, food with the potential to cause an adverse health effect (FAO, 1997).

GAP and GMP are formulated toreduce the risk of contamination of fruits and vegetables from the fieldand hence deliver fresh and safe food to consumers (CAST, 2009; Kirezieva *et al*., 2013).According to past surveys, majority of organic producers in the Philippines have a good compliance on food safety standards through these strategiesalthough only a few are certified (Sel and Chansopea, 2014; Kabigting, 2015). Additional local studieson fresh organic agricultural produce are needed to continuously monitor the impact of microbial contamination, especially of pathogenic microorganisms, in order to ensure consumer safety.

Two of the notorious pathogens known to cause disease outbreaks and are linked to the consumption of uncooked fresh produce are *Salmonella* spp.and *Escherichia coli* (Heaton and Jones, 2007; Vital *et al.*, 2014). In the past decade, these pathogens have been isolated from fresh fruits and vegetables used in salad preparations.

Salmonella spp.is an enteric pathogen that causes diarrhea in humans or even life-threatening typhoid fever (Jay, 1986 as cited by Brackett, 1994).Itis commonly present in the intestinal tract of warm and cold-blooded vertebrates with or without showing signs of illness. Carrier animals can shed *Salmonella* spp. through their feces leading to contamination of the surrounding environment: soil, surface of the plants and nearby source of water (Jay *et al.*, 2003; Heaton and Jones, 2007).

Coliforms are comprised of microorganisms such as *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter*. These are indicator microorganisms that provide a measure of fecal contamination in food. The largest concern under this group of microorganisms is the *Escherichia coli,* strain O157:H7, known to cause hemorrhagic colitis more popularly known as"bloody diarrhea."If this disease progresses, it causes detrimental effects such as serious kidney damage and even death (Oliveira *et al*., 2010; Connor, 2015). Like *Salmonella* spp., the common carriers of *E. coli* are soils and manure from livestock used as fertilizers in organic farming (Heaton and Jones, 2007;ICMSF, 2011).

The study aimed to determine the microbial loads at the farm and market level and identify points of microbial contamination along the value chain of organically-grown commodities by investigating the prevalence of food-borne pathogens namely *Salmonella* spp. and *Escherichia coli* in selected fresh organically-grownfruits and vegetables from different farms in Luzon, Philippines.

The effect of simple washing techniques commonly applied in the household was also investigated. This study provides benchmark information towards further improvement on the value chain in the major organic fruit and vegetable producers in the Philippines.

Materials and Methods

This study is composed of three parts:1) determination of the microbial loads of organically- and conventionally-grown vegetables at the farm and market level, 2)identification of the sources of contamination along the value chain of Romaine lettuce, and 3) determination of the effect of simple washing techniques on the microbial loads of Romaine lettuce.

Sample collection

The sampling site, located in the Luzon island group of the Philippines, is composed of 12 different farms and 6 local wet marketsand specialty storesin Benguet, Batangas, Cavite, Laguna, Manila and Quezon. Organicfarms are either third party certified or recognized through provincial guarantee systems. A total of 321 intact organically- and conventionally-grown vegetables that are commonly eaten raw or blanched such as broccoli; green and red cabbage; celery; cucumber; Green Ice, Red Sail, and Romaine lettuce; strawberry and cherry tomato; were obtained and analyzed for microbial load.The packaging material used by the organic fruit and vegetable suppliers (both for the wet market and supermarket) is low density polyethylene bag, the thickness of which ranges from 0.05 to 0.1 mm. Three sets of samples for each commodity (~300 g) except for cherry tomatoes and strawberries (250-400 g each) were collected at each sampling point.

The identification of hazards and critical control points as stated in the HACCP procedure was followed to trace the source of contamination in the value chain of Romaine lettuce. Lettuce samplingwas done sequentially, following the route of the batch of samples from harvesting, trimming, washing, retailing (first hour of retail exposure for the microbiological assessment along the lettuce value chain and third hour of retail exposure for the washing experiment) up to the consumer level.The sampler wore sterile disposable gloves and placed the samples in sterile re-sealable polyethylene bags. All samples were stored in an ice chest maintained at 5°C and immediately brought to the laboratory for analyses within 24 hours.

Surfaces of equipment where the lettuce come in contact with were sampled using a sterile cotton swab: cutting edge of stainless steel scissors, inside walls and bottom of the plastic crate, ceramic coated wash tub, plasticdryer and plastic display tray. Collected samples were asceptically placed inside 20 mL sterile serum capped tubes containing Amies transport medium (ATTC, 2008; USFDA, 2016).

Microbiologicalanalysis

Specimens obtained were submitted to the accredited laboratories nearest the location of experiment: Optimal Laboratories, Inc. in Batangas, DOST-CAR in Benguet, BIOTECH-UPLB in Cavite, Quezon, Laguna and Manila; for total coliform count, detection of *Escherichia coli*, and *Salmonella* spp. Conventional method by Vanderzant and Splihtoesser (1992) were used in Batangas and BIOTECH-UPLB while DOST-CAR used streak-plate method (Merck, 2001) and PetrifilmTM Method (USFDA, 2016). All these laboratories followed the Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods, FDA Circular No. 2013-010.

Washing techniques

Crops produced under organic system are in small quantities hence, washing experiment was done only in lettuce which is the most widely grown and consumed organic vegetable in the Philippines. Whether lettuceis bought washed or unwashed, consumers tend to wash them prior to eating. Simple washing techniques done in the household weresimulated: 1) washing once or 2) twice for 1 minute in a plastic basin of tap water with a chlorination level maintained at 0.02 mg/L (Arzadon, personal communication) (water temperature: 29-32°C); and 3) washingin a plastic basin of 100 ppm chlorine solution (Suslow, 2000)for 1 mins (temperature: 29-32°C).After thorough air drying, three lettuce heads from each washing technique were placed in sterile resealable polyethylene bags. These were stored in an ice chest maintained at 5°C and immediately brought to the analyzing laboratory for microbiological tests within 24 hours. Sample preparation and collection for the washing experiment were performed inside a 25°C laboratory.

Results and Discussion

The presence of *Salmonella* spp. and *Escherichia coli* in food eaten raw is deemed a serious microbial hazard. This indicates fecal contamination and unclean or improper handling and storage. *Salmonella* spp. detection in food also shows poor preparation practices in the form of inadequate cooking or cross-contamination (Carrasco *et al*., 2012; Yousif *et al*., 2013).

Ready-to-eat food, whether raw or processed, has a score ofsatisfactory if *Salmonella* spp. and *E. coli* were not detected, borderline if the type of food does not harbor the growth of these microorganisms, and unsatisfactory or unsafe for consumption when detected in 25 g of sample (Center for Food Safety, 2014).

There are no standards set for coliform levels on fresh fruits and vegetables because these commodities naturally have high levels of coliforms and sometimes belong to the food's natural microflora (Abadias *et al*., 2008). However, high levels of these can indicate high possibility of fecal contamination. For this study, the minimum acceptable limits set by the FDA (2013) for coliforms (<3 MPN/g) and *E. coli* (<3 MPN/g) were used to compare the values reported (Food and Drug Administration, 2013; Center for Food Safety, 2014).

Contamination at the farm and market level

E. coli was not detected in differentorganically-grown commodities sampled from the farm except cabbage (Table 1).On the other hand, *Salmonella* spp. was not detected on all freshly harvested commodities except strawberry. Hence, freshly harvested cabbage andstrawberry are marked unsatisfactory according to standards.

There was no *E. coli* contamination on all commodities at the market level. However, Salmonellaspp. was detected in Romaine lettuce at the market levelmaking it unsafe for consumption (Table 1).

Unless processed and handled properly, organic produce tend to have higher loads of pathogenic microorganisms as compared to the conventionally-grown ones.Organic fruits and vegetables are generally more exposed to carriers of pathogenic microorganisms due to surface contact to the natural sources used as fertilizers such as animal manure; wastes from fruits and vegetables like peels, seeds, uneaten portions, and weeds; and vermi-compost used during farming (Oliveira *et al*., 2010).

The conventional counterparts of broccoli (150 cfu/g), cabbage (590 cfu/g), celery (985 cfu/g), and Green Ice lettuce (955 cfu/g) from La Trinidad Market exhibited higher loads of total coliforms as compared to the organic ones: broccoli (50 cfu/g), cabbage (30 cfu/g), and celery (150 cfu/g). On the other hand, higher counts were observed in organic Romaine lettuce (60cfu/g) and strawberry (1405cfu/g). *E. coli* was not detected in all organic commodities. Conventionally-grown celery and Green Ice lettuce

Table 1. Detection of *E. coli* and *Salmonella* spp. inselected organically-grown crops produced and marketed in La Trinidad, Benguet.

Sample	Farm		Retail Local Market	
	E. coli	*Salmonella*spp.	*E. coli*	*Salmonella*spp.
Broccoli	-	-	-	-
Cabbage	+	-	-	-
Celery	-	-	-	-
Green Ice lettuce	-	-	-	-
Romaine lettuce	-	-	-	+
Strawberry	-	+	-	-

(-)-not detected; (+)-presence of *Salmonella* spp.detected

Table 2.Coliform count (confirmatory test) and detection of *Escherichia coli* and *Salmonella* spp. on fresh produce from La Trinidad Market, Benguet.

Sample	Total Coliform (cfu/g)		*Escherichia coli* (cfu/g)		*Salmonella* spp.	
	O	C	O	C	O	C
Broccoli	50	150	0	0	-	-
Cabbage	30	590	0	0	-	-
Celery	45	985	0	70	-	+
Green ice lettuce	150	955	0	345	-	-
Romaine	60	35	0	0	+	-
Strawberry	1405	115	0	0	-	+

O – organic; C – conventional; (-)-not detected; (+)-presence of *Salmonella* spp. detected

had 70 cfu/g and 345 cfu/g counts of *E. coli,* respectively, which mark it unsafe for consumption. Similarly, *Salmonella* spp. was detected in organic Romaine lettuce and conventionally-grown celery and strawberry which also make them unsafe for consumption. Organic commodities have lower coliform loads as compared to the conventionally-grown ones (Table 2).

From the organically-grown fruits and vegetables obtained from different markets in Cavite, Batangas, and Laguna, *E. coli* was detected in Green Ice lettuce and red cabbage while for *Salmonella* spp., Green Ice, Romaine, Red Sail, and Ice berg lettuce were tested positive (Table 3). Most of the contaminated samples are lettuces, which indicate its susceptibility to contamination. This is due to their large surface area which makes it easy for microbes to cling to. Among the lettuce varieties, higher counts of *E. coli* and higher frequency of contamination of *Salmonella* spp. were observed in Green ice lettuce. Its leaves have curly leaf structures which provide more accessible surfaces to bacteria.

In terms of market type, *E. coli* was detected in Green Ice lettuce in the Cavite specialty store and supermarket in Batangas while Romaine lettuce in all market types are *E. coli*-negative (Table 3). Moreover, Romaine and Green Ice lettuce from Cavite specialty stores are *Salmonella*-negative while those from Tanauan and Laguna supermarket are Salmonella-positive.

Lettuce from the supermarkets have higher frequency of harboring *E. coli* and *Salmonella* spp. than those in the specialty market. This can be due to the large volumes of crops that get mixed with each other in the supermarket leading to cross-contamination. On the other hand, there is a relatively lower volume of crops sold and displayed in specialty stores which makes the risk of cross-contamination lower (Soendjojo, 2012).

Contamination alongorganic lettuce value chain

The apparent risk of pathogenic contamination of lettuce in the retail market was followed up by tracing the point along the chain where contamination occurs. Romaine lettuce, being the most common in vegetable preparations was used. The critical control points (CCP) along the value chain of Romaine lettuce are the trimming and after 1 hour of retail exposure (Table 4).

Based on the initial findings, the coliform load of the newly harvested lettuce are above the acceptable limits set by the FDA (2013). However, *E. coli* was not detected at the harvest stage. Lower coliform counts were observed on samples obtained during trimming but *E. coli* was detected at this stage. Theprobable hazards are the equipment used for trimming, scissors and knives, the handler's hand, and the plastic crates used as containers for hauling and temporary storage.

High loads of coliform were observed in lettuce obtained at the succeeding points of the value chain. *E. coli* was again detected on samples obtained after 1 hour of retail exposure. At this stage, the possible hazards are the handler's hand, consumer's hands or those who touch the lettuce to check its quality, and the container used for display.

The hazards along the value chain of Romaine lettuce were verified through a more detailed sampling based on the previously identified CCPs. This time, the lettuce was intended for high end super markets in Manila and washing was performed as an additional preparatory step.

Table 3.Detection of *E. coli* and *Salmonella* on organically-grownvegetables from different retail markets in Cavite, Batangas, and Laguna.

Market Type/ Crop Origin	Sample	*E. coli*		*Salmonella*spp.	
		Count (MPN/g)	Remarks*	Detection (per 25g sample)	Remarks*
Specialty Store / Silang, Cavite	Romaine lettuce	<3	Passed	-	Passed
	Green Ice lettuce	7.4	Failed	+	Failed
	Tomato	<3	Passed	-	Passed
Specialty Store / Tagaytay, Cavite	Romaine lettuce	<3	Passed	-	Passed
	Green Ice lettuce	<3	Passed	-	Passed
Supermarket / Tanauan, Batangas	Romaine lettuce	<3	Passed	+	Failed
	Green Ice lettuce	3.6	Failed	+	Failed
	Tomato	<3	Passed	-	Passed
Supermarket / Majayjay, Laguna	Green Ice lettuce	<3	Passed	+	Failed
	Romaine lettuce	<3	Passed	+	Failed
	Red Sail lettuce	<3	Passed	+	Failed
	Iceberg lettuce	<3	Passed	+	Failed
	Cherry tomato	<3	Passed	-	Passed
	Strawberry	<3	Passed	-	Passed
	Cucumber	<3	Passed	-	Passed
	Red Cabbage	3.6	Failed	-	Passed

*based on the microbial limits set by the Food and Drug Administration (2013)

Coliform counts above the acceptable limit and *E. coli* were detected in Romaine lettuce obtained at the harvesting stage (Table 4). Swab samples collected from the equipment used (hazards): scissors and harvesting crates as well as farmer's hands showed no presence of *E. coli* and coliform counts were within the acceptable limits. The high levels of coliform and detection of *E. coli* at the harvesting stage can be attributed to the initial contact of the produce with soil which harbors the detected microorganisms.

E. coli was detected on stainless steel scissors and plasticcrates used during sorting, trimming and on trimmer's hand (Table 4). Despite the high levels of coliforms, *E. coli* was not detected on the wash tub, water used for washing, washings, as well as the packaging material. Washing may have possibly helped remove the adhering microorganisms. However due to the use of *E. coli*-contaminated dryer, handler's hand during retail preparation, and display tray, the end-product was still contaminated with *E. coli* rendering it unsafe for consumption.

Microbial quality of organic lettuce after washing

Disinfection is deemed to be one of the most important processing steps affecting the quality and safety of fresh fruits and vegetables (Olmez and Kretzschmar, 2009). Household washing methods are effective at detaching bacteria from the surfaces of the produce through the shear force applied.

Table 4. Critical control points along the value chain of washed organically-grown Romaine lettuce from Laguna sold at a local retail market and supermarket in Manila, respectively.

Critical Control Points	Coliform Count (MPN/g)
1. Lettuce at Harvesting	93*
2. Harvesting	-
- Scissors	<3
- Harvesting crates	<3
- Farmer's hands	<3
3. Sorting and Trimming	
- Scissors	>1,100*
- Trimmer's hands	<3*
- Holding crates	>1,100*
4. Washing	
- Wash tub	>1,100
- Wash water	12 MPN/100ml
- Washings	>23 MPN/100ml
5. Drying	
- Dryer	23*
6. Packaging Material	<3
7. Retail Exposure (0 hour)	-
- Handler's hand	<3*
- Display tray	>1,100*
8. Retail Exposure (1st hour)	-
9. Lettuce at Consumer Level	<3*

*-*Escherichia coli* detected; - – no data

However, the use of sanitizers and disinfectants are proven more effective in reducing microbial loads in commodities (Hadjok *et al*., 2008; Allende *et al*., 2009; Gil *et al*., 2009).

Chlorine is the most popular sanitizer widely used for its effectiveness on reducing pathogenic microorganisms and convenience for use at a very low cost (Rodgers *et al.,* 2004; Olmez and Kretzschmar, 2009). This is the reason why its use is widely practiced especially in developing countries.

The earliest and highest onset of coliform count was exhibited by unwashed lettuce (Table 5). Additionally, this is the only lot where *E. coli* was detected at the end stage. However, it must be noted that *E. coli* was not detected on all samples from harvesting up to retail exposure. Hence, contamination did not occur at the farm.

Lettuces washed twice with water have a relatively low coliform count as compared to the unwashed ones (Table 5). This indicates the slight removal of adhering coliforms from the surface of the samples. Moreover, the tap water used was also initially chlorinated and this may have a reducing effect on the microbial count. Although some microorganisms were removed, the remaining microorganisms thrived and increased on the succeeding sampling points. On the other hand, the coliform count of lettuce washed with chlorinated water showed a significant reduction

Table 5.Coliform count (confirmatory) and detection of *E. coli* on washed and unwashed Romaine lettuce from Laguna and local retail store.

Point of Sampling	Unwashed (MPN/g)	Water (2x) (MPN/g)	Chlorinated Water (100ppm) (MPN/g)
Harvest	29	29	29
Washing	>1,100	460	9
Retail exposure	>1,100	>1,100	1,100
Arrival at home	>1,100*	1,100	460

*-*E. coli* detected

of about 31% after washing. Lettuce washed with chlorinated water exhibited the lowest coliform count among the samples at the consumer level. This indicates the effectiveness of chlorine in the removal of undesirable microorganisms.

For the farms and markets in this study, the implementation of GAP would be very helpful in decreasing the risk of contamination among fruits and vegetables consumed fresh. However, it is noteworthy that GAP and GMP must be implemented at all the points in the chain in order to control re-contamination of the produce. GAP include proper site selection, water quality testing, runoff control, manure and compost management, domestic animal and wildlife control, worker health and hygiene monitoring, field sanitation of harvest equipment, and safe harvesting practices (CAST, 2009). This is best coupled with Standard Sanitation and Operating Procedures (SSOP) and GMP. Corrective action such as washing is very helpful but the produce is still at risk of contamination if proper operations will not be observed in the succeeding processes..

Conclusion

Freshly harvested organically-grown cabbages have high coliform counts and are *E.coli*-positive while strawberries are *Salmonella*-positive. In the market, all organically-grown produce are *E. coli*-negative. *E. coli* and higher loads of total coliforms were detected in broccoli, cabbage, celery and lettuce, being the most susceptible to contamination due to its large surface area where microbes can easily cling on.

The points of contamination along the lettuce value chain were at the trimming, drying, retailing and consumer stages. The critical control points at the trimming stage are the scissors, handler's hand and crates used which were *E. coli*-positive. Contamination was eliminated through washing however, re-contamination occurred as the produce got in contact with *E. coli*-positive dryer as well during the retailing stage where the customer hand and display trays were also found to be *E. coli*-positive.

The experiment on different washing techniques revealed that chlorinated waterwas the most effective in coliform reduction. Pathogenic microorganisms are found everywhere, thus, contamination can take place at any point in the value chain. Although control measures such as washing can be implemented, the produce is still at risk of contamination if proper handling will not be observed in the succeeding handling steps. Adoption of GAP and GMPcan create a positive impact on the microbial quality of the products from the farms and markets inLuzon, Philippines. The information from this study can helpthe stakeholdersadopt appropriate interventions for their operations to deliver fresh, nutritious, high quality and safe organic fruits and vegetables to consumers.

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