Comparison of the Microbiological Quality and Safety between Conventional and Organic Vegetables Sold in Malaysia

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Given the remarkable increase of public interest in organic food products, it is indeed critical to evaluate the microbiological risk associated with consumption of fresh organic produce. Organic farming practices including the use of animal manures may increase the risk of microbiological contamination as manure can act as a vehicle for transmission of foodborne pathogens. This study aimed to determine and compare the microbiological status between organic and conventional fresh produce at the retail level in Malaysia. A total of 152 organic and conventional vegetables were purchased at retail markets in Malaysia. Samples were analyzed for mesophilic aerobic bacteria, yeasts and molds, and total coliforms using conventional microbiological methods. Combination methods of most probable number-multiplex polymerase chain reaction (MPN-mPCR) were used to detect and quantify foodborne pathogens, including Escherichia coli O157:H7, Shiga toxin-producing E. coli (STEC), Listeria monocytogenes, Salmonella Typhimurium, and Salmonella Enteritidis. Results indicated that most types of organic and conventional vegetables possessed similar microbial count (P > 0.05) of mesophilic aerobic bacteria, yeasts and molds, and total coliforms. E. coli O157:H7 and S. Typhimurium were not detected in any sample analyzed in this study. Among the 152 samples tested, only the conventional lettuce and organic carrot were tested positive for STEC and S. Enteritidis, respectively. L. monocytogenes were more frequently detected in both organic (9.1%) and conventional vegetables (2.7%) as compared to E. coli O157:H7, S. Typhimurium, and S. Enteritidis. Overall, no trend was shown that either organically or conventionally grown vegetables have posed greater microbiological risks. These findings indicated that one particular type of farming practices would not affect the microbiological profiles of fresh produce. Therefore, regardless of farming methods, all vegetables should be subjected to appropriate post-harvest handling practices from farm to fork to ensure the quality and safety of the fresh produce.

Keywords: Escherichia coli O157:H7, salmonella, Listeria monocytogenes, fresh produce, organic farming
INTRODUCTION

Public awareness of healthy eating habits have been intensified in recent years and prompted an increased demand for fresh fruits and vegetables (Olaimat and Holley, 2012). Despite the health benefits derived from consuming fresh produce, the risk of microbiological contamination in vegetables is of concern as the contamination can possibly occur through the food chain, from farm to fork. Over the past decade, numerous foodborne disease outbreaks caused by Listeria monocytogenes, Escherichia coli O157:H7, and Salmonella spp. were related to the consumption of contaminated fresh vegetables (Beuchat, 2002; Centers for Disease Control and Prevention, 2011, 2012; Maffei et al., 2013).

Given people’s growing awareness of health and environmental sustainability, organic farming systems have become more well-received because conventional farming uses large amounts of synthetic pesticides and chemical fertilizers. Organic foods are perceived as safer and more healthful foods due to the chemical-free farming techniques used for their production as compared to conventionally produced foods (Somasundram et al., 2016). In Europe, the consumption of fresh organic produce has increased annually (Willer and Kelcher, 2009). In Malaysia, although the organic food remains a niche market and comprises only a small fraction of the food market, the demand for organic food has grown steadily. There were about 131 ha organic farms in Malaysia in 2001. However, the land area for organic farms was increased by 18-fold—2,367 ha, of which 962 ha are certified organic within a 5-year period. In Malaysia, the production of organic food is limited to vegetables and fruits only and most of the fresh organic produce are sold in domestic markets (Mohamad et al., 2014; Tiraiyari et al., 2014; Somasundram et al., 2016).

Despite the widespread consumers’ belief that organic foods are “safer” and “more healthful” than conventional foods, evidence to support this concept is difficult to determine. Microbiological quality and safety of organic produce remain to be controversial and debated (Maggkos et al., 2006). This issue emerged due to the lack of research and limited scientific data to reveal the actual scenario. The view that fresh organic produce is “safer” than conventionally grown food appears to be constructed on the perception that organic fruits and vegetables are free from chemical fertilizers and synthetic pesticides (Institute of Food Technologists, 2000; Somasundram et al., 2016). Conversely, previous studies have suggested that organic production practices, such as the use of manure may increase the risk of microbiological contamination. Manure may harbor foodborne pathogens, such as Salmonella spp., L. monocytogenes, and E. coli O157:H7 (Stephenson, 1997; McMahon and Wilson, 2001; Williams, 2002; Johannessen et al., 2004). Also, manure may introduce various pathogenic microorganisms that can persist for a long duration in the soil (Pell, 1997). However, it is difficult to conclude that the consumption of fresh organic produce would confer greater microbiological risk to consumers than conventional food. Other than cultivation method, microbial contamination can occur during harvesting, post-harvest handling or at any point along the food supply chain (Beuchat and Ryu, 1997).

The present study aimed to investigate and compare the microbiological status of different organic and conventional vegetables sold in the retail markets in Malaysia. To the best of our knowledge, this is the first comprehensive study on the comparison of microbiological quality and safety level between organically and conventionally grown vegetables in Southeast Asia.

MATERIALS AND METHODS

Sample Collection

A total of 152 organic and conventional vegetables, comprising of 77 organic (certified by competent national and overseas authorities) and 75 conventional, were randomly purchased from hypermarkets and wet markets in Kuala Lumpur, Selangor, and Putrajaya. The samples collected included: cabbage (Brassica oleracea), carrot (Daucus carota subsp. sativus), calamondin (× Citrofortunella microcarpa), cherry tomato (Solانum lycopersicum var. cerasiforme), Bird’s eye chili (Capsicum annuum), cucumber (Cucumis sativus), eggplant (Solanum melongena), winged bean (Psophocarpus tetragonolobus), Romaine lettuce (Lactuca sativa var. longifolia), Iceberg lettuce (Lactuca sativa var. capitata), Looseleaf lettuce (Lactuca sativa var. crispa), Butterhead lettuce (Lactuca sativa var. capitata), sweet potato (Ipomoea batatas), tomato (Solانum lycopersicum), and white radish (Raphanus sativus). Sampling was carried out over a 1-year period (November 2015 to October 2016). All samples (250–300 g each) were randomly collected from bulk quantities of vegetables, placed in sterile bags (BagMixer® 400 mL, Interscience, Saint-Nom-la-Bretèche, France), kept in an insulated box with ice packs and transported immediately to the Food Safety and Quality Laboratory, Universiti Putra Malaysia for microbiological analyses.

Microbiological Analysis

Twenty-five grams of each sample was cut into small pieces, weighed, placed in a sterile stomacher bag, and followed by homogenization using a stomacher machine (BagMixer® 400P, Interscience, Saint-Nom-la-Bretèche, France) with 225 mL of 0.1% (v/v) peptone water (OxoidTM, Basingstoke, Hampshire, UK) for 1 min. The pH of the bacterial culture broth was neutralized to pH 7.0 with 0.5 M NaOH solution. Mesophilic aerobic bacteria, total coliforms, and yeasts and molds were enumerated using conventional methods (Beuchat and Cousin, 2001; Kornacki and Johnson, 2001; Morton, 2001). Each sample was analyzed in triplicate and all the results were expressed as colony-forming units per gram (CFU/g).

Detection and Enumeration of Foodborne Pathogens by MPN-PCR Method

Most-Probable-Number (MPN)

Each sample was cut into small pieces, and then a total of 10 g of sample was mixed with 90 mL of Tryptic Soy Broth (TSB; Merck, Darmstadt, Hesse, Germany), Listeria Enrichment Broth (LEB; Merck, Darmstadt, Hesse, Germany), and Buffered Peptone Water (BPW; Merck, Darmstadt, Hesse, Germany) for detection of E. coli O157:H7, Listeria spp., and Salmonella spp.,
respectively, in sterile stomacher bag and homogenized using a stomacher machine (BagMixer® 400P, Interscience, Saint-Nom-la-Bretèche, France) for 1 min. The pH of the enrichment broths was adjusted to pH 7.0 with 0.5 M NaOH solution before incubation. For the three-tube MPN analysis, 1 mL of the 10-, 100-, and 1,000-fold dilutions of the enriched bacteria culture were incubated in MPN tubes for 24 h at 37°C for detection and enumeration of E. coli O157:H7 and Salmonella spp., and 48 h at 30°C for detection and enumeration of Listeria species.

Genomic DNA Extraction and Multiplex-PCR
All the incubated MPN tubes were subjected to DNA extraction using the boiled-cell method as described in Table 1. Multiplex-PCR assays and gel electrophoresis for the detection of Shiga toxin-producing E. coli (STEC), E. coli O157:H7, Listeria spp., L. monocytogenes, Salmonella spp., S. Enteritidis, and S. Typhimurium were performed based on the methods as summarized in Table 2.

Statistical Analysis
 Colony counts were converted into $\log_{10}$ CFU/g. The data were subjected to a one-way analysis of variance (ANOVA) analysis using Minitab 16.0 software (Minitab Inc., State College, Pennsylvania, U.S.A.) to evaluate if there were differences between the organic and conventional vegetables at $P \leq 0.05$ level of significance.

RESULTS

Microbiological Quality of Conventional and Organic Vegetables

Table 3 shows the microbial counts of mesophilic aerobic bacteria, yeasts and molds, and total coliforms in 12 types of organic and conventional vegetables. Among the 12 types of vegetables analyzed, no trend was shown that either organic or conventional vegetable has a greater microbial count of mesophilic aerobic bacteria, yeasts and molds, and total coliforms. However, cabbage, carrot, and winged bean showed significant differences ($P < 0.05$) in mesophilic aerobic population between organic and conventional samples. For mesophilic aerobic bacteria, the results varied from 3 to $>7 \log_{10}$ CFU/g for organic vegetables and 3 to $>8 \log_{10}$ CFU/g for conventional vegetables. Most of the samples had a mesophilic aerobic bacteria count that ranged from 5 to $7 \log_{10}$ CFU/g.

For total coliforms counts, results varied from 1 to 7 $\log_{10}$ CFU/g for organic and conventional vegetables. In this study, no coliform bacterium was detected in calamondin samples and carrot was the only sample that showed significant differences ($P < 0.05$) in coliform populations between organic and conventional samples. Overall, greater microbial counts (mesophilic aerobic bacteria, yeasts and molds, and total coliforms) were detected in chili samples whereas lower microbial counts were found in cherry tomato and tomato samples.

The yeasts and molds counts in the vegetables were lower compared to mesophilic aerobic bacteria, ranged from 1 to $>6 \log_{10}$ CFU/g for organic vegetables and 0.5 to $>6 \log_{10}$ CFU/g for conventional vegetables. The yeasts and molds counts of most samples varied from 3 to $6 \log_{10}$ CFU/g. In this study, all conventionally and organically grown vegetable samples showed comparable yeast and mold counts except for conventional winged bean which showed a higher count compared to the organic counterpart.

Microbiological Safety of Conventional and Organic Vegetables

As shown in Tables 4–6, there was obviously not much difference in the prevalence of foodborne pathogens between conventional and organic vegetables. Therefore, statistical analysis for comparison of the positive-negative data for foodborne pathogens between conventional and organic vegetables was not conducted in this study. In this study, E. coli O157:H7 and S. Typhimurium were not detected in 152 vegetable samples (Tables 4, 5). Of the 152 samples analyzed, only the conventional lettuce (Table 4) and organic carrot (Table 5) were contaminated with STEC and S. Enteritidis, respectively. L. monocytogenes were more frequently detected in both organic and conventional vegetables as compared to E. coli O157:H7, S. Typhimurium, and S. Enteritidis (Tables 4–6). The prevalence of L. monocytogenes in conventional and organic vegetables was 9.1% (seven positive samples out of 77 samples) and 2.7% (two positive samples out of 77 samples).

### TABLE 1 | Procedures for extraction of pathogens genomic DNA from conventional and organic vegetables.

| Target pathogens | Genomic DNA extraction procedures | References |
|------------------|-----------------------------------|------------|
| STEC O157:H7 and STEC non-O157 | Each MPN tube was centrifuged at 13,400 × g for 1 min. The supernatants were discarded and the pellets were resuspended with 200 μL of Tris-EDTA buffer. The mixture was then boiled for 10 min and cooled at −20°C immediately for another 10 min before it was centrifuged at 13,400 × g for 2 min. The supernatant was used as a template DNA in PCR assays. | Loo et al., 2013 |
| Listeria spp. and L. monocytogenes | Each MPN tube was centrifuged at 13,400 × g for 1 min. The supernatants were discarded and the pellets were resuspended in 200 μL of Tris-EDTA buffer. The mixture was then boiled for 10 min and cooled at −20°C immediately for another 10 min before it was subjected to centrifugation at 13,400 × g for 3 min. The supernatant was used as a template DNA in PCR assays. | Kuan et al., 2013 |
| Salmonella spp., S. Enteritidis, and S. Typhimurium | Each MPN tube was centrifuged at 15,000 × g for 3 min. The supernatants were discarded and the pellet was resuspended in 200 μL Tris-EDTA buffer and boiled for 10 min. The mixture was then cooled at −20°C immediately for another 10 min, followed by centrifugation at 15,000 × g for 3 min. The supernatant was used as a template DNA in PCR assays. | Pui et al., 2011 |
TABLE 2 | Target genes, primer sequences, PCR preparations, thermocycling conditions, and gel electrophoresis conditions used in this study.

| Target genes (stx1 and stx2) were targeted for the detection of STEC | Primer sequences | PCR preparations* | Thermocycling conditions | Gel electrophoresis conditions | Amplicon size | Reference(s) |
|---|---|---|---|---|---|---|
| Shiga-toxin genes (stx1 and stx2) | stx1-F: 5′-ATA AAT CGC CAT TOG TTG ACT AC-3′ | 5 µL × PCR buffer, 2 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1 U Taq DNA Polymerase, 0.5 µM for each primer, 10.3 µL of sterile distilled water and 2 µL of extracted DNA. | Initialisation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s, and followed by final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.5% (w/v) agarose gel at 100 V for 28 min. | 180 bp (stx1) and 255 bp (stx2) | Çadir et al., 2010; Loo et al., 2013 |
| fliCh7 and rfbO157 genes were targeted for the detection of O antigen and flagellar antigen in E. coli O157:H7 | rfbO157-F: 5′-CGG ACG TCG TGA TAG TAC TCC TG-3′ | 5 µL × PCR buffer, 2 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1 U Taq DNA Polymerase, 0.5 µM for each primer, 10.3 µL of sterile distilled water and 2 µL of extracted DNA. | Initialisation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s, and elongation at 72°C for 1 min and followed by a final elongation at 72°C for 10 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.5% (w/v) agarose gel at 100 V for 28 min. | 259 bp (rfbO157) and 625 bp (fliCh7) | Hashemi et al., 2010; Loo et al., 2013 |
| 16S rRNA gene was targeted for the detection of Listeria spp. | U1: 5′-CTC CAT AAA GGT GAC CCT-3′ | 5 µL × PCR buffer, 1.5 µL 25 mM MgCl2, 0.2 µL 10 mM deoxynucleoside triphosphate (dNTP) mix, 1.5 U Taq DNA Polymerase, 0.5 µM for each primer LM1 and LM2, 1.0 µM of each primer U1 and LI1, 14 µL of sterile distilled water and 2 µL of extracted DNA. | Initialisation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 1 min, and elongation at 72°C for 2 min, and followed by a final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.0% (w/v) agarose gel at 100 V for 28 min. | 938 bp | Border et al., 1990; Kuan et al., 2013 |
| hlyA gene was targeted for the detection of L. monocytogenes | LM1: 5′-CCT AAG AGC CGA ATG GAA-3′ | 5 µL × PCR buffer, 2.5 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1.5 U Taq DNA Polymerase, 0.5 µM primer mix, 0.2 µM for ST11 and ST15, 1.2 µM for ST11, Typ04, ENT, and ENTR, 14.2 µL sterile distilled water, and 2 µL of extracted DNA. | Initialisation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, elongation at 72°C for 1 min, and followed by a final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.0% (w/v) agarose gel at 100 V for 40 min. | 429 bp | Soumet et al., 1999; Alvarez et al., 2004; Pui et al., 2011; Thung et al., 2016 |
| Random fragment was targeted for the detection of Salmonella spp. | ST11: 5′-GCC AAC CAT TGC TAA ATT GGC GCA-3′ | 5 µL × PCR buffer, 2.5 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1.5 U Taq DNA Polymerase, 0.5 µL primer mix, 0.2 µM for ST11 and ST15, 1.2 µM for ST11, Typ04, ENT, and ENTR, 14.2 µL sterile distilled water, and 2 µL of extracted DNA. | Initialisation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, elongation at 72°C for 1 min, and followed by a final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.5% (w/v) agarose gel at 100 V for 28 min. | 429 bp | Soumet et al., 1999; Alvarez et al., 2004; Pui et al., 2011; Thung et al., 2016 |
| SdrI gene was targeted for the detection of S. Enteritidis | ENTF: 5′-TGT GTT TTA TTA GAT GAA AGA-3′ | 5 µL × PCR buffer, 2.5 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1.5 U Taq DNA Polymerase, 0.5 µL primer mix, 0.2 µM for ST11 and ST15, 1.2 µM for ST11, Typ04, ENT, and ENTR, 14.2 µL sterile distilled water, and 2 µL of extracted DNA. | Initialisation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, elongation at 72°C for 1 min, and followed by a final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.5% (w/v) agarose gel at 100 V for 40 min. | 304 bp | Soumet et al., 1999; Alvarez et al., 2004; Pui et al., 2011; Thung et al., 2016 |
| fliC gene was targeted for the detection of S. Typhimurium | FliI 5′-CGG TGT TGC CCA GGT TOG TAA T-3′ | 5 µL × PCR buffer, 2.5 µL 25 mM MgCl2, 0.5 µL 10 mM dNTP mix, 1.5 U Taq DNA Polymerase, 0.5 µL primer mix, 0.2 µM for ST11 and ST15, 1.2 µM for ST11, Typ04, ENT, and ENTR, 14.2 µL sterile distilled water, and 2 µL of extracted DNA. | Initialisation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, elongation at 72°C for 1 min, and followed by a final elongation at 72°C for 7 min. | An aliquot (5 µL) of the amplified PCR product was loaded and electrophoresed on 1.5% (w/v) agarose gel at 100 V for 28 min. | 620 bp | Soumet et al., 1999; Alvarez et al., 2004; Pui et al., 2011; Thung et al., 2016 |

*Amplification of DNA was performed in 25 µL reaction mixtures.
TABLE 3 | Microbial counts (log_{10} CFU/g) of mesophilic aerobic bacteria, yeasts and molds, and total coliforms in different organic and conventional vegetables purchased at retail markets in Malaysia.

| Samples         | Mesophilic aerobic bacteria | Total coliforms | Yeasts and molds |
|-----------------|-----------------------------|-----------------|-----------------|
|                 | Conventional | Organic | Conventional | Organic | Conventional | Organic |
| Cabbage         | 6.97 ± 0.51  | 5.82 ± 0.71* | 4.83 ± 2.89  | 2.28 ± 2.57  | 6.36 ± 1.07  | 5.40 ± 0.74  |
| Calamondin      | 5.10 ± 0.61  | 4.56 ± 1.62  | NDa            | NDa            | 4.86 ± 0.79  | 4.84 ± 1.43  |
| Carrot          | 5.57 ± 0.35  | 6.29 ± 0.41* | 4.22 ± 0.89  | 5.62 ± 0.45*  | 5.59 ± 0.70  | 6.03 ± 0.51  |
| Cherry tomato   | 3.90 ± 2.17  | 3.17 ± 2.46  | 2.09 ± 2.73  | 1.89 ± 2.10  | 1.51 ± 1.89  | 3.15 ± 2.50  |
| Chili           | 8.06 ± 0.38  | 7.91 ± 0.55  | 7.04 ± 0.85  | 6.94 ± 0.61  | 5.95 ± 0.55  | 6.48 ± 1.18  |
| Cucumber        | 5.55 ± 0.60  | 6.24 ± 0.53  | 2.48 ± 2.36  | 2.67 ± 3.07  | 3.35 ± 1.58  | 4.58 ± 0.91  |
| Eggplant        | 6.89 ± 0.74  | 6.23 ± 0.49  | 5.18 ± 2.33  | 4.13 ± 2.09  | 5.53 ± 0.81  | 4.78 ± 0.76  |
| Winged bean     | 7.41 ± 0.35  | 6.91 ± 0.17* | 4.73 ± 2.22  | 3.49 ± 1.75  | 6.05 ± 0.30  | 5.61 ± 0.08* |
| Lettuce         | 6.70 ± 0.58  | 7.14 ± 0.60  | 4.61 ± 0.88  | 4.06 ± 2.14  | 5.77 ± 0.66  | 6.30 ± 0.70  |
| Sweet potato    | 7.09 ± 0.28  | 6.50 ± 1.42  | 5.70 ± 0.69  | 4.05 ± 2.34  | 5.48 ± 0.35  | 4.84 ± 0.92  |
| Tomato          | 4.90 ± 1.50  | 3.83 ± 3.02  | 1.00 ± 1.71  | 1.70 ± 2.77  | 0.48 ± 1.26  | 1.37 ± 2.14  |
| White radish    | 7.76 ± 0.77  | 7.13 ± 0.39  | 6.11 ± 1.01  | 5.01 ± 1.35  | 6.90 ± 0.76  | 6.28 ± 0.60  |

Mean comparisons between conventional and organic within mesophilic aerobic bacteria, total coliforms and yeasts and molds by one-way ANOVA at P ≤ 0.05. The results were expressed as mean ± SD (log_{10} CFU/g) of three measurements.

*aSignificantly different at P ≤ 0.05.

TABLE 4 | Prevalence of STEC O157:H7 and STEC non-O157 in conventional and organic vegetables purchased at retail markets in Malaysia using the MPN-PCR method.

| Types of samples | STEC O157:H7 | STEC non-O157 |
|------------------|--------------|---------------|
|                  | Conventional | Organic | Total | Conventional | Organic | Total |
|                  | (n_p/n_t) %  | (n_p/n_t) %  | (n_p/n_t) % | (n_p/n_t) %  | (n_p/n_t) %  | (n_p/n_t) %  |
| Cabbage          | (0/6) 0      | (0/6) 0      | (0/11) 0    | (0/6) 0      | (0/6) 0      | (0/11) 0      |
| Calamondin       | (0/6) 0      | (0/7) 0      | (0/13) 0    | (0/6) 0      | (0/7) 0      | (0/13) 0      |
| Carrot           | (0/6) 0      | (0/6) 0      | (0/13) 0    | (0/6) 0      | (0/6) 0      | (0/13) 0      |
| Cherry tomato    | (0/7) 0      | (0/6) 0      | (0/13) 0    | (0/7) 0      | (0/6) 0      | (0/13) 0      |
| Chili            | (0/7) 0      | (0/6) 0      | (0/13) 0    | (0/7) 0      | (0/6) 0      | (0/13) 0      |
| Cucumber         | (0/7) 0      | (0/6) 0      | (0/13) 0    | (0/7) 0      | (0/6) 0      | (0/13) 0      |
| Eggplant         | (0/7) 0      | (0/6) 0      | (0/13) 0    | (0/7) 0      | (0/6) 0      | (0/13) 0      |
| Winged bean      | (0/7) 0      | (0/6) 0      | (0/13) 0    | (0/7) 0      | (0/6) 0      | (0/13) 0      |
| Lettuce          | (0/6) 0      | (0/6) 0      | (0/13) 0    | (1/6) 16.7   | (0/7) 0      | (1/13) 7.7    |
| Sweet potato     | (0/6) 0      | (0/6) 0      | (0/12) 0    | (0/6) 0      | (0/6) 0      | (0/12) 0      |
| Tomato           | (0/6) 0      | (0/6) 0      | (0/13) 0    | (0/6) 0      | (0/6) 0      | (0/13) 0      |
| White radish     | (0/6) 0      | (0/6) 0      | (0/12) 0    | (0/6) 0      | (0/6) 0      | (0/12) 0      |
| Total            | (0/77) 0     | (0/75) 0     | (1/152) 1.3 | (3/75) 4.0   | (1/152) 0.7  | 0.7           |

n_p, Total number of positive samples confirmed by MPN-PCR; n_t, Total number of samples; %, Percentage of positive sample.

out of 75 samples), respectively. The microbial load of L. monocytogenes in vegetable samples ranged between < 3 and 6.0 MPN/g (Table 7). Overall, the microbial load for most positive samples was ranged from < 3 to 3.0 MPN/g.

DISCUSSION

Since vegetables are grown in soil and exposed to different kind of environmental conditions and hazards, these conditions would be reflected in the mesophilic aerobic count. Therefore, the mesophilic aerobic count can be used as an indicator to access the microbiological quality of foods (Pianetti et al., 2008). Brackett and Splittstoesser (1992) found that mesophilic aerobic counts in vegetables can be as high as 7 log_{10} CFU/g. A previous study suggested that fruits and vegetables grown without or under low level of pesticides can be contaminated with larger microbial population since some pesticides have been found to inhibit the growth of some microorganisms (Guan et al., 2001). Oliveira et al. (2010) also reported that
TABLE 5 | Prevalence of Salmonella spp., S. Enteritidis, and S. Typhimurium in conventional and organic vegetables purchased at retail markets in Malaysia using the MPN-PCR method.

| Types of samples | Salmonella spp. | | | S. Enteritidis | | | S. Typhimurium | | |
| | Conventional | Organic | Total | Conventional | Organic | Total | Conventional | Organic | Total |
| | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % |
| Cabbage | (0/5) | 0 | (0/6) | 0 | (0/11) | 0 | (0/6) | 0 | (0/11) | 0 |
| Calamondin | (0/6) | 0 | (1/7) | 14.3 | (1/13) | 7.7 | (0/6) | 0 | (1/7) | 14.3 | (1/13) | 7.7 |
| Carrot | (0/6) | 0 | (1/7) | 14.3 | (1/13) | 7.7 | (0/6) | 0 | (1/7) | 14.3 | (1/13) | 7.7 |
| Cherry tomato | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/6) | 0 | (0/13) | 0 |
| Chili | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/6) | 0 | (0/13) | 0 |
| Cucumber | (0/7) | 0 | (1/6) | 16.7 | (1/13) | 7.7 | (0/6) | 0 | (0/6) | 0 | (0/13) | 0 |
| Eggplant | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/6) | 0 | (0/13) | 0 |
| Winged bean | (0/7) | 0 | (1/6) | 16.7 | (1/13) | 7.7 | (0/6) | 0 | (0/6) | 0 | (0/13) | 0 |
| Lettuce | (0/6) | 0 | (0/7) | 0 | (0/13) | 0 | (0/6) | 0 | (0/7) | 0 | (0/13) | 0 |
| Sweet potato | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 |
| Tomato | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/6) | 0 | (0/6) | 0 | (0/13) | 0 |
| White radish | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 |
| Total | (0/77) | 0 | (3/75) | 4.0 | (3/152) | 2.0 | (0/77) | 0 | (1/75) | 1.3 | (1/152) | 0.7 |

n_p, Total number of positive samples confirmed by MPN-PCR; n_t, Total number of samples; %, Percentage of positive sample.

TABLE 6 | Prevalence of Listeria spp. and L. monocytogenes in conventional and organic vegetables purchased at retail markets in Malaysia using the MPN-PCR method.

| Types of samples | Listeria spp. | | | L. monocytogenes | | | | |
| | Conventional | Organic | Total | Conventional | Organic | Total | Conventional | Organic | Total |
| | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % | (n_p/n_t) | % |
| Cabbage | (1/5) | 20.0 | (1/6) | 16.7 | (2/11) | 18.2 | (1/5) | 20.0 | (1/6) | 16.7 | (2/11) | 18.2 |
| Calamondin | (1/6) | 16.7 | (0/7) | 0 | (1/13) | 7.7 | (1/6) | 16.7 | (0/7) | 0 | (1/13) | 7.7 |
| Carrot | (1/6) | 16.7 | (0/7) | 0 | (1/13) | 7.7 | (1/6) | 16.7 | (0/7) | 0 | (1/13) | 7.7 |
| Cherry tomato | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 |
| Chili | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 |
| Cucumber | (1/7) | 14.3 | (0/6) | 0 | (1/13) | 7.7 | (1/7) | 14.3 | (0/6) | 0 | (1/13) | 7.7 |
| Eggplant | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 |
| Winged bean | (1/7) | 14.3 | (0/6) | 0 | (1/13) | 7.7 | (1/7) | 14.3 | (0/6) | 0 | (1/13) | 7.7 |
| Lettuce | (1/6) | 16.7 | (2/7) | 28.6 | (3/13) | 23.1 | (1/6) | 16.7 | (2/7) | 28.6 | (3/13) | 23.1 |
| Sweet potato | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 | (0/6) | 0 | (0/6) | 0 | (0/12) | 0 |
| Tomato | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 | (0/7) | 0 | (0/6) | 0 | (0/13) | 0 |
| White radish | (0/6) | 0 | (2/6) | 33.3 | (2/12) | 16.7 | (0/6) | 0 | (2/6) | 33.3 | (2/12) | 16.7 |
| Total | (9/77) | 11.7 | (5/75) | 6.7 | (14/152) | 9.2 | (7/77) | 9.1 | (2/75) | 2.7 | (9/152) | 5.9 |

n_p, Total number of positive samples confirmed by MPN-PCR; n_t, Total number of samples; %, Percentage of positive sample.

Organic lettuce contained larger mesophilic aerobic population than conventional lettuce. Surprisingly, nine out of 12 types of organically and conventionally grown vegetables in this study showed comparable and no significant difference (P > 0.05) in mesophilic aerobic population.

Our findings were in tandem with the data obtained from previous studies (Oliveira et al., 2010; Maffei et al., 2013) in which yeasts and molds counts were lower than mesophilic aerobic bacteria counts. Yeasts and molds, depending on genus and species, are the main culprit in most fresh produce spoilage and can be pathogenic. These microbial groups can invade fresh produce in the field prior to harvest and during storage. The presence of yeasts and molds not only link to food spoilage problems in vegetable, they can also pose health risks due to mycotoxins production (Tournas, 2005; Tournas and Katsoudas, 2005). Diseases caused by exposure to mycotoxins include allergic reactions, immunosuppressive diseases, and possibly cancers (Kovács, 2004; Buyukunal et al., 2015).
Coliform bacteria are commonly used as an indicator of sanitary quality of foods or to check potential contamination of pathogenic microorganisms (Kornacki and Johnson, 2001). In this study, the presence of coliform bacteria in vegetable samples suggested the deterioration of the quality of vegetable due to fecal contamination. It may be caused by different contamination sources, such as the use of polluted irrigation water during pre-harvest, transportation, improper storage conditions, or poor handling practices along the entire food chain (National Advisory Committee on Microbiological Criteria for Foods, 1999). Despite most of the coliform bacteria do not cause disease, uncommon strain, such as E. coli O157:H7 is pathogenic to human which contributed toward many foodborne disease outbreaks (Delaquissi et al., 2007; Cieslik and Bartoszcze, 2011; Chang et al., 2013). Interestingly, no coliform bacterium was found in both organic and conventional calamondin samples. This might due to the bioactive compounds on the peel of calamondin that protect it from microbial deterioration (Jeong et al., 2004; Rubiatiul et al., 2015). Also, the acidic internal environment of calamondin does not favor the growth of coliform bacteria. Although most of the pathogens are distributed on the surface of fresh produce, contamination might occur through the internalization of opportunistic pathogens or spoilage bacteria into fresh produce. Montville and Matthews (2008) and Ryser et al. (2009) pointed out that microorganisms can gain access to the internal tissues of fresh produce via stomata, lenticels, trichomes, lesions caused by plant pathogens, and stem scars. Internalization of pathogens, such as Salmonella spp. and E. coli O157:H7 in fresh produce have also been widely reported (Bordini et al., 2007; Deering et al., 2012; Ge et al., 2012; Najwa et al., 2015; Nicholson et al., 2015).

In this study, emphasis was given to the detection of S. Typhimurium and S. Enteritidis among 2,463 serovars of Salmonella species. This was mainly due to S. Typhimurium and S. Enteritidis have been reported to be the most prevalent serovars and common causes of human of salmonellosis (Herikstad et al., 2002; Bangtrakulnonth et al., 2004; Rabch et al., 2013; Najwa et al., 2015). Hence, it is our interest to investigate the occurrence of these two serovars in Malaysia. Also, based on the previous prevalence studies and epidemiological data, S. Typhimurium and S. Enteritidis are the common foodborne pathogens detected in Malaysia (Modarressi and Thong, 2010; Nillian et al., 2011; Pui et al., 2011; Adzitey et al., 2012; Najwa et al., 2015; Thung et al., 2016). It is worth noting that E. coli O157:H7 and S. Typhimurium were not detected in any samples. According to the study by Ryu et al. (2014), neither E. coli O157:H7 nor Salmonella spp. were detected in conventional and fresh organic produce. In another study conducted by Mukherjee et al. (2004), organic and conventional fresh produce in Minnesota, United States were found to be negative for Salmonella but positive for E. coli O157:H7. Although E. coli O157:H7 was not detected in all the samples, one of the conventional lettuce samples was contaminated with STEC. STEC are well-known as important pathogenic bacteria causing many foodborne illness outbreaks that are linked to consumption of raw vegetables (Loo et al., 2013). STEC can produce Shiga toxin which causes severe bloody diarrhea and results in life-threatening complications, such as

| Types of vegetables | STEC non-O157:H7 | L. monocytogenes | S. Enteritidis |
|---------------------|------------------|-----------------|----------------|
|                     | Prevalence (%)   | Microbial load (MPN/g) | Prevalence (%)   | Microbial load (MPN/g) | Prevalence (%)   | Microbial load (MPN/g) |
| Cabbage             | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Carrot              | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Cherry tomato      | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Chilli              | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Cucumber            | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Eggplant            | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| Lettuce             | 16.7             | <3.0–3.0        | 16.7           | <3.0–3.0        | 16.7           | <3.0–3.0        |
| Tomato              | 0                | <3.0            | 0              | <3.0            | 0              | <3.0            |
| White radish        | 0                | <3.0–1100       | 0              | <3.0            | 0              | <3.0            |
| Total               | 1.3              | <3.0–3.0        | 2.7            | <3.0–6.0        | 1.3            | <3.0–3.0        |
haemolytic-uremic syndrome (HUS) (Mead and Griffin, 1998; Sarimehmetoglu et al., 2009).

The overall prevalence of foodborne pathogens in fresh produce (including conventional and fresh organic produce) were 0.7, 9.2, 5.9, 2.0, and 0.7% for STEC non-O157, Listeria spp., L. monocytogenes, Salmonella spp., and S. Enteritidis, respectively, which were comparatively lower as compare to previous local studies (Arumugaswamy et al., 1994; Jeyaletchumi et al., 2010; Chang et al., 2013; Loo et al., 2013; Najwa et al., 2015). These findings are also contrary to the findings by Oliveira et al. (2010) that no pathogen was found in 72 organically and conventionally grown lettuces. In this study, contaminations by Listeria spp. and L. monocytogenes in both conventional and organic vegetables were obviously observed, and being slightly higher in the conventional vegetables than in the organic vegetables. Although there was a low microbial load of L. monocytogenes in the fresh produce (ranging between < 3 and 6.0 MPN/g) and listeriosis cases are also rarely reported in Malaysia, it may pose a safety risk to consumers as a warm and humid environment may encourage proliferation of L. monocytogenes to a dangerous level in vegetables (Steinbruegge et al., 1988).

In this study, the comparison of microbiological quality and safety of organic and conventional vegetables showed no trend whether conventional fresh produce is more or less safe than organic ones. Regardless of the cultivation methods, fresh produce can be contaminated starting from the pre-harvest stage, for example, through the use of fresh or non-composted animal manure, irrigation water, wild animals, pests, and insects (Beuchat and Ryu, 1997; Mandrell, 2009; Talley et al., 2009; Mishra et al., 2017). Post-harvest handling activities, such as selection, trimming, precooling, washing, grading, packaging, storage, and transportation can exacerbate the situation (Mandrell, 2009; Buchholz et al., 2012; Maffei et al., 2013).

Additionally, the differences in the contamination levels of vegetables can be affected by farm location, weather or climatic conditions, and types of vegetable crops (e.g., leafy and salad, bulb and stem, root and tuber, flower and flower buds, seed and fruit) (Ryu et al., 2014). In Malaysia, the great difference in price between organic and conventional fresh produce by as much as 100–300%, indirectly may affect the microbiological quality of vegetables. Since fresh organic produce is sold at higher prices compared to those of conventional produce, farmers or retailers tend to use better post-harvest handling practices and higher quality packaging materials for organic produce. As a result, quality and safety of organic vegetables can be preserved.

CONCLUSIONS

Findings in this study indicated that regardless of farming methods, either organic or conventional, raw vegetables can act as a potential vehicle for transmission of Salmonella, L. monocytogenes, and E. coli O157:H7 and thus, pose a health risk to consumers. Although the use of composted manure as a nutrient source for fresh produce in organic farming is believed to pose a greater risk of microbial contamination, this research found that one particular type of cultivation practices would not affect the microbiological status of fresh produce. However, more works are required to verify the observations in this study. Environmental factors, such as weather conditions and the post-harvest handling practices along the entire food chain should also be taken into account in future studies, since they may also affect the microbial level of organic and conventional fresh produce. The present study provides baseline information on the microbial profiles of organically and conventionally grown vegetables in Malaysia. Meanwhile, the data obtained in this study also serves as useful information in future risk assessment.

AUTHOR CONTRIBUTIONS

CHK, YR, SA, CW, and SR developed the study design. CHK, CSK, and SY co-ordinated the collection of samples in retail markets required for this study. CHK, TT, JP, WC, YL, CT, OR, and SM conducted the microbiological analysis of food samples and carried out the PCR confirmation of specific foodborne pathogens, for example, Listeria spp. and L. monocytogenes, STEC, E. coli O157:H7, Salmonella spp., S. Enteritidis, and S. Typhimurium. MN provided culture media, PCR reagents, and technical advice on the study. CHK interpreted the data, drafted the manuscript, and revised the manuscript. YR, SA, CW, CSK, SY, and SR vetted the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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