UNIVERSITI PUTRA MALAYSIA

MIGRATION ASSESSMENT AND MODELLING OF RESIDUAL STYRENE MONOMER MIGRATED FROM POLYSTYRENE FOOD CONTACT MATERIALS INTO SELECTED FOODS

NAZIRUDDIN BIN MAT ARIFFIN

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By

NAZIRUDDIN BIN MAT ARIFFIN

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Chairman : Maimunah Binti Sanny, PhD
Faculty : Food Science and Technology

Polystyrene food contact materials can be tainted with unreacted residual styrene monomer from the manufacturing process, which later migrate into food when both materials come into contact. Ingestion of styrene is the main concern as it has been classified in group 2B substance (possibly carcinogenic to humans) by International Agency for Research on Cancer IARC (2002). The risk of styrene monomer migration into Southeast Asian cuisines is imminent as the foods are inherently fatty, often prepared by high-heat cooking methods, and commonly serve at hot state. Additionally, data of styrene monomer migration from High Impact Polystyrene (HIPS) into food at prolong storage is rarely modelled. In this study, the effects of fat content, temperature and contact time on styrene monomer migration from General-Purpose Polystyrene (GPPS) into selected Malaysian dishes and beverage were determined. Distilled water, 3% acetic acid, 10% ethanol and palm olein were prepared in addition to the beef soup, curry noodles and pulled tea at four different amounts of fat-contributing ingredients. Two-sided contact migration cell was filled with food simulants and samples at temperature ranging from 40°C to 70°C at different times. Headspace Solid-Phase Micro Extraction (HS-SPME) coupled with Gas Chromatography–Mass Spectrometry (GC-MS) was used to extract, detect and quantify styrene. Styrene levels in palm olein were found as the highest and significantly differed from other food simulants. Styrene levels in food samples with the highest fat content resulted in the highest levels and significantly differed from other formulations. Food simulants and food samples exposed to 70°C for 2hr were found to have the highest styrene levels compared to other conditions of contact. Subsequently, diffusion model based on Fick's law was used to examine the migration profile of styrene monomer from HIPS pot into yoghurt at different storage periods. Three different brands of plain stirred yoghurt packed in HIPS pot (Y_1, Y_2, and Y_3) were purchased from the established local manufactures. About 18 pots of yoghurt from the same brand and batch were collected during a production

month. Migration experiments were conducted at refrigerated condition (±4°C) and samples were instantly analysed during the collection day and every 4 days throughout storage until the product expired (approximately 28 days). Styrene monomer in samples were analysed by performing similar extraction, detection, and quantification method as formerly mentioned. All yoghurts exhibited significant increase in styrene migration levels before it reached the plateau after a certain time period depending on yoghurt. Finally, diffusion coefficient and partition coefficient of the migration process were estimated by means of migration model. Although styrene levels increased substantially throughout storage, its migration into yoghurts was limited as indicated by partition coefficients values (5.181×10^{-5} to 3.464×10^{-3}). The resulted diffusion coefficients were 2.920×10^{-18} cm^2 s^{-1}, 1.606×10^{-18} cm^2 s^{-1}, and 1.325×10^{-18} cm^2 s^{-1} in yoghurt Y_1, Y_2, and Y_3 respectively. Results revealed that the migration of styrene monomer into foods is strongly depended on fat content, temperature and contact time.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENILAIAN MIGRASI DAN PEMODELAN SISA MONOMER STIRENA YANG BERMIGRASI DARI BAHAN-BAHAN KONTAK MAKANAN POLISTIRENA KE DALAM MAKANAN TERPILIH

Oleh

NAZIRUDDIN BIN MAT ARIFFIN

Jun 2020

Pengerusi : Maimunah Binti Sanny, PhD
Fakulti : Sains dan Teknologi Makanan

Bahan-bahan kontak makanan polistirena boleh dicemari oleh sisa monomer stirena yang tidak bereaksi ketika proses pembuatannya, yang kemudiannya bermigrasi ke dalam makanan apabila kedua-dua bahan ini kontak secara langsung. Konsumsi stirena secara tidak sengaja adalah menjadi kebimbangan kerana stirena telah diklasifikasikan dalam kumpulan bahan 2B (berkemungkinan karsinogenik kepada manusia) oleh International Agency for Research on Cancer (IARC, 2002). Risiko migrasi monomer stirena ke dalam masakan-masakan Asia Tenggara adalah dijangka berlaku disebabkan oleh wujudnya lemak, penyediaan yang menggunakan kaedah masakan bersuhu tinggi, dan kebiasaanya disajikan ketika ianya masih panas. Tambahan lagi, data migrasi monomer stirena daripada *High Impact Polystyrene (HIPS)* ke dalam makanan pada masa yang lama adalah jarang dimodelkan. Dalam kajian ini, kesan-kesan kandungan lemak, haba dan masa kontak terhadap migrasi monomer stirena dari *General-Purpose Polystyrene (GPPS)* ke dalam masakan-masakan dan minuman Malaysia yang terpilih telah ditentukan. Air suling, 3% asid asetik, 10% etanol dan olein kelapa sawit telah disediakan berserta sup daging, mi kari dan teh tarik yang menggunakan empat jumlah bahan-bahan kontribusi lemak yang berbeza. Sel migrasi dua sisi kontak telah diisi dengan simulan-simulan makanan dan sampel-sampel pada jangka suhu haba 40°C ke 70°C pada masa yang berbeza. *Headspace Solid-Phase Micro Extraction (HS-SPME)* berpasangan dengan *Gas Chromatography–Mass Spectrometry (GC-MS)* telah digunakan untuk mengekstrak, mengesan dan kuantifikasi stirena. Tahap-tahap stirena dalam olein kelapa sawit telah ditemui sebagai yang tertinggi dan sangat berbeza daripada simulan-simulan makanan yang lain. Sampel-sampel makanan yang mengandungi kandungan lemak yang tertinggi menyebabkan tahap-tahap stirenanya tertinggi dan sangat berbeza daripada formulasi-formulasi yang lain. Simulan-simulan dan sampel-sampel makanan yang terdedah kepada suhu 70°C selama 2 jam telah ditemui untuk mengandungi tahap-tahap stirena yang tertinggi berbanding kondisi kontak yang

lain. Seterusnya, model difusi berdasarkan hukum Fick telah digunakan untuk memeriksa profil migrasi monomer stirena dari pot diperbuat daripada HIPS ke dalam yogurt pada tempoh penyimpanan yang berbeza. Yogurt jenis *plain stirred* yang dipek di dalam pot HIPS (Y_1, Y_2, and Y_3) daripada tiga jenama yang berbeza telah dibeli daripada pengeluar tempatan yang berlainan. Sebanyak 18 pot yogurt daripada jenama dan kumpulan produksi yang sama telah diambil ketika bulan produksi. Eksperimen migrasi telah dijalankan pada kondisi sejuk ($\pm$4°C) dan semua sampel dianalisis dengan serta-merta ketika hari persampelan dan setiap 4 hari sepanjang penyimpanan sehingga produk luput (kira-kira 28 hari). Monomer stirena di dalam sampel-sampel telah dianalisa dengan menggunakan kaedah mengekstrak, mengesan dan kuantifikasi yang sama seperti dinyatakan sebelum ini. Semua yogurt mempamerkan peningkatan tahap migrasi stirena yang ketara sebelum mendatar selepas tempoh tertentu bergantung kepada yogurt. Akhirnya, pekali difusi dan pekali sekatan untuk proses migrasi telah dianggarkan melalui model migrasi. Walaupun tahap stirena meningkat dengan ketara sepanjang penyimpanan, migrasinya ke dalam yogurt-yogurt adalah terhad seperti yang ditunjukkan oleh nilai pekali sekatan (5.181×10^{-5} ke 3.464×10^{-3}). Pekali difusi yang yang diperoleh adalah 2.920×10^{-18} cm^2 s^{-1}, 1.606×10^{-18} cm^2 s^{-1} dan 1.325×10^{-18} cm^2 s^{-1} bagi yogurt Y_1, Y_2, and Y_3. Keputusan-keputusan ini mendedahkan migrasi monomer stirena ke dalam makanan-makanan sangat bergantung kepada kandungan lemak, haba dan masa kontak.

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"Those who can imagine anything, can create the impossible."– Alan Turing.

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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:

Maimunah Binti Sanny, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Hjh Rabiha Binti Hj Sulaiman, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PHD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 October 2020

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature : _____

Name of Chairman of
Supervisory Committee : Assoc. Prof. Dr. Maimunah binti Sanny

Signature : _____

Name of Member of
Supervisory Committee : Assoc. Prof. Dr. Rabiha Binti Sulaiman

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflection
ATSDR	Agency for Toxic Substances and Disease Registry
CAR/PDMS	Carboxen/Polydimethylsiloxane
CFR	Code of Federal Regulation
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPS	Expandable Polystyrene
FAP	Food Additive Petition
FCN	Food Contact Notification
FCMs	Food Contact Materials
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared Spectroscopy
GC-FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography – Mass Spectrometry
GPPS	General-Purpose Polystyrene
GRP	Glass Reinforced Plastic
HIPS	High Impact Polystyrene
HS-SPME	Headspace Solid-Phase Micro Extraction
IARC	International Agency for Research on Cancer
ILSI	International Life Sciences Institute
LOD	Limit of Detection

LOQ	Limit of Quantification
MPOB	Malaysian Palm Oil Board
MyFCD	Malaysian Food Composition Databases
NTP	National Toxicology Program
NIOSH	National Institute of Occupational Safety and Health
OML	Overall Migration Limit
RoC	Report on Carcinogens
RSD	Relative Standard Deviation
SIM	Single Ion Monitoring
SML	Specific Migration Limit
SSE	Sum of Squared Errors
TDI	Tolerable Daily Intake
Tukey's HSD	Tukey's Honest Significant Difference
WHO	World Health Organization
XPS	Extruded Polystyrene Foam

CHAPTER 1

INTRODUCTION

1.1 Background

Food Contact Materials (FCMs) are extensively used in daily life in array of form such as food packaging, tableware, kitchen utensils and food preparation surface (Karamfilova, 2016). FCMs can be manufactured from varieties of polymer to tailor different uses in the industry and customer needs. Chemicals in the forms of monomers, plasticizers, stabilizers, and pigments might exist in the manufacturing process and inevitably presence as residues from polymerization reactions. Hence, depending on its properties and composition, these polymer probably transfer its constituents when in contact with food (Christian Block et al., 2017). Consequently, this issue sparks health concern to numerous consumers and legislators all over the world. Polystyrene is a common type of FCMs and it was the first mouldable clear rigid plastics to reach commercial market in large volume due to its thermoplastic properties (Robertson, 2012). General-Purpose Polystyrene (GPPS) and High Impact Polystyrene (HIPS) are among the main form of polymer resin that exist in market place (PlasticsEurope, 2019). GPPS and HIPS has been widely used for food packaging such as disposables cups and bowls, yoghurt pots, meat trays and cutleries. Nonetheless, migration of unwanted substances such as residual styrene monomer may cause various food safety problems when foods in direct contact with polystyrene. Evaluation studies have underlined possible health risks due to exposure of styrene that are inimical to humans health and well-being (Moradi et al., 2016). Styrene was re-classified to group 2B (possibly carcinogenic to humans) substance which formerly in group 3 (non-carcinogenic) by International Agency for Research on Cancer (IARC) based on register-based researches, together with new animal evidence (IARC, 2019). Due to proven risk related to styrene, European Commission has specified in Commission Regulation (EU) 2019/1338 (European Commission, 2019) that the migrated styrene monomer from polystyrene may not exceed the overall migration limit expressed as 10 mg of total constituent released per dm^2 of food contact surface (mg/dm^2).

1.2 Problem Statement

Surging trend of eating out and robust demand for packaged food by urban population in Southeast Asia contributes to prevalent usage of polystyrene. Due to its low cost, many local foods service establishment use GPPS as takeaway container and disposable food contact material. Meanwhile, HIPS is prominently used as packaging for dairy products such as yoghurt due to its enhanced

physical properties. Regardless of its popularity, GPPS and HIPS are vulnerable to degradation induce by prolong exposure to temperature, time and fat content of food in which might causing synergistic effect to food (Christian Block et al., 2017; Food and Environment Hygiene Department, 2008; Robertson, 2012). In Southeast Asia, cuisines are commonly prepared by high-heat cooking methods such as boiling, frying, and steaming (Jiamyangyuen & Ooraikul, 2007). Concomitantly, high fat ingredients such as oil and coconut milk are also pivotal in most dishes as it augment consistency and taste (Raji et al. 2017). Problems surfaced when GPPS food containers commonly used to serve these foods especially at a high contact temperature (Choi et al., 2005). Subsequently, as yoghurt pots are widely made from HIPS, migration from HIPS pot into yoghurt is anticipated to happen. Although temperature is not a concerning factor for styrene migration process into yoghurt, influence of storage time is inherent and noteworthy to be investigated. Additionally, increased in storage time has been proven to elevate rate of styrene migration into yoghurt before it reaches an equilibrium state due to partitioning effect (Antonella et al., 2010; Muncke, 2016). Migration from packaging materials into food are a predictable process and a better migration profile can be interpreted by means of migration model (Begley et al., 2005; Yining & Rubino, 2015). Nevertheless, many researchers applied data from styrene migration into various food simulants as an input for the migration models. These data not necessarily portray the actual styrene migration profile into foods and prone to give poor estimation due to complexity of food matrices. Therefore, the effects of the aforementioned styrene migration factors and modelling approached based on polystyrene-food migration data will be investigated in this study.

1.3 Significant of Study

In this study, the effect of fat contents, temperature and contact time on styrene migration from GPPS into selected Malaysian cuisine can be observed. Besides that, kinetic data of styrene migration from HIPS pot into yoghurt will provide clarity in migration process and testified the existing migration model. Outcomes from this study could contribute to better public health by improving the knowledge and practice regarding the usage of polystyrene FCMs. In addition, the findings also could provide an insight for industry player and policy maker to mitigate the risk of styrene migration into food.

1.4 Scope of Study

The scope of research covers migration testing of GPPS and HIPS at predetermined temperature-time combinations and applicability of migration model to describe the migration process. Styrene monomer was the analyte of interest and its migration from polystyrene FCMs was evaluated. Migration test of polystyrene FCMs were conducted by exposing the materials to foods and food simulants at temperature-time combinations which representing the actual practice. In the case of GPPS migration test, selected Southeast Asian cuisines were chosen and prepared at varying degree of fat contents to emphasize the

effect. Meanwhile, for HIPS migration test, three different brands of plain stirred yoghurt packed in HIPS pot was chosen and kept in the chiller until the products expired. Yoghurt samples were periodically taken for analysis throughout storage. HS-SPME method was employed to extract styrene and the detection and quantification of styrene was performed by using GCMS. Subsequently, migration model based on Fick's law was used to fit data obtained from styrene monomer migration into yoghurt. Migration profiles of each yoghurt were interpreted by the means of diffusion coefficient and partition coefficient values obtained from the model.

1.5 Limitation of the Study

In this study, executing an experimental design with a triplicate replication have proven difficult in practice, due to large number of samples and complexity in preparation of samples. A total of four food simulants, 12 formulations of food samples (four formulations for each food type), and 54 pots of yoghurt were utilised for the migration test of both polystyrene FCMs. Preparation of food samples were done in-house in which the processes were tedious and time consuming. All the ingredients need to be meticulously weighed and need to be procured from the same batch in order to minimize variability in experimental results. Subsequently, GCMS used in this study was leased for the analysis and the usage was confined by the leasing agreement. Cost of procuring the ingredients, samples, and leasing charge were substantial in the financial budget of this research. Therefore, the author had to resort to duplicate replication to resolve the complications. Furthermore, the author confident that duplicate replication sufficed the significance of the studied experimental factors.

1.6 Objectives

Therefore, the objectives of this study were:

i. To determine the effects of fat content, temperature and contact time on migration of styrene monomer from GPPS and HIPS into selected foods.
ii. To examine the migration profile of migrated styrene monomer from HIPS pots into yoghurt at different storage periods by modelling approached based on Fick's law.

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BIODATA OF STUDENT

Naziruddin Bin Mat Ariffin was born in Alor Setar, Kedah on 7 December 1992. He received his primary education for six years at Sekolah Rendah Kebangsaan Haji Idris, Alor Setar. Afterward, he continued his secondary education at Sekolah Menengah Kebangsaan Jabi, Alor Setar from form 1 until form 5. Then, he continued his study in Kedah Matriculation College for a two years matriculation programme. In 2012, he enrolled in a Bachelor of Science (Food Study) at Universiti Putra Malaysia and graduated his Bachelor degree in 2016. After graduation, he worked as a research assistant before registering for Master degree in February 2017.

LIST OF PUBLICATIONS

Manuscript

Naziruddin, M. A., Sulaiman, R., Abdul Halim Lim, S., Jinap, S., Nurulhuda, K., & Sanny, M. (2020). The effect of fat contents and conditions of contact in actual use on styrene monomer migrated from general-purpose polystyrene into selected fatty dishes and beverage. *Food Packaging and Shelf Life, 23*, 100461. (Accepted)

Naziruddin, M. A., Sulaiman, R., Jinap, S., Nurulhuda, K., & Sanny, M. Migration study of residual styrene monomer migration from high impact polystyrene pots into yoghurt at different storage. (In preparation)

Oral presentation

Naziruddin, M. A. The effect of fat contents and conditions of contact in actual use on styrene monomer migrated from general-purpose polystyrene into selected fatty dishes and beverage. International Postgraduate Seminar. FOSREC Mobility Programme. Bogor, Indonesia. 29th October-11th November 2018

Naziruddin, M. A. The effect of fat contents and conditions of contact in actual use on styrene monomer migrated from general-purpose polystyrene into selected fatty dishes and beverage. Postgraduate Oral Presentation. MIFT 11th National Food Science & Technology Competition. Universiti Tunku Abdul Rahman, Kampar, Perak. 6th-7th April 2019

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