

ORIGINAL ARTICLE

The Heavy Metals and Microbial Profile of Organic Fruits Sold at Retail in Malaysia

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ABSTRACT

Introduction: Food safety of fruits has become public health concern worldwide as they can be contaminated with heavy metals and microbes. This study investigated the levels of heavy metals and microbiological quality of selected organic and conventional common fruits sold in retail around Shah Alam, Malaysia. **Methods:** Organic and conventional kiwi, apple and plum were analysed for heavy metals (cadmium, copper and zinc) and microbes (*Escherichia coli*, coliform, *Staphylococcus aureus* and *Salmonella*). **Results:** The concentration of heavy metals (mg/kg) in these fruits were within the following range: 1.317~4.423, 0.610~0.930 and 0.070~0.163 for Zn, Cu and Cd respectively. All three types of fruits show significant differences in zinc concentration in organic and conventional fruits. All the fruits were in the permissible limits of heavy metals. Total coliform was found in 88.9% of conventional fruits and 66.7% in organic fruits. Total coliform counts ranged from 0 to 7.08 log₁₀ cfu/g and from 0 to 7.09 log₁₀ cfu/g in organic and conventional fruits, respectively. Only kiwi has been found to have significant differences between organic and conventional in total coliforms. The prevalence of *Staphylococcus aureus* was 72.2% and 47.4% respectively in conventional and organic fruits. Both *Escherichia coli* and *Salmonella* which are the indicators of fecal contamination in foods had 0 log₁₀ cfu/g in all fruits. **Conclusion:** All fruits are safe for consumption and specific method of farming activity does not have an effect on the heavy metals and microbiological status of the fruits.

Keywords: Heavy metals, Microbes, Food Safety, Organic fruits

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INTRODUCTION

Fruits are always known as the healthy component of a well-balanced diet, as a source of high energy and prosperous in nutrients, minerals, vitamins and fibre (1). Fruits represent the essential food group that has been linked to the prevention of various chronic diseases and promoting general well-being (2, 3, 4). Despite its multiple health benefits, food safety due to contamination is always a concern. Fruit quality can be affected by the level of pesticide, heavy metal, microbes and fertilizer (5). Permissible limits for microbes by "PHLS Guidelines for the Microbiology Quality of ready-to-eat foods sampled at point of sales" and "Microbiological Guidelines for Food by Food and Environment Hygiene" can be used as reference. As for heavy metals, permissible or

tolerable daily intake recommended by the Institute of Medicine (US) Panel on Micronutrients can be used. In the recent years, reported cases of toxicity and food-borne outbreak has increased worldwide relating to the consumption of contaminated fresh produce (3). The surging trend of organic produce in the market is contributed by the positive perception of the consumers since it is believed to be "safer" and "healthier" than the conventional produce. Organic produce is marketed as to have more beneficial nutrients like antioxidants, free from chemicals, pesticides and preservatives and can reduce chronic diseases, allergic and cancer (6).

The heavy-metals-enriched ecosystem due to various industrial and agricultural activities, resulted in heavy metals toxicity in fresh produce (7). Previous studies found that multiple heavy metals were discovered in the fresh produce in significantly high level (8, 9). The entry of these heavy metals into the food chain as a result of heavy rains will cause metal uptake by the roots of crops, with the remainder potentially ending

up in open water bodies. The heavy metal dissipation increased susceptibility and exposure of toxicity to local population and causes serious health problems because of the excessive uptake of dietary heavy metals. Food contaminated with heavy metals can deplete some vital nutrients in the body and led to retardation of intrauterine growth, loss of function of immunological defences, weakened psychosocial behaviours, malnutrition-related disabilities and a higher prevalence of upper gastrointestinal cancer (7, 10).

Organically grown fresh produce had increased risk of microbiological contamination compared to the conventionally grown fresh produce (11). This is due to the usage of organic fertilizers in organic farming which may introduce various pathogenic microorganisms such as *Escherichia coli*, *Salmonella spp.* and total coliforms into the fresh produce, which can cause foodborne outbreaks (4, 11, 12, 13). Microbial contamination can occur at any point of the food chain from the farm to the fork continuum which may all have potential fatal outcomes. Therefore, the quality and safety of the fruits either conventional or organic have become a concern among consumers. Hence, in this study we investigated the levels of heavy metals and microbiological quality of selected organic and conventional fruits sold in Malaysia to understand the food safety aspect of the fruits.

METHODS AND MATERIALS

Heavy metals in organic and conventional fruits

Three organic and conventional fruit samples that were berry kind: *Actinidia deliciosa* (kiwi), the stone kind: *Prunus domestica* (plum) and the pome kind: *Malus pumila* (apple) were purchased from a highly frequented hypermarket with an organic line in Shah Alam, Malaysia. The three types of fruit were selected for their availability in both conventional and organic origin and all the three fruits represented different physical features in fruit retail. The samples were prepared according to Amin et al (14) method. A minimum of 100 g of each fruit sample or usually a consumer-size container of product unit were randomly packed in sterile plastic bags and kept in an insulated ice box with icepack to transport to laboratory. Within 24 hours upon collection, the samples were analysed. The surface of the packaging was sterilised with ethanol swabs before the samples were taken out to prevent cross-contamination. Then, all possible contact surfaces and tools, such as knife and cutting board, were sterilized by wiping with 70% ethanol. The samples were diced into small pieces by using sterilized knife on a sterilized chopping board and each sample was weighed into approximately 25 grams.

The samples were dried in the oven at 105°C for 24 hours before ground to powder and stored in a fresh and clean container for acid digestion following Amin et al (14) method suitable for perishable food like fruits

and vegetables. The powdered samples from each dried fruit sample (10 gram each) were accurately weighted and digested by using 10 ml of concentrated nitric acid (HNO₃). The samples were heated for 15 minutes before reaching boiling point. The samples were added with concentrated nitric acid after they had cooled. Next, the samples were evaporated until the volume reduced to about 1 mL. The samples were cooled for several minutes. After that, 2 mL of distilled water and 3 mL 30% Hydrogen Peroxide (H₂O₂) were added into the samples. An additional Hydrogen Peroxide (H₂O₂) was added until the effervescence ceased. The process was continued until the volume of the sample reduced to less than 5 mL. The samples were cooled for several minutes. Distilled water (10 ml) was added into the samples and filtered by using filter paper (Whatman 41). The filtrates of 30 samples of each conventional and organic fruit were stored in the vials and analysed by using PinAAcle 900T Atomic Absorption Spectrometer (AAS). Quality control check standards were prepared at the calibration curve midpoint concentration for each heavy metal with detection limit of between 1–100 µg L⁻¹. The data recorded was analysed by using SPSS (Statistical Package for the Social Sciences) to evaluate the concentration of heavy metals in the fruit samples. Independent t-test was used to determine the significant difference (p < 0.05) between the means of the organic and conventional fruits.

Microbiological quality of organic and conventional fruits

The samples for microbiological test were prepared according to Seow et al (4) method. Stomacher bag was used to place 25 grams of each sample. Later, each of sterile stomacher bag was filled with sterile 225 ml of 0.1% buffered peptone water (BPW) and then homogenized for two minutes using a stomacher machine. Then, the sample in the stomacher bag was then agitated for another two minutes to suspend the surface microbes. The sample mixture from the enrichment of the sample was diluted in 10 serial dilutions where 1 ml of the original bacterial culture mixture was diluted with 9 ml of buffered peptone water (BPW). Enumeration of the microbes was performed by placing 0.1 ml of the rinsed fluid on the agar plate using the spread plate technique. The enumeration of number of bacteria (CFU) per milliliter or gram of sample was by dividing the number of colonies by the dilution factor. For coliform and *E. coli* test, 0.1 ml of the sample was spread onto brilliance *E. coli*/coliform agar (positive control of *Escherichia coli* ATCC 25922- good growth; purple colonies and negative control of *Staphylococcus aureus* ATCC 25923 - inhibited). As for *Staphylococcus aureus*, Mannitol Salt Agar was used to spread the plate (positive control: *Staphylococcus aureus* ATCC 6538, medium-sized yellow colonies and negative control: *Escherichia coli* ATCC 25922; Partial to Complete Inhibition). The plates were incubated at 37°C for 24 hours. For enumeration of for *Salmonella*, the serial dilutions were spread-plate

Table I: The heavy metals concentration in organic and conventional fruits

Heavy metal	Permissible limit	Fruits sample	Variables	Mean	SD	F	p-value
Zinc (Zn)	0.6 mg/kg	Apple	Organic	1.480	0.040	0.361	0.012*
			Conventional	1.317	0.051		
		Kiwi	Organic	4.423	0.029	1.841	0.000*
			Conventional	3.507	0.057		
		Plum	Organic	2.620	0.035	4.780	0.000*
			Conventional	2.010	0.096		
Copper (Cu)	10 mg/kg	Apple	Organic	0.930	0.010	6.760	0.195
			Conventional	0.887	0.047		
		Kiwi	Organic	0.913	0.016	7.224	0.000*
			Conventional	0.610	0.043		
		Plum	Organic	0.830	0.036	0.026	0.000*
			Conventional	0.463	0.035		
Cadmium (Cd)	0.2 mg/kg	Apple	Organic	0.087	0.012	3.226	0.374
			Conventional	0.070	0.026		
		Kiwi	Organic	0.143	0.047	4.654	0.524
			Conventional	0.163	0.015		
		Plum	Organic	0.153	0.035	2.063	0.402
			Conventional	0.133	0.012		

*Significant at p value <0.05
Permissible limit by WHO/FAO and Institute of Medicine (US) Panel on Micronutrients

onto Xylose lysine deoxycholate (XLD) agar. Selective enrichment was then done by transferring 1 ml of pre-enrichment to 9 ml of tetrathionate broth and the plates were incubated at 37°C for 24-48 hours. All colonies on the plate were counted and identified. Colony counts per 1 g of samples were converted to base 10 logarithms and recorded as log 10 cfu/g. Parametric test of t-test and one-way analysis of variance (ANOVA) subject to Tukey test were used to determine any statistically significant difference (p < 0.05) in the mean of the microbial count for organic and conventional fruits.

RESULTS

Heavy metals in organic and conventional fruits

Table I shows the heavy metals concentration in three different types of fruits that were kiwi, plum and apple for both conventional and organic origin. The mean of the heavy metals' concentration (cadmium, copper and zinc) in the organic fruits is slightly higher than the conventional fruits except for kiwi. Zinc concentration in all three types of fruits between organic and conventional fruits were statistically significant. Meanwhile, all three types of fruits show no significant difference between organic and conventional fruits in terms of cadmium concentration. Copper concentration in organic and conventional kiwi and plum show significant differences. However, there is no significant difference between organic and conventional apple for cadmium.

Microbiological quality of organic and conventional fruits

Table II and Table III tabulated the prevalence and mean counts of each microorganisms with the different

Table II: Prevalence of total coliform, *S. aureus*, *E. coli* and *Salmonella* in organic and conventional fruits.

Types of samples	Total Coliform			<i>S. aureus</i>			<i>E. coli</i>			<i>Salmonella</i>		
	Conv.	Org.	Total									
	(n _p /n) %											
Kiwi	(6/6)	(2/6)	(8/12)	(3/6)	(3/6)	(6/12)	(0/6)	(0/6)	(0/12)	(0/6)	(0/6)	(0/12)
	100	33.3	66.7	50.0	50.0	50.0	0	0	0	0	0	0
Apple	(6/6)	(6/6)	(12/12)	(4/6)	(3/6)	(7/12)	(0/6)	(0/6)	(0/12)	(0/6)	(0/6)	(0/12)
	100	100	100	66.7	50.0	58.3	0	0	0	0	0	0
Plum	(4/6)	(4/6)	(8/12)	(4/6)	(3/6)	(7/12)	(0/6)	(0/6)	(0/12)	(0/6)	(0/6)	(0/12)
	66.7	66.7	66.7	66.7	50.0	58.3	0	0	0	0	0	0
Total	(16/18)	(12/18)	(28/36)	(13/18)	(9/18)	(20/36)	(0/18)	(0/18)	(0/36)	(0/18)	(0/18)	(0/36)
	88.9	66.7	77.8	72.2	47.4	55.6	0	0	0	0	0	0

Note: Conv., Conventional; Org., Organic; n_p, Total number of positive samples; n_t, Total number of samples; %, Percentage of positive samples.

Table III: Mean counts of total coliform and Staphylococcus aureus in conventional and organic fruits.

Types of samples	Total Coliform		<i>S. aureus</i>		<i>E. coli</i>		<i>Salmonella</i>	
	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic
Kiwi	6.88±0.18*	1.80±2.78*	2.97±3.26	2.77±3.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Apple	6.87±0.12	6.69±0.29	3.60±2.80	2.73±2.99	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Plum	3.72±2.91	3.80±2.97	2.60±2.85	2.55±2.80	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Results expressed as mean ± SD (log₁₀ cfu/g).

*Significant differences ($p < 0.05$).

0.00±0.00 in 25 g.

types of fruit samples. Prevalence was conducted in many microbiological qualities study to show that contamination of ready-to-eat foods with pathogenic microorganisms remains a public health risk that must be addressed in order to protect the health and well-being of consumers (11, 13). No trend was found suggesting whether conventional or organic fruit had a higher prevalence of microbial counts. There was no obvious difference in the prevalence of total coliform between organic and conventional fruits. A percentage of 88.9% of the conventional fruits and 66.7% of organic fruit were identified with total coliform. Slightly higher percentage of *S. aureus* was found on conventional fruits compared to organic fruits. *E. coli* and *Salmonella* were not identified during the study.

DISCUSSION

Presence of heavy metals in organic and conventional fruits

Fresh produce contains both essential and toxic metals over range of concentrations that reported to have both positive and negative effect in human (2, 14). Contamination of food commodity with heavy metals is one of the worldwide problems as the emergence of heavy metals came from human industrialization, agricultural chemistry and urban activities. The presence of harmful elements allows them to spread in the atmosphere by eating these harmful elements through food (15). This condition has caused the world to take precaution with a set of maximum permissible levels of toxic metals in human food with national and international regulations (15).

Zinc is one of the most abundant trace minerals in our bodies, and it plays an important role in cell division and development. Zinc is also fundamental to skin health, helps in the boost of immune system, speed wound healing, decrease inflammation, DNA synthesis and protein production (16). Furthermore, this study finds out all the reading of zinc concentration in the bound

of Recommended Dietary Allowance (RDA) where for adults is 8 mg/day for women and 11 mg/day for men (17). In other words, the fruit supply to customer either from organic or conventional origin seems safe to be taken. Organic fruits in this study shows to contain more zinc compared to conventional fruits which may seem to be more nutritious.

Copper also like zinc, serves as an essential micronutrient that functions as a biocatalyst to bind iron, maintains a healthy central nervous system and prevents anaemia (2). Copper concentration in organic and conventional fruits show significant differences in kiwi and plum but no significant difference between organic and conventional apple. Organic fruits contain more copper than conventional which may indicate more nutrition. Recommended Dietary Allowance (RDA) of copper for adult men and women is 0.9 mg/day (18). All the fruits are within the RDI limit but organic apple (0.930 mg/kg) and organic kiwi (0.913 mg/kg) are excess in a small value. Thus, it is recommended to be taken with caution. Unlike zinc and copper, cadmium is a non-essential element in our foods and water because it can build up in the kidneys and liver (2). It usually came from phosphate fertilizer sewage sludge, plated/galvanized equipment, industrial emissions, enamels and glazes. It can cause chronic effects to human health such as lung cancer, pulmonary adenocarcinomas, kidney dysfunction and prostatic proliferative lesions. In this study, all three types of fruits shows that there is no significance differences between organic and conventional fruits in cadmium concentration. Cadmium concentration allowed in fruits is 0.2 mg/kg (19, 20). Therefore, all the fruit samples in this study in within the safe values as the results were below 0.2 mg/kg. Metal contamination and microbial content in fruits may have been affected by environmental factors ranging from fruit growth to collection, handling, storage, and transportation (15).

Microbes in organic and conventional fruits

Epidemiological research has shown that the consumption of fresh produce associated with direct

and indirect faecal contamination is the main source of most foodborne disease outbreaks (3, 13). Thus, the total coliform count is widely used as a sanitary quality measure of food or to assess the risk of pathogenic microorganism contamination. Total coliform count is useful as a hygiene measure indicator since they were naturally found in fruits, vegetables and other food products, but their presence does not imply that pathogens are present in food. Most coliform bacteria do not cause harm and disease, but rather unusual strains such as *E. coli* O157: H7 is a human health pathogen that plays a role as a source of many foodborne disease outbreaks (12). The higher the number of coliforms, the greater the potential presence of pathogenic microorganisms. This analysis shows that the prevalence of total coliform is 77.8% in all 36 samples with a microbial count range of 5.00 to 7.08 log₁₀ cfu/g and 5.30 to 7.09 log₁₀ cfu/g in conventional and organic fruits respectively. Kiwi was the only sample that showed significant differences in total coliform populations between organic and conventional samples. The means of coliform were 6.87 log₁₀ cfu/g and 6.69 log₁₀ cfu/g for conventional and organic apple respectively. Lower means were recorded by previous analysis by Mukherjee et al. (21) where the mean result obtained was 2.7 log₁₀ MPN/g from both conventional and organic apple. The same study also revealed that 92% of all samples tested positive for the presence of total coliform bacteria, with an overall average of 2.9 log MPN/g in both organic and conventional produces. A study in Istanbul, Turkey discovered that coliform counts ranged between 4 to 7 log₁₀ cfu/g in fruits sold in the supermarkets (22). Since these commodities naturally have high levels of coliforms and often belong to the food's natural microflora, there are no uniform set of guidelines for microbiology quality of coliform levels on fresh fruits and vegetables (23).

Staph food poisoning is a gastrointestinal disease caused by eating food contaminated with toxins produced by the bacteria *Staphylococcus aureus*, namely *S. aureus* enterotoxins. *Staphylococcus aureus* is found on the skin and nose of around 25% of people and animals, but it seldom causes disease in healthy people (24). However, *Staphylococcus aureus* is an opportunistic pathogen that can cause a wide variety of infections. The presence of moisture on food or wash water influences the survival and growth of cell multiplication. Fruits that are consumed raw like kiwi, apple and plum pose higher food safety issue of pathogenic microbes (25).

This study found that 55.6% of the samples tested were positive and exceeded the unacceptable level of *Staphylococcus aureus* which is more than > 10⁴ or 10,000 cfu/g by available voluntary guidelines (26, 27). The highest count of *Staphylococcus aureus* for both conventional and organic were found in conventional kiwi. This may be due to the hairy structure on the kiwi's peel that increase the adherence of *Staphylococcus aureus* compared to apple and plum that have smooth

peel skin. Consumers can be extremely vulnerable to Staphylococcal poisoning when the presence of *Staphylococcus aureus* is in an excessive range. This situation endangers public health in Malaysia, particularly among those who never or rarely wash their fruits before eating them. *Staphylococcus aureus* can be a source of numerous diseases, including skin lesions, toxic shock syndrome and staphylococcal food poisoning, which is the most serious disease that may cause diarrhoea, abdominal cramping, and nausea (24). None of the samples for tested positive with *E. coli* and *Salmonella spp.* which reflect satisfactory level of food hygiene.

CONCLUSION

The study aims is to establish the profile of heavy metal concentration and microbial count in organic as well as their counterpart in conventional fruits. The findings show the presence of cadmium, copper and zinc are within the acceptable level for consumption. The findings in microbes study suggested that, regardless of organic or conventional farming practices, raw fruits can serve as a potential vehicle for pathogen transmission. The environmental conditions during growth to the processing, handling, storage and transportation of fruits may have influenced the metal contamination and microbial content in fruits. The study also found that a specific method of farming activity does not have an effect on the heavy metals and microbiological status of the fresh produce. It is also recommended to further the analysis on pesticide in fruits as organically grown fruits are supposed to restrict the use of pesticides.

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