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Microbiological aspect of fresh produces as retailed and consumed in West Java, Indonesia

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Abstract

A total of 50 fresh vegetables typically produced, marketed and consumed by people in West Java, Indonesia were quantitatively analysed for the presence of predominant bacteria including the total aerobic bacteria, *Salmonella* spp., *Bacillus cereus*, *Staphylococcus aureus, Eschericia coli*, coliform bacteria, *Shigella* spp. and lactic acid bacteria (LAB). The results revealed that the incidence levels of the above mentioned bacteria were highly varied from 8 to 100%. The microbial load of the samples were in the range of 3.65-10.61; 1.30-3.95; 2.00-4.30; 2.30-6.36; 2.30-6.30; 2.30-7.65; 3.65-4.87; and 4.48-7.11 $\log_{10}$ cfu/g for total plate counts, *Salmonella* spp., *B. cereus, S. aureus, E. coli,* coliform bacteria, *Shigella* spp. and LAB, respectively. It was observed that microbial contaminant was quite high especially in thai lemon basil of which the TPC was >9 $\log_{10}$ cfu/g. Furthermore, the incidence level and microbial load in fresh vegetables procured from supermarket/grocery stores and local traditional market was higher in comparison to vegetables collected from packing house operations and homeyard. This could be probably attributed to a longer holding time of the vegetables at supermarket (longer period between harvesting and selling), the lack of cold storage facilities during transportation, a more frequent exposure to the air, as well as a more handling steps to go through such as sorting, grading, trimming which consequently more frequently exposed to the hands. Furthermore, in case of fresh vegetables obtained from local traditional market, this could be contributed from improper sanitation practices, lack of hygiene as well as better facilities during the handling and selling of fresh vegetables. The outcome of this study assumes importance as the information provided here would give a specific boost for producers, food handlers and consumers for considering food safety as a first priority during fresh produces handling. Furthermore, it is also important for hygiene officials to pay attention on what is offered to consumers and specify acceptable handling practices.

Introduction

Considering for their nutritional value and health benefits, fresh products (fruits and vegetables) become more and more significantly influence the human diets. The demand for fresh products continues to increase yearly along with the increase in consumer's awareness on health benefits of consuming fresh and natural products. The shift in human lifestyle also has driven demand for fruits and vegetables since consumers look for healthier and more nutritious options but also convenience for their diets, such as fresh-cut fruits and vegetables, ready-to-eat salads, etc. The trend in increased consumption of fresh fruits and vegetables is expected to continue through 2020 with fruit consumption increasing by 24 to 27% and vegetable consumption increasing by 19 to 24% (Raicevic *et al*., 2010).

Scientific studies in the field of health related dietary have shown that many nutrients and bioactive compounds are naturally present in fruits and vegetables, such as folate which is mostly found in green leafy vegetables (such as spinach and lettuce), carotenoids in yellow and orange fruits and vegetables (such as carrot and pumpkin), dithiolthiones and isothiocyanates in cruciferous vegetables (such as cabbages and broccoli), and vitamin C in citrus (Wang and Bohn, 2012). Next to this, the health benefits from consuming these fresh products have been extensively studied (Block *et al*., 1992; Steinmetz *et al*., 1996; Williams *et al*., 1999; Liu *et al*., 2000; He *et al*., 2004). It was suggested that consumption of a minimum of 400 g of fruits and vegetables per day significantly prevent against some chronic diseases such as heart disease, hypertension, cancer, diabetes and obesity (WHO, 2003).

Although fruits and vegetables are regarded as an important part of a healthy diet and considered good

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for human health due to the high content of vitamins, minerals, and fibers, they also may represent a hazard for human health. It was reported in many studies that fresh fruit and vegetables are highly correlated to the unwanted foodborne outbreaks because they are potentially to be contaminated with a range of microbial and chemical contaminants (Abadias *et al*., 2008; Eni *et al*., 2010; Hannan *et al*., 2014; Jeddi *et al.*, 2014). The freshness of fruits and vegetables as well as the nutritious compounds in these products are favorable for the growth of microorganisms, either food spoilage microorganisms or food pathogenic microorganisms (Ray and Bhunia, 2008).

Microorganisms frequently detected and tend to dominate the bacterial population in fresh fruits and vegetables include *Enterobacter* spp. and other coliforms, *Salmonella, Shigella, Bacillus,* and *Lactobacillus*. Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks (Buck *et al*., 2003). And recently even an outbreak associated with raw cantaloupes has been linked to *Listeria monocytogenes* strain (CDC, 2011). The disease caused by these bacteria is usually mild and has a short duration, however the frequent of the occurrences of the cases are quite often. Outbreaks of human infection associated with the consumption of raw fruit and vegetables often occur in developing countries and have become more frequent in developed countries over the past decade (Hedberg, 1994; Altekruse and Swerdlow, 1996; Beuchat, 1996, Beuchat, 2002). Although, the majority of microorganisms associated with fresh fruits and vegetables are non-pathogenic, the present of these microorganisms indicates the low quality and microbial safety of the products (Hayes, 1992). Thus, instead of obtaining the nutritional and health benefits, the health of consumers can be adversely affected by consumption of microbiologically unsafe fruit and vegetables.

Although it is still far below the minimum level recommended by Food and Agriculture Organisation (FAO has recommended a minimum consumption of vegetables of 91.25 per capita per year), the consumption rate of fresh vegetables in West Java Province is among the highest compared to other provinces in Indonesia. According to the data from Indonesia Food Security Agency, the consumption of fresh vegetables in West Java in 2011 is estimated at 48.80 kg per capita per year (BKP, 2012), while at the national level, as reported by Indonesian Statistic Bureau, it is 37.52 kg per capita per year (BPS, 2012). Furthermore, the local people of West Java (Sundanese) usually consume vegetables as a raw or by cooking them with minimal processing. They believe that consuming vegetables as a raw will give better affects to human health compared to when they are cooked. Taking into consideration that fresh vegetables are the high consumption rate combined with the way how they consume it, may generate serious health risk to them.

To the best of our knowledge, limited data was available for local studies on the microbiological quality of fresh vegetables in Indonesia. The present study is the first study which samples were directly collected from various sources along the production line and fresh produce supply chain of local fresh vegetables in West Java, Indonesia. The main objective of the study was to evaluate the microbiological qualities of fresh vegetables, originated from different sources, and to provide baseline information on the prevalence and level of microbial contamination of fresh vegetables. Results generated in this study is expected to be useful for consumers health awareness as well as the local governing agency to implement appropriate food safety measures to minimize the risk factors associated.

Materials and Methods

Sample collection

Fifty samples of fresh vegetables were collected from different sampling sites in West Java province-Indonesia during April-October 2013. These samples included thai lemon basil, lettuce, tespong leaves, caisim leaves, leaf lettuce, water spinach, spinach, chinese kale, zucchini/ courgette, chinese cabbage, yardlong bean, handelin leaves, local cucumber, brussels sprouts, white cabbage, tomato, black nightshade, eggplant, cayenne pepper. The sampling sites included a homeyard in location of sustainable food reserve garden program (Kawasan Rumah Pangan Lestari - KRPL) in Subang district, two packing house operations (PHO) in Lembang district, a local market (LM) and three selected supermarkets (SM) in Tangerang district. List of tested product types is presented in Table 1. Each sample taken from sampling site was put separately into a steril HDPE bag, inserted into a cool box and transported to the laboratory. No additional washing steps were applied to the samples after collection. Samples were stored at 5-8°C until the analysis was performed. Analysis was performed within 48 h of collection.

Sample preparation

Each sample (25 g) was put into a sterile Erlenmeyer flask, soaked and homogenized by shaking thoroughly with 225 ml of buffered peptone water (BPW) solution. From these, serial dilutions

Tabel 1. List of tested product type

Product type		n (%)							
General name	Local term	Total	KRPL	PHO-1	PHO-2	LM	SM-1	SM-2	SM-3
Thai lemon basil	kemangi	5(10)		1 (2)		1 (2)	1 (2)	1 (2)	1 (2)
Ocimu(m × citriodorum)									
Lettuce	selada kepala	3(6)		1 (2)				1 (2)	1 (2)
(L. sativa var. Capitata)									
Tespong leaves	tespong	1(2)		1 (2)					
Oenanth(e javavica D.C)									
Caisim leaves	caisim	2(4)	1 (2)	1 (2)					
Brassic(a rapa var. parachinensis L)									
Leaf lettuce	selada keriting	5(10)		1 (2)		1 (2)	1 (2)	1 (2)	1 (2)
(L. sativa var. Crispa)									
Water spinach	kangkung	1(2)		1 (2)					
(Ipom oea aquatic)									
Spinach	bayam	1(2)		1 (2)					
(Spinacia oleracea)									
Chinese kale	kai-lan	1(2)		1 (2)					
(Brassica oleracea cultivar Alboglabra)									
Zucchini/ courgette	timun jepang	4(8)			1 (2)		1 (2)	1 (2)	1 (2)
(Cucurbita pepo)									
Chinese cabbage	pak choy	1(2)		1 (2)					
B(. rapa cultivar chinensis)									
Yardlong bean	kacang panjang	4(8)		1 (2)		1 (2)	1 (2)	1 (2)	
(Vigna unguiculata subsp. Sesquipedalis)									
Hanjeli leaves	hanjeli	1(2)	1 (2)						
(Coixlachrym a - Jobi L.)									
Local cucumber	timun lokal	5(10)		1 (2)		1 (2)	1 (2)	1 (2)	1 (2)
(Cucum is sativus)									
Brussels sprouts	baby kol putih	2(4)		1 (2)				1 (2)	
(Brassica olevacea var. gemmifera DC.)									
White cabbage	kol putih	2(4)		1 (2)			1 (2)		
(Brassica oleracea cultivar capitata var. *alba* L.)									
Tomato	tomat	7(14)		1 (2)	2 (4)	1 (2)	1 (2)	1 (2)	1 (2)
Solanu(m lycopersicum)									
Black nightshade	leunca	1(2)	1 (2)						
(Solanum nigrum L.)									
Eggplant	terung	3(6)				1 (2)	1 (2)		1 (2)
(Solanum m elongena)									
Cayenne pepper	cabai	1(2)					1 (2)		
(Capsicum annuum)									
Total		50 (100)	3 (6)	14 (28)	3 (6)	6 (12)	9 (18)	8 (16)	7 (14)

were prepared (10^1 to 10^8) using sterile BPW as the diluent.

Microbial enumerations, isolations and identifications

Microbial evaluation was carried out based on the standard protocol and isolated colonies were identified based on growth in selective media and biochemical tests.

Total Plate Count (TPC)

For total plate count (TPC), the pour plate method was used by following the procedure described in American Public Health Association (APHA, 1992). One milliliter volume of certain three dilutions was inoculated into a Petri dish. This was followed by pouring a volume of 12 ml molten plate count agar (PCA, Oxoid) and mixing it. Petri dish were then placed to allow being set, and incubated at 37°C for 24 ± 2 h. PCA plates showing number of colonies between 30 to 300 were selected and counted using electronic colony counter, and the number of colonies was recorded.

Salmonella *spp.*

Referring the methods described in the American Public Health Association (APHA, 1992), the pour plate method was used for determination of *Salmonella* spp.. One milliliter volume of certain three prepared dilutions was inoculated into a Petri dish. A volume of 12 ml of molten selective and differential plating medium xylose-lysine desoxycholate agar (XLD, Oxoid) was then poured into the Petri dish, mixed, and placed to allow being set. Plates were incubated at 37°C for 24 ± 2 h. Typical colonies of *Salmonella* spp. are black, 2-3 mm in diameter. Following incubation, the number of colonies was recorded.

Bacillus cereus

Bacterial colony counts of *B. cereus* were made on Mannitol Egg Yolk Polymyxin Agar (MYP, Oxoid) using spread plate technique (Bacteriological Analytical Manual, 1998). A volume of 0.1ml of each dilution sample was inoculated onto the surface of MYP agar plate and spread using L-shaped glass rod (often called a "hockey stick") to allow the inoculum to soak into the agar. Plates were incubated at 30°C for 24 ± 2 h. Typical colonies of *B. cereus* are rough and dry with a bright pink background surrounded by an egg yolk precipitate.

Staphylococcus aureus

Spread plate technique referring the method described in Bacteriological Analytical Manual, 1998 were used for the enumeration of *S. aureus*, and the media of Baird Parker Agar (BPA, Oxoid) was used as the selective medium. A volume of 0.1ml of diluted samples was inoculated onto the surface of BPA agar plate and were spread using a sterile, L-shaped glass rod. Plates were incubated at 37°C for 24 ± 2h. After incubation, plates were examined for the number of colonies. Plates having 20-200 colonies were selected, and the number of typical *S.*

aureus colonies was recorded. Typical colonies of *S. aureus* are black, shiny, convex colony with entire margins and clear zones.

Eschericia coli *and coliform bacteria*

Total coliforms and total *E. coli* were enumerated by following the method described in Bacteriological Analytical Manual, 1998. The selective and differential media Eosin Methylene Blue Agar (EMBA, Oxoid) were used for the isolation of *E. coli* and coliform bacteria. One milliliter volume of certain three dilutions was inoculated into a Petri dish, followed by pouring approximately 12 ml of molten selective and differential plating medium Eosin Methylene Blue Agar (EMBA, Oxoid). Media were then mixed and placed to allow being set. Incubation of the plates was done at 37°C for 24 ± 2 h. Following incubation, the number of colonies was recorded for each plate. *E. coli* colonies typically are dark centered and usually have a green metallic sheen. While colonies of coliform appear as pink with a dark/black center.

Shigella *spp.*

Highly selective medium of *Salmonella-Shigella* Agar (SSA, Oxoid) was used for the isolation and enumeration of Shigella spp. Enriched samples were inoculated on SSA and incubated at at 37°C for 48 ± 2 h. Typical colony of *Shigella* spp. produce colorless, translucent colonies on SSA (Bacteriological Analytical Manual, 1998).

Lactic acid bacteria (LAB)

Lactic acid bacteria count was conducted by pour plating method with an over layer on De Man Rogosa Sharpe Agar (MRSA, Oxoid). One milliliter of the appropriate dilution was inoculated into a Petri dish, followed by pouring approximately 15 ml of MRSA. Plates were then mixed and placed to allow being set. Subsequently, plates were overlaid with further MRSA to cover the surface, placed to allow being set and inverted incubated under aerobic conditions at 37°C for 48 ± 2 h (Bacteriological Analytical Manual, 1998).

Results

Description of the sampling sites

The sampling site of homeyard was a small plot or plots around the home, managed by household members, where a variety of crops including vegetables, fruits, legumes, tubers, non-food plants, e.g., medicinal herbs, spices, are grown throughout the year and often livestock and fish are raised, primarily for household consumption. They typically use low-cost inputs and indigenous varieties, as well as local knowledge and practices and community participation. The samples represented local products of a homeyard is taken from the location of sustainable food reserve garden program (Kawasan Rumah Pangan Lestari - KRPL). KRPL is a program developed by Indonesian Ministry of Agriculture which involves women in the family as the main actor to use homeyard optimally with vegetable and potential fruits planting and/or poultry and small ruminant raising.

The sampling site of PHO-1 was described as a big moderated PHO, where separate units are available for receiving, trimming, washing, and packing of the produces. The sampling site of PHO-2 was described as a small non-sophisticated PHO, where a minimal facility was available and the same area was utilized for receiving, cleaning and packing the produces. No trimming or washing activities were done.

The sampling site of local market was described as a closed wet market, appears to be less clean, and has less equipped facilities (no air conditioning). Vegetables were sold unpacked and a bulky size of each vegetable was placed on a plastic crate/bulk trays and they are located close each other. The sampling site of supermarkets were described as modern markets having a clean place and surrounding, and have well-equipped facilities such as air conditioning and display shelves with cooling system. Although there are some produces sold in bulky form, most of them are sold in packed. The packed produces were placed on the display shelves equipped with cooling system. Furthermore, the three supermarkets are different in term of level of cleanliness of place, facilities, and convenience. The SM-2 is better than SM-1, also SM-3 is better than SM-2.

Incidence level and microbial load of the fresh vegetables samples

Figure 1 shows the average of incidence levels of total aerobic bacteria and indicator bacteria of all samples analysed. All the fifty fresh vegetables sampled in the present study were contaminated. Among the indicator bacteria analysed, coliform bacteria group (84%), *S. aureus* (78%), and LAB (80%) were the most commonly present in the samples, while *Shigella* spp. (13.3%) *Salmonella* spp. (10%) were the least frequently present (Figure 1). The incidence levels of *B. cereus* and *E.coli* in all samples were 16 and 34%, respectively.

Furthermore, the microbial load of each fresh vegetable varied with type. The microbial load of the fresh vegetables were in the range of 3.65-10.61;

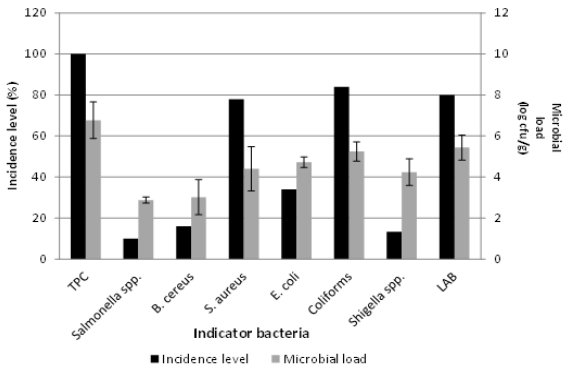

Figure 1. Incidence levels (%) and microbial load (log_{10} cfu/g) of TPC and indicator bacteria of all samples tested

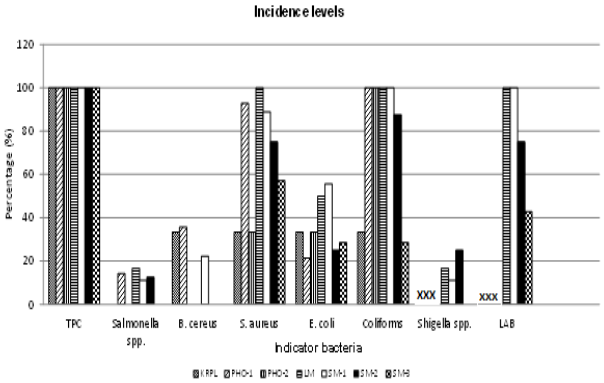

Figure 2. Incidence levels (%) of TPC and indicator bacteria classified by sample origin (X=not analysed)

1.30-3.95; 2.00-4.30; 2.30-6.36; 2.30-6.30; 2.30-7.65; 3.65-4.87; and 4.48-7.11 log_{10} cfu/g for total plate counts, *Salmonella* spp., *B. cereus, S. aureus, E. coli,* coliform bacteria, *Shigella* spp. and LAB, respectively (Table 2). While the average microbial load were 6.77; 2.88; 3.02; 4.40; 4.72; 5.25; 4.24; and 5.44 log_{10} cfu/g for TPC, *Salmonella* spp., *B. cereus*, *S. aureus, E. coli,* coliform bacteria, *Shigella* spp. and LAB, respectively (Figure 1).

Regarding the distribution of the contaminated samples, with an exception of TPC, *S. aureus* and coliform bacteria group, the highest proportion of contaminated samples fell in the microbial load below 2 log_{10} cfu/g. The highest incidence level of samples being contaminated with TPC fell in the range of 6 log_{10} to 8 log_{10} (40%), although a quite large proportion also observed for microbial load of 4 to 6 log_{10} (30%) and 8 to 10 log_{10} (24%). While for *S. aureus* and coliform bacteria group, the highest proportion of contaminated samples fell in the range of 4 to 6 log_{10} cfu/g, which were 46% and 42%, respectively. Moreover, a quite equal proportion was observed for the samples having LAB counts <2 log_{10} (52%) and 4 to 6 log_{10} (40%) (Table 2).

Incidence level and microbial load of the fresh vegetables as grouped by sample origin

Figure 2 presents the incidence levels of total aerobic bacteria and indicator bacteria of examined fresh vegetables grouped by sampling site. All samples were found to be contaminated disregarding the origin of the samples. Identical results also observed for the incidence of contamination by *S. aureus*, coliform bacteria group and *E.coli*. However, the level of incidence varied with location sampling. *S. aureus* was the most frequently isolated from fresh vegetables taken from PHO-1, LM, SM-1, and SM-2, of which most of them had incidence level of more than 80%. Coliform bacteria was present in all fresh vegetables obtained from PHO-1, PHO-2, LM, SM-1, and SM-2, and in more than 80% samples from SM-2. LAB was detected in all fresh vegetables sampled from LM and SM-1, and in most of the samples collected from SM-2 (75%). Although it was not as frequent as contamination by *S. aureus,* coliform bacteria, and LAB, a low incidence related to contamination by *E.coli* was found in samples obtained from the seven different sampling sites. Salmonella spp. was present in low number of the fresh vegetables obtained from PHO-1 (14%), LM (16%), SM-1 (11%), and SM-2 (12.5%), but not in any of fresh vegetables obtained from KRPL and SM-3. *B. cereus* were detected in quite high number of fresh vegetables obtained from KRPL (33%), PHO-1 (35%), and SM-1 (22%), but not in any of fresh vegetables taken from PHO-2, LM, SM-2 and SM-3. While incidence of contamination by *Shigella* spp. was observed in low number of fresh vegetables collected from LM (16%), SM-1 (11%) and SM-2 (25%), but not in any of fresh vegetables obtained from PHO-1, PHO-2, and SM-3. Remarkably, samples from KRPL, PHO-1, and PHO-2 were not analysed for the incidence of *Shegella* spp. and LAB.

The number of TPC and other indicator bacteria in the fresh vegetables grouped by sample origin are presented in Figure 3. As shown in Figure 3, the TPC of fresh vegetables collected from LM, SM-1, SM-2, and SM-3 was found to be much higher than those of fresh vegetables from KRPL, PHO-1, and PHO-2. Most of them were more than 8 log_{10} cfu/g. Similiar results were observed for the number of *S. aureus, E.coli*, and coliform bacteria. Quite high number of *Shigella* spp. and LAB were also found in the fresh vegetables obtained from LM, SM-1 and SM-2. Again, it should be noted that samples from KRPL, PHO-1, and PHO-2 were not analysed for the incidence of *Shigella* spp. and LAB.

Table 2. Distribution of contaminated samples

Microorganisms	Number of samples analysed	Microbial load (log cfu/g)			Percentage of contaminated samples in the indicated interval					
		min	max	mean	<2	2 - <4	4 - <6	6 - <8	8 - <10	>10
TPC	50	3.65	10.61	6.77	0	4	30	40	24	2
Salmonella spp	50	1.30	3.95	2.88	92	8	0	0	0	0
B. cereus	50	2.00	4.30	3.02	84	14	2	0	0	0
S. aureus	50	2.30	6.36	4.40	22	22	46	10	0	0
E. coli	50	2.30	6.30	4.72	66	10	14	10	0	0
Coliforms	50	2.30	7.65	5.25	16	16	42	26	0	0
Shigella spp.	30*	3.65	4.87	4.24	92	4	4	0	0	0
LAB	30*	4.48	7.11	5.44	52	0	40	8	0	0

* samples from LM, SM-1, SM-2, and SM-3 were not analysed

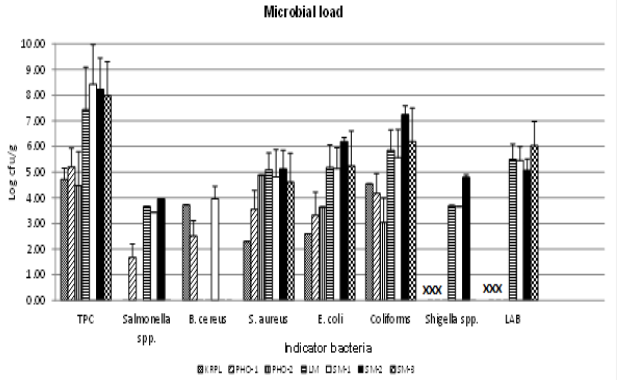

Figure 3. Microbial load (log_{10} cfu/g) of samples classified by sample origin (X=not analysed)

Discussion

Despite their nutritional and health benefits, fresh vegetables can also act as a major source and vehicle for the transmission of foodborne disease as they are normally widely exposed to microbial contamination throughout the supply chain, from production stage on the farm to the selling stage in the market, even at the serving preparation stage by consumers before consume it (Soriano *et al.* 2006). There are many factors may contribute to the microbial contamination on fruits and vegetables. This microbial contamination can occur directly or indirectly during pre-harvest, harvesting as well as post-harvest handling. A study by Sangshin *et al*. (2012), reported that the agricultural water for irrigation was one of the most predominant potential of pre-harvest sources of microbial contamination on fruits and vegetables. Other sources of contamination are mainly from animals or insects, soil, manures, as well as equipments used during growing. During harvest, fruits and vegetables may be bruised resulting in the release of nutrients which ultimately results in providing nutrients for microorganisms present on the surface of the fruits and vegetable to grow (Bell *et al*., 2005). Furthermore, fresh vegetables are also considered as high-risk foods as usually most of fresh vegetables are typically for raw eaten without any heating or processing treatment prior to consumption (Beuchat, 1998; de Rover, 1998; Nguyen-the and Carlin, 2000). As what observed, the local people of West Java (Sundanese) have a habit of consuming vegetables as a raw or with minor processing treatments. Consumption of these contaminated products is very risky and might cause foodborne outbreaks. These such conditions is one of contributing factors to the remarkably increase in incidence of foodborne outbreaks recently (Sivapalasingam *et al*., 2004).

There are many studies conducted in different countries reported the microbiological quality of fresh produces as well as the risk associated to consumption of these contaminated produces (van Ha *et al*., 2008; Bohaychuk *et al*., 2009; de Oliveira *et al*., 2011; Halablab *et al*., 2011; Seow *et al.*, 2012; Goja and Mahmoud, 2013; Mngoli and Manani, 2014; Vital *et al*., 2014). However, such data on the microbial contamination on fresh vegetables in Indonesia are very limited. To our knowledge, this study is the first study to address the incidence level as well as the microbial load of fresh vegetables of which the samples were collected from a variety sources along the fresh produce supply chain. The data obtained from this study could be useful to give an overview as well as to compare the safety quality of the fresh vegetables on every stage of different produce handling and distribution. It is expected that the data collected in this study could give valuable information for farmers, food handlers, industries, policy makers, and consumers in order to improve quality and microbial safety of fresh produces. A better understanding on these issues could lead to better microbiological quality of fresh produces.

All samples analysed in the present study were found to be contaminated. Furthermore, the bacterial count was also quite high. Similar findings to these results were also reported in many other previous studies. Study conducted by Abadias *et al*. (2008) reported all vegetables samples purchased from four supermarkets in Spain harboured TPC. Furthermore, a quite high loads of TPC was observed in these samples, up to 8.9; 9.2; and 8.0 log_{10} cfu/g in fresh-cut vegetables, sprouts, and whole vegetables,

respectively. Other study by Allen *et al*. (Allen *et al.*, 2013) has detected microbial contaminants on all samples of imported vegetable produce available at retail in Canada. Study by Eni *et al.* (2010) reported all fresh vegetables and fruit samples collected from three vendors in Sango Ota, Nigeria were highly contaminated with microbial load ranged from 5.95 to 7.48 $\log_{10}$ cfu/g. Other study by Hannan *et al*. (2014) which examined 50 different ready-to-eat salads sampled from vendors and restaurants in Lahore, Pakistan found a bacterial contamination level of up to 7.76 $\log_{10}$ cfu/g. Study on the microbial quality of fresh-cut vegetables, ready-to-eat salads, wheat and mung sprouts collected from four supermarkets in Tehran, Iran found all examined samples were contaminated with an average microbial loads of 6.4, 6.7, 6.9, and 7.0 $\log_{10}$ cfu/g, respectively (Jeddi *et al.*, 2014). The results from all these studies demonstrated that even though fresh vegetables contain high nutritional compounds and offer potential health benefits for human, they are also a good substrate for microbial growth and hence considered as reservoir of microbial contamination. Remarkably, thai lemon basil samples had a quite high TPC counts which was >9 $\log_{10}$ cfu/g (data not shown). As also observed in other previous study, it was suggested that leafy vegetables had a tendency to have high microbial load (Abadias *et al*., 2008, Valentin-Bon *et al*., 2008; WHO/FAO, 2013).

Incidence levels of *Salmonella* spp., *B. cereus, S. aureus, E. coli*, coliform bacteria, *Shigella* spp. and LAB were 10; 16; 78; 34; 84; 8; and 48%, respectively. While the load of these indicator bacteria ranged from 1.30-3.95; 2.00-4.30; 2.30-6.36; 2.30-6.30; 2.30-7.65; 3.65-4.87; and 4.48-7.11 $\log_{10}$ cfu/g. A remarkable result were observed in the contamination incidence and microbial load by *Shigella* spp.. The incidence level of the fresh vegetables being contaminated by *Shigella* spp. is quite lower (13.3%). However, the microbial load of this contaminated samples is quite high (4.24 $\log_{10}$ cfu/g). It was reported that the present of this microorganism is highly related to fecal contamination and unhygienic practices of food handlers. Furthermore, although usually this microorganism is transmitted from person to person, transmission through contaminated food products, including fresh vegetables and salads, is also greatly possible (Crowe *et al*., 1999; Berger *et al*., 2010).

The indicator bacteria analysed in the present study are commonly found in soil and water as well as on the surface of fresh produce (Adams and Moss, 2008; Leff and Fierer, 2013). Hence, it was suggested that the contamination might be already occurred during plantation when the produces exposed to the soil and irrigation water, although postharvest contamination could not be ignored. The postharvest contamination might take place during postharvest handling, transporting, and storage of the produces. Handling of the produces with contaminated equipment and contaminated hands, exposing the produces extensively to the open air during transportation, as well as storing the produce in unhygienic places will lead and increase the produces being contaminated. An extra care during handling of fresh produces, from harvesting, cleaning, sorting, packaging, transport and storage, as well as proper cleaning and sanitation system and individual hygiene of food handler, are among the best ways to reduce the risks for food-borne illness (Beuchat, 1998).

Furthermore, it was hypothesized that samples obtained from supermarkets would have less prevalence and microbial load due to the cleanliness of the place, implementation such supporting instruments such as cooling system on the display shelves, as well as adequate hygienic practices such as the use of gloves by the handler. Contrary to the hypothesis, higher incidence level and microbial load were observed in the samples collected from the supermarkets compared to those obtained from homeyard and PHO. The TPC and bacterial counts of the contaminated samples collected from supermarkets were much higher compared to those associated with samples obtained from homeyard and PHO. This could be probably attributed to a longer holding time for the vegetables at the supermarkets compared to the corresponding time for samples obtained from homeyard and PHO and therefore giving more time for the microorganisms to grow. It was observed that oftentimes the fresh vegetables had to be transported and distributed to the market over long distances from the production areas. Due to the lack of cold storage facilities, certain types of microorganisms especially the mesophilic ones may continue to grow and proliferate during transport from field to supermarkets. Another possibility was due to more handling steps to go through by the fresh vegetables obtained from supermarkets, such as sorting, grading, trimming. Consequently, they are more frequently exposed to the hands, which could further increase the microbial load. However, a study by Soendjojo (2012) reported a much higher number of bacteria on some fresh vegetables available at the farmers' market compared to those obtained at the grocery store. It was suggested that it could be due to an effective sterilization treatments applied by the grocery store in reducing the microbial population on the produces in order to increase the shelf life of

the produces. Another study by Vital *et al.* (2014) which compared the microbial quality of fresh produces from open air markets and supermarkets in Philippines revealed a relatively higher incidence levels and microbial loads observed in samples obtained from supermarkets compared to those for samples obtained from open air, although statistically these differences was not significant. In general, it can be concluded that microbial contamination on fresh produces can be occurred at any stage of supply chain, from production, harvesting, distribution, selling point and domestic preparation, as what observed in the present study.

Conclusion

Results obtained in this study showed a quite high level of microbial contamination of fresh vegetables along the supply chain in West Java, Indonesia. Furthermore, the incidence levels as well as the microbial load of the contaminants in the samples obtained from supermarkets and local market was relatively higher compared to those obtained from homeyard in location of sustainable food reserve garden program and packing house operation. This might be caused by a longer holding time between harvesting and selling, the lack of cold storage facilities during transportation, as well as a more handling steps to go through for samples obtained from supermarkets. The absence of good handling practices and sanitation system in the local traditional market, at which the samples were taken, could have contributed to the high microbial load observed in samples obtained from site. Proper washing and disinfection of vegetables before consumption is strongly advised. Also, personal hygiene of the vegetables handlers should always be implemented. Furthermore, vegetables handlers should adhere to GMP and SSOP.

Finally, since this study has been conducted amongst only 50 samples representing the fresh vegetables products, a study with greater number of samples would probably provide more information which may confirm the results of this study.

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