Food Control 22 (2011) 337–342

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Short communication

Simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits using multiplex PCR

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ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form
23 May 2010
Accepted 29 May 2010

ABSTRACT

This study aimed to determine the prevalence and quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits from hawker stalls and hypermarkets in Malaysia. Analysis was carried out using the most probable number (MPN) – multiplex polymerase chain reaction (PCR) method. The prevalence of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in 210 samples of sliced fruits examined were 23.3%, 7.6% and 3.8%, respectively with estimated quantity varying from 0 to 19 MPN/g. This study urged the authority to look into the biosafety of sliced fruits in Malaysia.

1. Introduction

Salmonella spp. are Gram-negative, facultatively anaerobic and rod-shaped bacteria. So far there are more than 2500 serovars of *Salmonella* with typhoid fever by *Salmonella* Typhi and gastroenteritis by *Salmonella* Typhimurium being the most dominant serovars in public health (Namimatsu et al., 2000; Park et al., 2009). *Salmonella* Typhi causes enteric fever in humans whereas *Salmonella* Typhimurium causes systemic disease in mice which closely resembles typhoid fever in humans (Lin et al., 2007). In the United States, approximately 40,000 cases of salmonellosis are reported each year which cause about 600 deaths annually (Albufera, Bhugaloo-Vial, Issack, & Jaufeerally-Fakim, 2009). In Malaysia, it is difficult to evaluate the status of salmonellosis due to the lack of detailed epidemiological studies by the public health and veterinary sector. Nevertheless, 171 salmonellosis patients with two deaths were reported due to consumption of ready-to-eat foods in Kelantan in 2005 (Tunung et al., 2006). The surveillance program by Ministry of Health Malaysia (Communicable Disease Control Section, 2008) indicated that the incidence of typhoid fever in Malaysia from 1996 to 2006 was in the range of 0.71–4.50 per 100,000 populations.

Recently there is a sharp increase in the consumption of fresh produce worldwide due to their nutritional benefits to humans and changes in diet. The reported cases of foodborne outbreaks associated with fresh produce have also increased (Abadias, Usall, Anguera, Solsona, & Vinas, 2008). Yaun, Sumner, Eifert, and Marcy (2004) reported that the major outbreaks associated with fresh produce have been linked with foodborne pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Shigella* spp. Although fresh produce are less frequently implicated as vehicles for salmonellosis, multistate outbreaks of salmonellosis caused by apple, watermelon, lettuce, melon, mango and tomato consumption have been reported (Bordini, Ristori, Jakabi, & Gelli, 2007). To the best of our knowledge, there is no published data on the prevalence and number of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits in Malaysia.

The gold standard for the detection of *Salmonella* spp. from food usually involves pre-enrichment, selective enrichment, isolation on selective agar media and confirmation of presumptive positive colonies using biochemical and serological tests. This conventional cultural method is very expensive and time consuming (Schonenbrucher, Mallinson, & Bulte, 2008). Consequently, polymerase chain reaction (PCR)-based methods for *Salmonella* spp. detection have gained popularity. The multiplex PCR applied in this study, gives best results with number of positive results similar to those obtained by conventional method. This further reduces the

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doi:10.1016/j.foodcont.2010.05.021

labour, reagent cost and testing time for multiple bacterial pathogens (Cortez, Carvalho, Ikuno, Burger, & Vidal-Martins, 2006). However, PCR-based methods are limited to qualitative determination. Borowsky, Schmidt, and Cardoso (2007) stated that the quantification of microorganisms present in the food samples is important to assess the risk to consumers. Therefore, the most probable number (MPN) method which estimates the number of microorganism based on the probability is incorporated for the quantification of the PCR products (Mantynen, Niemela, Kaijalainen, Pirhonen, & Lindstrom, 1997).

Periodic surveillance to determine the prevalence and quantity of *Salmonella* spp. in foods is important to control human salmonellosis. As a consequence, the objective of this study was to determine the prevalence and quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits to assess the level of consumer exposure to *Salmonella*. Another objective was to compare the occurrence of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits from hawker stalls and hypermarkets in a limited geographical location in Malaysia. This study will be the first biosafety assessment of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits in Malaysia which will serve as an important monitoring program of Food Safety and Quality Division in the Ministry of Health Malaysia.

2. Materials and methods

2.1. Sample collection

A total of 210 samples of sliced fruits were purchased randomly from 3 hawker stalls at Seri Kembangan, hypermarket A at Kajang and hypermarket B at Kuala Lumpur. In this study, seven types of fruits were analyzed (Table 1). However, it was not possible to obtain all the samples at each sampling location and on each sampling occasion as this was dependent on their availability during particular sampling visit. All the samples were transported to the laboratory in an ice box and examined within 2 h after purchase.

2.2. Most probable number (MPN) method

A 10 g of sliced fruit sample was aseptically weighed and transferred into a sterile stomacher bag. The sample was pummeled in a stomacher for 60 s with 90 mL of buffered peptone water (BPW; Merck, Darmstadt, Germany). A three-tube most probable number (MPN) method was employed where 100 fold and 1000 fold dilutions of the stomacher fluids were prepared. Each dilution of the fluids was transferred into three tubes, with 1 mL in each tube. All the tubes were incubated at 37 °C for 24 h. After incubation, MPN tubes were checked for turbidity where turbid tubes were subjected to DNA extraction followed by multiplex PCR.

Table 1
Species and the number of fruits examined for the prevalence and quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium.

English name	Local name	Scientific name	Number of samples (n)
Papaya	Betik	*Carica papaya*	30
Watermelon	Tembikai	*Citrullus lanatus*	40
Mango	Mangga	*Mangifera indica*	20
Sapodilla	Ciku	*Manilkara zapota*	20
Jackfruit	Nangka	*Artocarpus heterophyllus*	20
Dragon fruit	Buah naga	*Hylocereus undatus*	40
Honeydew	Tembikai susu	*Cucumis melo*	40
Total			210

2.3. Plating method

The bacteriological method used for culturing *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium was modifications of the methods of Rall, Rall, Aragon, and Silva (2005) and Tunung et al. (2006). A 1 mL of pre-enriched BPW was transferred to 10 mL Selenite Cystine Broth (SC; Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. A loopful of the broth culture was then plated onto CHROMagar *Salmonella* (CHROMagar Microbiology, Paris, France) and Xylose Lysine Deoxycholate Agar (XLD; Eiken Chemical Co., Tochigi, Japan). The plates were incubated at 37 °C for 24 h and the presumptive colonies obtained were purified by the streak and isolation method using the Tryptic Soy Agar (TSA; Merck, Darmstadt, Germany).

2.4. DNA extraction

DNA was extracted from the turbid MPN tubes and presumptively identified colonies from agar plates using a modified boiled cell method (Chai et al., 2007; Tang, Mohamad Ghazali, Saleha, Nishibuchi, & Son, 2009). A 1 mL portion of each broth was centrifuged at 15,000×g for 3 min. The pellet was suspended in 500 μL sterile distilled water and vortexed vigorously. The cell suspension was boiled for 10 min, cooled at −20 °C for 10 min, centrifuged again at 15,000×g for 3 min. The supernatant was used as the DNA template solution in the multiplex PCR as described below. DNA extraction from the presumptively identified colonies from agar plate was the same except that the initial cell pelleting step was omitted and a loopful of colony was suspended in distilled water.

2.5. Multiplex PCR

Three sets of primers targeting a randomly selected-sequence of unknown function but is specific to *Salmonella* spp., 23*S* rRNA gene specific to *Salmonella* Typhi and *fliC* gene specific to *Salmonella* Typhimurium were used in the multiplex PCR assay (Table 2). All oligonucleotide primers were synthesized by 1st BASE Laboratories, Malaysia. The reference *Salmonella* Typhi and *Salmonella* Typhimurium strains included as positive controls in each PCR assay were obtained from Institute for Medical Research, Malaysia.

Multiplex PCR amplification was carried out using Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). We optimized the multiplex PCR reaction conditions by a series of preliminary experiments so that the three independent PCR reactions can be performed in the same tube (data not shown). The optimized multiplex PCR reaction was performed in 50 μL reaction mixtures containing 10 μL of 5× PCR buffer, 0.2 mM of deoxynucleoside triphosphate mix, 0.2 μM of ST11 and ST15 primers, 1.2 μM for Fli15, Typ04, sty-1 and sty-2 primers, 2.5 mM $MgCl_2$, 1.5 U of Taq DNA polymerase and 4 μL of DNA template. All the materials used in the PCR were purchased from Vivantis Technologies, Selangor, Malaysia. The thermocycler conditions were initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 53 °C for 1 min and extension at 72 °C for 1 min. Lastly, a final extension at 72 °C for 7 min and indefinite holding period at 4 °C was employed.

For visualization of PCR products, 5 μL of PCR products was loaded on 1.2% agarose gel using 0.5 × TBE buffer (pH 8.0) run at 90 V for 40 min. The gel was then stained with ethidium bromide and viewed under ultraviolet (UV) light. A DNA-molecular ladder (100 bp ladder) (Vivantis Technologies, Selangor, Malaysia) was included in each gel as molecular weight markers.

3. Results

The target genes specific to *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium produced amplicons at 429 bp,

Table 2
Primer sequences for the detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium using a multiplex PCR.

Primer designation	Target		Primer sequence 5′ to 3′	Length (bp)	Amplicon size (bp)	Reference
	Species	Gene				
ST11 ST15	*Salmonella* spp.	Sequence of unknown function	GCC AAC CAT TGC TAA ATT GGC GCA GGT AGA AAT TCC CAG CGG GTA CTG G	24 25	429	Soumet et al., 1999
sty-1 sty-2	*Salmonella* Typhi	23*S* rRNA gene	TGC CGG AAA CGA ATC T GGT TGT CAT GCC AAT GCA CT	16 20	300	Zhu, Lim, & Chan, 1996
Fli15 Typ04	*Salmonella* Typhimurium	*fliC* gene	CGG TGT TGC CCA GGT TGG TAA T ACT GGT AAA GAT GGC T	22 16	620	Soumet et al., 1999

300 bp and 620 bp, respectively. Fig. 1 shows the result of gel electrophoresis comparing various combinations of the PCR primer sets and verifying the multiplex PCR established for the current study consists of three independent and specific PCR reactions.

The prevalence of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in 210 samples of sliced fruits examined were 23.3%, 7.6% and 3.8%, respectively. Simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in 210 samples of sliced fruits using multiplex PCR are summarized in Table 3. It was shown that the prevalence of *Salmonella* spp. in total sliced fruits from hawker stalls (30%) were three times higher than those from hypermarkets (10%) with 140 and 70 samples examined, respectively. *Salmonella* Typhi and *Salmonella* Typhimurium were detected in sliced fruits from hawker stalls with prevalence for total fruits being 11.4% and 5.7%, respectively. None of the samples from hypermarket was contaminated by *Salmonella* Typhi and *Salmonella* Typhimurium. Out of all the seven species of fruits examined, dragon fruits from hawker stalls showed the highest prevalence of *Salmonella* spp. (75%), *Salmonella* Typhi (40%) and *Salmonella* Typhimurium (25%). On the other hand, papayas from hypermarkets showed zero contamination of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in all 10 samples examined.

The estimated quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits varied from 0 to 19 MPN/g (Table 4). The highest quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits from hawker stalls and hypermarkets were 19 MPN/g. Most of the samples from hawker stalls and hypermarkets showed a minimum of 3 MPN/g and a maximum of 19 MPN/g of *Salmonella* spp. The quantity of *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits from most locations were 0 MPN/g, but their presence in dragon fruit from hawker stalls reached a maximum of 19 MPN/g.

Table 5 demonstrates the difference between MPN-multiplex PCR and MPN-plating method in the sensitivity in detecting *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits. *Salmonella* spp. were detected in 49 of 210 samples (23.3%) using the MPN-multiplex PCR whereas only in 20 samples (9.5%) using the MPN-plating method. The samples judged positive by the MPN-plating method were always positive by the MPN-multiplex PCR method whereas the opposite was not true, indicating higher sensitivity of the MPN-multiplex PCR method.

4. Discussion

The three primer sets were used simultaneously to assure the identification of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in the same reaction tube even in the presence of other related and non-related bacteria. *Salmonella* Typhi and *Salmonella* Typhimurium were chosen because they are the most dominant serotypes among those isolated from the patients who

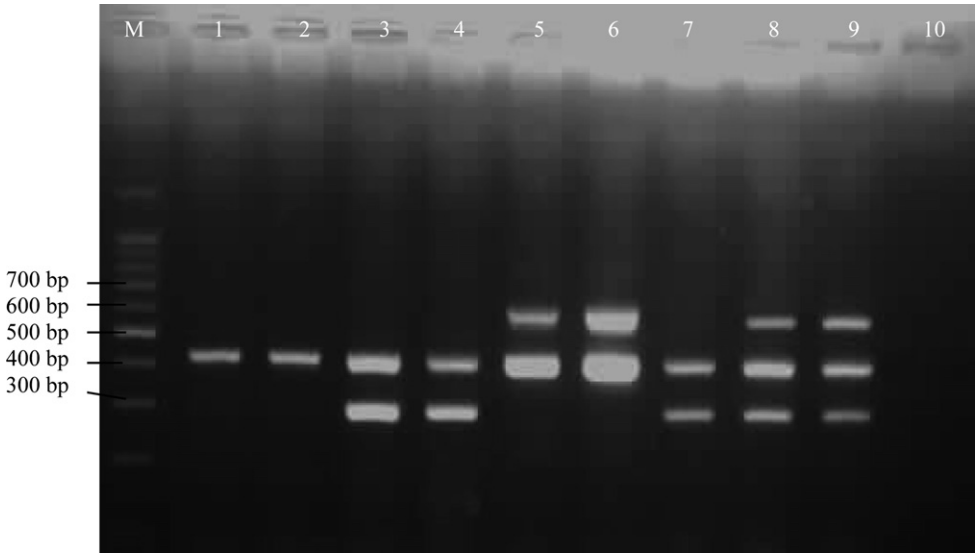

Fig. 1. Representative amplification of random sequence, 23*S* rRNA gene and *fliC* gene for the identification of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium, respectively. Lanes 1 and 2 show the PCR amplicons specific to *Salmonalla* spp. at 429 bp. Lanes 3, 4 and 7 show the PCR amplicons specific to *Salmonalla* spp. at 429 bp and *Salmonella* Typhi at 300 bp. Lanes 5 and 6 show the PCR amplicons specific to *Salmonella* spp. at 429 bp and *Salmonella* Typhimurium at 620 bp. Lanes 8 and 9 show the PCR amplicons specific to *Salmonalla* spp. at 429 bp, *Salmonella* Typhi at 300 bp and *Salmonella* Typhimurium at 620 bp. Lane M shows the 100 bp DNA ladder, (1) to (8) representative positive samples, (9) positive control and (10) negative control.

Table 3
Prevalence of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits from hawker stalls and hypermarkets.

Fruit	Hawker stalls						Hypermarkets					
	Salmonella spp.		*Salmonella* Typhi		*Salmonella* Typhimurium		*Salmonella* spp.		*Salmonella* Typhi		*Salmonella* Typhimurium	
	No.[a]	%[b]	No.[a]	%[b]	No.[a]	%[b]	No.[a]	%[b]	No.[a]	%[b]	No.[a]	%[b]
Papaya	6/20	30.0	3/20	15.0	0/20	0.0	0/10	0.0	0/10	0.0	0/10	0.0
Watermelon	6/20	30.0	1/20	5.0	0/20	0.0	4/20	20.0	0/20	0.0	0/20	0.0
Mango	2/20	10.0	1/20	5.0	0/20	0.0	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
Sapodilla	6/20	30.0	0/20	0.0	0/20	0.0	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
Jackfruit	2/20	10.0	0/20	0.0	1/20	5.0	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
Dragon fruit	15/20	75.0	8/20	40.0	5/20	25.0	1/20	5.0	0/20	0.0	0/20	0.0
Honeydew	5/20	25.0	3/20	15.0	2/20	10.0	2/20	10.0	0/20	0.0	0/20	0.0
Average	42/140	30.0	16/140	11.4	8/140	5.7	7/70	10.0	0/70	0.0	0/70	0.0

[a] Number of positive samples/number of samples examined.
[b] Frequency (in %) of positive samples among the samples examined.
[c] Sample was not available.

presented enteric fever and gastroenteritidis in humans (Namimatsu et al., 2000). In Malaysia, typhoid fever is also recognized as an endemic problem which affects all age groups (Thong, Cheong, Puthucheary, Koh, & Pang, 1994). Our results support these previous observations.

In this study, MPN-PCR method was employed which allowed the detection and quantification of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits to be completed within 2 days. This was definitely an advantage as compared with the MPN method coupled with conventional biochemical test-based tests which involved much workload, great amount of material and 7–10 days for complete identification (Martin, Jofre, Garriga, Hugas, & Aymerich, 2004). If direct PCR was to be performed on food samples, false positive results might have occurred due to the dead cells (Saroj, Shashidhar, Karani, & Bandekar, 2008). This problem was solved by the incorporation of MPN method which allowed only viable cells to grow so as to complement multiplex PCR method for the accurate detection of *Salmonella*.

In Malaysia, many studies on the incidence of *Salmonella* spp. from food samples have been reported. These includes contamination of *Salmonella* spp. in raw and ready-to-eat cooked foods (Arumugaswamy, Rusul, Abdul Hamid, & Cheah, 1995), broilers (Rusul, Khair, Son, Cheah, & Yassin, 1996), raw vegetables (Salleh et al., 2003) and street food and clinical samples (Tunung et al., 2006). This is the first prevalence study reporting simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits in Malaysia. The result highlighted the concern of the presence of *Salmonella* spp. (23.3%), *Salmonella* Typhi (7.6%) and *Salmonella* Typhimurium (3.8%) with majority showing the maximum quantities (19 MPN/g) in the sliced fruits. Johannessen, Loncarevic, and Kruse (2002) pointed out that *Salmonella* spp. had been isolated from fresh produce, mostly in low levels. This was supported by Salleh et al. (2003) who reported that the prevalence and contamination levels of *Salmonella* spp. were lower in fresh vegetables and fruits than in meat. However, the maximum number of 19 MPN/g in 4 types of sliced fruits examined is considered high as fruits are usually eaten raw. Hence the risk of acquiring salmonellosis becomes higher than consuming meat.

The prevalence of *Salmonella* spp. in sliced fruits from hawker stalls was three times higher than those from hypermarkets; *Salmonella* Typhi and *Salmonella* Typhimurium were detected only from hawker stalls. This might be attributed to the attitude and food safety knowledge of hawkers in Malaysia. Hawkers have been considered to be poor, uneducated and lack appreciation for safe food handling. An informal study by City Hall, known as Dewan Bandaraya Kuala Lumpur (DBKL) confirmed a lack of knowledge of good food handling and failure of hawkers to fulfill the health requirements (Toh & Birchenough, 2000). In fact, the contamination of the fresh fruits may take place while farmers grow them in fields or orchards, during harvesting and post harvesting, processing and distribution. The use of animal manure as fertilizers, fecal contamination by animals and employees, presence of domestic and wild animals, use of contaminated water in irrigation and human manipulation are known to be potential pre-harvest sources of the pathogens. The post harvest contamination sources include contaminated rinsing water or ice, human manipulation, animals, contaminated equipment or transportation vehicles, cross-contamination and high storage temperatures (Beuchat, 2002; Bordini et al., 2007; Johannessen et al., 2002). As a result, it can be concluded that there is a wide contamination route which results in unsafe fresh produce unsuitable for consumption without heat treatment.

Table 4
Quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium (MPN/g) in sliced fruits from hawker stalls and hypermarkets.

Location	Fruit	*Salmonella* spp.			*Salmonella* Typhi			*Salmonella* Typhimurium		
		Min[a]	Med[b]	Max[c]	Min[a]	Med[b]	Max[c]	Min[a]	Med[b]	Max[c]
Hawker stalls	Papaya	6.2	14.2	19.0	6.2	9.2	9.3	0.0	0.0	0.0
	Watermelon	3.0	5.2	7.4	3.0	3.0	3.0	0.0	0.0	0.0
	Mango	3.0	3.0	3.0	3.0	3.0	3.0	0.0	0.0	0.0
	Sapodilla	3.0	12.7	19.0	0.0	0.0	0.0	0.0	0.0	0.0
	Jackfruit	3.0	3.0	3.0	0.0	0.0	0.0	3.0	3.0	3.0
	Dragon fruit	3.0	9.3	19.0	3.0	4.5	19.0	11.0	15.0	19.0
	Honeydew	3.0	5.2	16.0	3.0	3.0	3.0	3.6	7.3	11.0
Hypermarkets	Papaya	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Watermelon	9.3	19.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mango	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
	Sapodilla	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
	Jackfruit	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
	Dragon fruit	15.0	15.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0
	Honeydew	3.0	3.3	3.6	0.0	0.0	0.0	0.0	0.0	0.0

[a] Minimum MPN/g value.
[b] Median MPN/g value.
[c] Maximum MPN/g value.
[d] Sample not available.

From our observation during sampling, the hawkers were found to be less hygienic in the preparation of sliced fruits as compared to handlers in hypermarkets. The hawkers seldom wear gloves in contrast to handlers in hypermarket who normally wear gloves during handling of sliced fruits; although wearing gloves, does not always guarantee safe food unless gloves are frequently changed. Hawkers usually pack the sliced fruits in plastic bags without sealing and use crushed ice to cool the sliced fruits during display. On the other hand, the sliced fruits in hypermarkets are sealed using polystyrene packaging and displayed in chiller. Besides, hawkers normally wash fruits, hands, cutting board and knives in the buckets with water. Viswanathan and Kaur (2001) pointed out that the running water is seldom available at the vendor's stand, foods are ineffectively protected from flies and hence the sanitation of street vending operations required much concerns. They also emphasized that the vendors might contaminate the fresh produce during slicing, chopping and hand mixing. Apart from that, the shedding of *Salmonella* spp. by human carriers during handling of food can be recognized as another source of contamination (Hatha & Lakshmanaperumalsamy, 1997).

This study showed the MPN-multiplex PCR method was considered to be more successful in detecting *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium than MPN-plating method (Table 5). This was consistent with the findings by Mahon, Murphy, Jones, and Barrow (1994) who compared the multiplex PCR technique and standard bacteriological technique for the detection of *Salmonella* on the chicken skin. Out of the 10 positive samples detected by multiplex PCR, only 5 of them were positive by culture. They concluded that PCR may have advantages over standard culture methods for the detection of *Salmonella* in poultry samples. Busse (1995) stressed that the process of isolating *Salmonella* from food is sometime prone to failure as they can be lost during enrichment even though a contaminated portion has been drawn. Apart from that, *Salmonella* spp. can enter viable but nonculturable state (VBNC) under unfavourable condition which contributes to difficulty in culturing though many different conventional culture media and enrichment regimes have been proposed for isolation of *Salmonella* from food and environmental samples (Way et al., 1993). Smith, Newton, Harwood, and Barer (2002) also claimed that many major pathogens, including *Salmonella enterica*, are able to enter a VBNC state where they cannot be recovered by standard culture methods but retain viability and can resuscitate under favourable conditions.

In conclusion, this study demonstrated distribution of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits in Malaysia with hawker stalls showing considerably higher contamination frequency than hypermarkets. Therefore, sliced fruits in Malaysia pose a health risk to consumers. It is important to carry out the risk assessment of *Salmonella* in fresh produce and establish control measures that can ensure the original sensory quality and nutritional values of the fresh fruits.

Table 5
Comparison between the MPN-multiplex PCR and MPN-plating method in sensitivity in detecting *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits.

Fruit	Number of samples	MPN-multiplex PCR method						MPN-plating method					
		Salmonella spp.		*Salmonella* Typhi		*Salmonella* Typhimurium		*Salmonella* spp.		*Salmonella* Typhi		*Salmonella* Typhimurium	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Papaya	30	6/30	20.0	3/30	10.0	0/30	0.0	6/30	20.0	0/30	0.0	0/30	0.0
Watermelon	40	10/40	25.0	1/40	2.5	0/40	0.0	3/40	7.5	0/40	0.0	0/40	0.0
Mango	20	2/20	10.0	1/20	5.0	0/20	0.0	0/20	0.0	0/20	0.0	0/20	0.0
Sapodilla	20	6/20	30.0	0/20	0.0	0/20	0.0	4/20	20.0	0/20	0.0	0/20	0.0
Jackfruit	20	2/20	10.0	0/20	0.0	1/20	5.0	0/20	0.0	0/20	0.0	0/20	0.0
Dragon fruit	40	16/40	40.0	8/40	20.0	5/40	12.5	4/40	10.0	0/40	0.0	0/40	0.0
Honeydew	40	7/40	17.5	3/40	7.5	2/40	5.0	3/40	7.5	0/40	0.0	0/40	0.0
Average	210	49/210	23.3	16/140	11.4	8/140	5.7	20/210	9.5	0/140	0.0	0/140	0.0

Acknowledgements

This study was supported by Science Fund (project no. 545260) from Ministry of Science, Technology and Innovation, Malaysia and in part by a Grant-in-Aid for Science Research (KAKENHI 19101010) from the Japan Society for the Promotion of Sciences.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foocont.2010.05.021.

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