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The Heavy Metals and Microbial Profile of Organic Vegetables in Malaysian Retail

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ABSTRACT

Organic foods including organic vegetables become more popular among the consumer because of the perception that the production of organic food is safer, healthier and environmental friendly. There is limited scientific study to understand the food safety aspect of organic vegetables in Malaysia. Therefore, this paper aimed to provide insights on organic food safety by focusing on three comparative scientific studies conducted on vegetables in Malaysian retail. All the vegetable samples analyzed did not detect lead concentration. There was a significant difference between the mean concentrations of zinc in organic and conventional vegetables (p=0.01) while there was no significant difference between mean concentrations of cadmium in organic and conventional vegetables (p=0.11). Zinc concentration in leafy and fruit-like vegetables was significant (p=0.00). As for local and imported organic vegetables, only copper was detected and only tomato shows significant difference between the origins of the vegetables (p=0.02). This study also revealed 13.9% (*E. coli*) and 90.3% (*Staphylococcus aureus*) did not meet the Public Health Laboratory Service (PHLS) guidelines for microbiological quality. In overall, no substantial trend was shown in the difference in crop farming system (organic or conventional) or type of origin in organic vegetables (local or imported) to pose greater heavy metal risks however difference in organic certification influenced microbiological quality status. This study has profiled a reference point for organic vegetables from Malaysian retail to further understand the farming and its produce.

1. INTRODUCTION

Production of organic food has been increasing significantly worldwide. The International Federation of Organic Agriculture Movement (IFOAM) indicated that the organic agriculture has been widely practiced in 186 countries all over the world with the land devoted to organic agriculture of 71.5 million hectares in 2018 [1]. In Malaysia, organic production remains a niche market but is slowly growing. Since 2001, the

Malaysian government has supported organic farming by the establishment of national regulations for the Malaysian Organic Certification Program known as "Sijil Organik Malaysia" (SOM). The certification was later rebranded to Malaysian Organic (myORGANIC) in 2003 to recognize farms that practice organic farming complying with the Malaysian Standard MS 1529:2015 [2]. By 2025, Malaysia targeted 200 million of organic food production to cope with the high demand locally as well as for export market [3]. Despite the dramatic increase in the sale of organic food, the available scientific evidence is unable to support claim that organic food is safer in terms of heavy metals and microbiological contamination. Consumers, however continue to perceive organic food as a safer and healthier option [4], [5]. Generally, organic produce costs more than conventional produce and also higher price often translated as the product having greater value. Organic food consumption may also trigger 'feel good' sensation that what they are eating is good food and will positively result in better health condition [6]. There were few inferences that claimed to be sourced from conventional farming involving certain types of cancer that have been identified with exposure to some pesticides [7]. Due to those concerns, food safety becomes the strongest motivation for consumers to choose organic food.

This study integrated three comparative scientific studies to profile the heavy metals and microbial status of organic vegetables from retailers in Malaysia. Firstly, heavy metals in leafy and fruit-like organic and conventional vegetables were assessed. Then, the organic vegetables from local and imported producers were examined for heavy metal contents. The sources of heavy metals in crops could be from the atmospheric deposition, irrigation water, municipal waste, industrial effluents and pesticide [8]. Finally, the microbial status for certified and non-certified organic producers were also examined. There is an extensive establishment of uncertified organic farms by the farmers who claim to practice organic farming method. By not being certified, there is no guarantee that farmers are using the method defined by the national organic standard hence may raise food safety and fraud issue on organic produce. The findings of this study should be able to provide an overview of the status of organic vegetables in Malaysian retail.

2. Materials and methods

2.1 Heavy metals in organic and conventional vegetables

Organic and conventional vegetable samples of four leafy vegetables that are mustard, water spinach, spinach and lettuce and four fruit-like vegetables that were tomato, cucumber, French bean, bitter gourd were purchased from a retail store in Puncak Alam, Selangor. [9] method was used to prepare the samples. The samples were washed with deionized water to get rid of air born pollutants. The vegetables were cut into small size before being dried in the oven at 70-80oC for 24 hours to remove all moisture. Dried samples were powdered using pestle and mortar. Wet digestion method was used, followed by atomic absorption spectrophotometric analysis as stipulated in the Perkin-Elmer manual for atomic absorption spectrophotometer. For each analysis, 0.5 g of dried samples was digested with 1 ml of hydrogen peroxide (H2O2) and 4 ml of nitric (HNO3). The samples were allowed to cool and the contents were filtered off by using Whatman 42 filter paper. The filtrate was made to 50 ml with distilled water and analysed for heavy metals (Cd, Zn and Pb) by atomic absorption spectrophotometric (AAS). The data obtained were statistically analysed using analysis of variance (ANOVA) using SPSS Static Version 23.0 (Statistical Package for the Social Sciences).

2.2 Microbiological quality in certified and non-certified organic vegetables

Lettuce, pakchoi, mustard, cucumber, white radish and winged bean were randomly collected from two organic retailers selling vegetables from certified and non-certified producers. Certified producers may include vegetables with any local or foreign organic certification. Non-certified producers may include small scale organic producers in which the land they cultivate on may or may not meet the requirement of the organic standard. Samples were collected in sterile plastic bags and labelled with a number and date of sample collection. Then, the samples were transported in an ice box at 4°C to the laboratory and further analysis were conducted within 24 hours upon collection. For analyses for coliforms, E. coli and Staphylococcus aureus, 25 g of each sample were placed in a sterile stomacher bag containing 225 ml of sterile 0.1% buffered peptone water (BPW) and homogenized using a stomacher for two minutes, and serially diluted with 9 ml of buffered peptone water according to [10] method. Then, 0.1 ml of diluted samples were inoculated on brilliance E. coli / coliform and mannitol salt agar to determine numbers of coliforms; E. coli and Staphylococcus aureus respectively. The plates were incubated at 37°C for 24 h before the colonies were counted.

Isolation of Salmonella used 25 g of each sample mixed with 225 ml of sterile 0.1% buffered peptone water (BPW) and pummeled in a stomacher for two minutes. The 1 ml sample was serially diluted with 9 ml of BPW and 0.1 ml of the diluted sample was placed in 10 ml Rappaport-Vassiliadis Soy Broth and 1 ml of sample dilution in 10 ml Tetrahionate broth and were incubated at 37°C for 24 hours and at 41.5°C for 24 hours. Then, the samples were inoculated into two selective media of xylose lysine deoxycholate and brilliant green agar plate at 37°C for 24 hours. A loop from each agar plate was streaked on the nutrient agar and biochemical tests (Indole test, Lysine decarboxylase, ONPG (B-galactosidase) test, triple-sugar iron test, urea broth test and Voges Proskauer test) were carried out to identify Salmonella's presence. Results expressed as CFU/g were converted to decimal logs. The statistical tests conducted were descriptive analysis, Mann Whitney test and chi square test using SPSS Statistic Version 23.0 (Statistical Package for the Social Sciences).

3. Results and Discussion

3.1 Heavy metals in organic and conventional vegetables

The mean concentration of cadmium and zinc in all leafy and fruit-like vegetable from organic and conventional origin is presented in Table 1. Lead concentration, however was not detected in all vegetable samples.

Table 1: Comparison of metals concentration in organic, conventional, leafy and fruit vegetables.

Heavy metals	Type of vegetable	Mean ± standard deviation (mg/kg)	F-stats (df)	p-value
Cd	Organic	1.12 ± 0.30	2.63 (1)	0.11
	Conventional	1.25 ± 0.35		
	Leafy	1.33 ± 0.32	17.55 (1)	0.00*
	Fruit-like	1.03 ± 0.26		
Zn	Organic	37.06 ± 13.68	6.60 (1)	0.01*
	Conventional	54.23 ± 35.25		
	Leafy	55.73 ± 35.18	9.48 (1)	0.00*
	Fruit-like	35.56 ± 11.60		

* significant at p-value < 0.05

Cadmium concentrations were higher in conventional vegetables compared to organic vegetables. Leafy vegetables recorded higher cadmium concentrations compared to fruit-like vegetables. Zinc concentrations were higher in conventional vegetables. Zinc concentrations in fruit-like vegetables were lower than leafy vegetables. Unlike lead and cadmium, element like zinc is essential to human life but with moderation. Only daily intake of 150-450 mg of zinc is considered excessive and have been related to reduction of copper utilization, alteration of iron function, reduction of immune function, as well as the reduction of high-density lipoprotein (HDL) level [11].

There was a significant difference between the mean concentrations of zinc in organic and conventional vegetables while there was no significant difference between mean concentrations of cadmium in organic and conventional vegetables. [12] reported lower range concentration of cadmium in vegetables. In this study, lead in the organic sample was not detected meanwhile, high concentrations of lead were recorded by [13]. The results obtained from this study are similar with the finding by [13], which stated that the heavy metals in conventional vegetables production had shown higher concentration of metals in leafy vegetables compared to fruit-like vegetables. This study found out that there was a significant difference between concentration of zinc and cadmium in leafy and fruit vegetables. Leafy vegetables may have higher heavy metal concentration as leaves are considered as entry points of heavy metals from air. Leafy vegetables have uneven surfaces to harbor more heavy metals compared to smoother surface of fruit-like vegetables [14].

3.2 Heavy metals in local and imported organic vegetables

All the analyzed samples for both local and imported organic vegetables did not detect concentration of lead and cadmium. Table 2 shows the only concentration detected which was Copper (Cu) for both local and imported vegetables.

Table 2: Copper concentration in local and imported organic vegetables.

Vegetable	Origin	Mean	p-value
Carrot	Local	0.21 ± 0.11	0.27
	Import	0.21 ± 0.09	
Tomato	Local	0.02 ± 0.01	*0.02
	Import	0.05 ± 0.08	
Cabbage	Local	0.11 ± 0.02	0.18
	Import	0.12 ± 0.04	

*Significant at p value < 0.05

The highest copper concentration was in carrot, followed by cabbage and the lowest copper concentration was in tomato. Only tomato shows the significant differences between the origins of the vegetables. The concentration of copper in both imported and local carrots were the same with 0.21 mg/kg. The concentration of copper in imported organic tomato was higher than the local organic tomato. The overall results obtained shows that the imported vegetables has slightly higher concentration of copper.

3.3 Microbiological quality in certified and non-certified organic vegetables

The prevalence of microbial organisms (E. coli and coliform) between certified and non-certified organic vegetables is presented in Table 3.

Table 3: Prevalence of Escherichia coli, coliform, salmonella and Staphylococcus aureus in different varieties of certified and non-certified vegetables

Types of samples	*Escherichia coli*			Coliforms			*Salmonella*			*Staphylococcus aureus*		
	Certified	Non-certified	Total	Certified	Non-certified	Total	Certified	Non-certified	Total	Certified	Non-certified	Total
	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %	(np/nt) %
Lettuce	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
Pakchoi	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
Mustard	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
Cucumber	(1/6) 16.7	(3/6) 50	(4/12) 33.3	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
White radish	(0/6) 0	(2/6) 30.3	(2/12) 16.7	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
Winged bean	(1/6) 16.7	(3/6) 50	(4/12) 33.3	(6/6) 100	(6/6) 100	(12/12) 100	(0/6) 0	(0/6) 0	(0/12) 0	(6/6) 100	(6/6) 100	(12/12) 100
Total	(2/36) 5.56	(8/36) 22.2	(10/72) 13.9	(36/36) 100	(36/36) 100	(72/72) 100	(0/36) 0	(0/36) 0	(0/72) 0	(36/36) 100	(36/36) 100	(72/72) 100

E. coli prevalence was detected in 22.2% and 5.56% of non-certified and certified organic vegetables. The findings were consistent with a study conducted in Minnesota by [15] that reported 12% and 59% of certified and non-certified organic farms respectively were contaminated with *E. coli*. Prevalence of coliform were 100% in all 72 samples analyzed. [16] obtained 84% and 70% of coliform prevalence. Incidence percentage of Staphylococcus aureus was 100% for both 36 certified and non-certified organic vegetables. The prevalence of Staphylococcus aureus prevalence and count in this study were greater than reported by [17] which isolated Staphylococcus aureus in 83.9% of fresh cut organic vegetables. [18] also reported lower prevalence of Staphylococcus aureus (8%) in organic vegetables grown in Korea. Salmonella contamination was not found in any samples of certified and non-certified organic vegetables. Greater incidence percentage and microbial load of coliform, E. coli and Staphylococcus aureus in non-certified organic vegetables suggest potential contamination due to several factors. Animal manure used in non-certified organic farming may not compose in the period of 90 – 120 days and with an internal temperature of 55º to 77ºC [15]. Besides, the source of irrigation water used in certified organic farming undergoes inspection at least once in 2 years and employees involved in handling and packaging process in certified organic production were subjected to hygiene practice [2].

Based on the Public Health Laboratory Service (PHLS) Microbiological Guidelines [19], 13.9% of the organic vegetables samples were in unsatisfactory condition for *E. coli* contamination. Presence of *E. coli* in ten samples indicate that faecal contamination has occurred during the overall agriculture practices [20]. The study found 90.3% of organic vegetables were in unacceptable for condition for *Staphylococcus aureus* count. S. aureus contamination mainly came from the skin and nose of those who handle the foodstuff [17]. Meanwhile, 100% of organic vegetables were in satisfactory condition for Salmonella.

4. Conclusion

Findings from the three comparative studies of organic produce focusing on different vegetables had profiled the risk of heavy metal and microbiological contamination. The findings shed some light that the type of farming is not a factor to assure no heavy metal concentration in vegetables. The results of the study of heavy metal in local and imported organic vegetables conclude that the origin of the vegetables cannot firmly determine the heavy metal concentration in organic vegetables. Findings in the microbiological quality study of certified and non-certified organic vegetables show the microbial count of coliform and S. aureus were found significantly higher in non-certified vegetables compared to certified organic vegetables which supports the idea that organic certification might further reduce the likelihood of fecal or pathogenic contamination. The findings also found that organic vegetables contain non-considerable levels of *E. coli* and majority of vegetables had an unacceptable level of *S. aureus*. These findings had pointed out that there is possibility the source of microbial contamination had occurred throughout organic agricultural system phase or unhygienic practice at the distribution level. Future studies of heavy metal and microbiological assessment involve organic produce could look into the farm practices to affirm these findings.

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