UNIVERSITI PUTRA MALAYSIA

PREVALENCE AND RISK ASSESSMENT OF SALMONELLA SPP. IN SLICED FRUIT

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PREVALENCE AND RISK ASSESSMENT OF *SALMONELLA* SPP. IN SLICED FRUIT

By

PUI CHAI FUNG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

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Dedicated to my beloved mum (Chu Su Chey), family and friends for their eternity love and endless support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Master of Science

PREVALENCE AND RISK ASSESSMENT OF *SALMONELLA* SPP. IN SLICED FRUIT

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PUI CHAI FUNG

March 2011

Chairman: Professor Son Radu, PhD

Faculty: Food Science and Technology

Fresh fruits and vegetables are increasingly recognized as a source of salmonellosis outbreaks in many parts of the world. Such products are always eaten raw or with minimal processing and if contaminated with *Salmonella*, they may represent a health hazard to the consumers. The public health importance of *Salmonella* in man led the present study to determine the prevalence and quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits. In domestic kitchen, the food contact surfaces are sometimes improperly cleaned which ease the formation of biofilm by *Salmonella*. Subsequent detachment from the biofilm layer can be at risk for contamination of fresh fruits and vegetables. Hence, the quantification of biofilm formed by *Salmonella* Typhi and *Salmonella* Typhimurium on plastic cutting board used in domestic kitchen was

determined in this study prior to analyzing the transfer rate of these two pathogens to the fruit model.

The most probable number (MPN)-multiplex polymerase chain reaction (PCR) was used for the prevalence and quantification studies. A multiplex PCR was optimized for the simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced papaya, watermelon, mango, sapodilla, jackfruit, dragon fruit and honeydew. 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 varied from 0 to 19 MPN/g. On the other hand, the prevalence of *Salmonella* spp. discovered from the total sliced fruits of hawker stalls were three times higher than those from hypermarkets at 30% and 10% respectively at $p<0.05$. *Salmonella* spp. were detected in 23.3% fruit samples using MPN-multiplex PCR and at 9.5% using MPN-plating method.

Out of the seven types of fruits examined, dragon fruit from hawker stalls showed the highest prevalence of contamination with 75% *Salmonella* spp., 40% *Salmonella* Typhi and 25% *Salmonella* Typhimurium. Hence, it was used as the fruit model to study the biofilm formation by *Salmonella* Typhi and *Salmonella* Typhimurium on plastic cutting board and their subsequent transfer to dragon fruit. By using crystal violet assay, it was found that

biofilm formation of *Salmonella* Typhi and *Salmonella* Typhimurium on plastic cutting board was the highest at 12 h when they are incubated in physiological saline solution at 28°C. On the transfer of these two pathogens from plastic cutting board to dragon fruit, results showed their mean transfer percentages were 92.38 and 91.30% respectively for *Salmonella* Typhi and *Salmonella* Typhimurium. These highlighted that biofilm can cross-contaminate dragon fruit and contribute to cross-infection. As a conclusion, there is a need for the health authority to look into the risk assessment of sliced fruits in Malaysia. The risk of microbial transfer from food contact surface to fresh fruits should also be of concern to prevent foodborne disease outbreaks.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PREVALEN DAN PENILAIAN RISIKO *SALMONELLA* SPP. DALAM BUAH HIRISAN

Oleh

PUI CHAI FUNG

Mac 2011

Chairman: Profesor Son Radu, PhD

Faculty: Sains dan Teknologi Makanan

Buah-buahan dan sayur-sayuran segar semakin diakui sebagai sumber wabak salmonelosis di kebanyakan bahagian dunia. Produk-produk ini selalu dimakan mentah atau melalui pemprosesan minima dan jika dikontaminasi oleh *Salmonella*, ini membahayakan kesihatan pelanggan. Kepentingan kesihatan masyarakat *Salmonella* ke atas manusia telah mendorong kajian ini dilaksanakan supaya prevalen dan kuantiti *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium dalam buah-buahan hirisan boleh ditentukan. Di dapur rumah tangga, permukaan sentuh makanan kadang-kadang tidak dibersihkan dengan betul dan ini memudahkan pembentukan biofilem oleh *Salmonella*. Penyingkiran selanjutnya lapisan biofilem berisiko ini dapat mengkontaminasi buah-buahan dan sayur-sayuran segar. Oleh itu, kuantiti biofilem yang

dihasilkan oleh *Salmonella* Typhi dan *Salmonella* Typhimurium pada permukaan papan potong plastik yang digunakan di dapur ditentukan terlebih dahulu, sebelum menganalisa kadar pemindahan kedua-dua patogen ini kepada model buah.

Most probable number (MPN)-multiplex polymerase chain reaction (PCR) telah digunakan untuk kajian prevalen dan kuantifikasi. Multiplex-PCR dioptimumkan untuk mengesan *Salmonella* spp., *Salmonella* Typhi dan *Salmonella* Typhimurium secara serentak dalam hirisan betik, tembikai, mangga, ciku, nangka, buah naga dan tembikai susu. Prevalen *Salmonella* spp., *Salmonella* Typhi dan *Salmonella* Typhimurium pada 210 sampel buah-buahan hirisan yang dikaji adalah masing-masing 23.3%, 7.6% dan 3.8% dengan jangkaan kuantiti berbeza daripada 0 hingga 19 MPN/g. Selain daripada itu, didapati bahawa prevalen *Salmonella* spp. pada buah-buahan hirisan dari gerai penjaja adalah tiga kali ganda lebih tinggi berbanding dengan buah-buahan dari pasar raya besar, masing-masing sebanyak 30% dan 10% pada $p<0.05$. Dengan menggunakan kaedah MPN-multiplex PCR, sebanyak 23.3% sampel telah dikesan mengandungi *Salmonella* spp. dan, hanya 9.5% apabila menggunakan kaedah MPN-plating.

Daripada tujuh jenis buah-buahan yang dikaji, buah naga dari gerai penjaja menunjukkan prevalen kontaminasi tertinggi dengan 75% *Salmonella* spp.,

40% *Salmonella* Typhi dan 25% *Salmonella* Typhimurium. Oleh itu, buah naga telah digunakan sebagai model buah untuk mengkaji pembentukan biofilem oleh *Salmonella* Typhi dan *Salmonella* Typhimurium di atas papan potong plastik dan juga kadar pemindahannya kepada buah naga. Dengan menggunakan ujian crystal violet, didapati bahawa pembentukan biofilem oleh *Salmonella* Typhi dan *Salmonella* Typhimurium atas papan potong plastik adalah tertinggi pada 12 jam inkubasi dalam larutan garam fisiologis pada suhu 28°C. Bagi pemindahan kedua-dua patogen dari papan potong plastik kepada buah naga, keputusan menunjukkan bahawa purata peratus pemindahan adalah 92.38 dan 91.30%, masing-masing untuk *Salmonella* Typhi dan *Salmonella* Typhimurium. Ini menunjukkan bahawa biofilem boleh mengkontaminasi buah naga secara bersilang dan menyumbang kepada jangkitan silang. Kesimpulannya, pihak berkuasa kesihatan perlu menilai risiko buah-buahan hirisan di Malaysia. Risiko pemindahan mikroorganisma dari permukaan sentuh makanan kepada buah-buahan segar seharusnya diberi perhatian untuk mencegah wabak penyakit yang berpunca daripada makanan.

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I certify that a Thesis Examination Committee has met on 24 March 2011 to conduct the final examination of Pui Chai Fung on her thesis entitled "Prevalence and Risk Assessment of *Salmonella* Spp. in Sliced Fruit" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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Date: 24 May 2011

APPROVAL

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is nor concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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PUI CHAI FUNG

Date: 24 March 2011

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