



DEVELOPMENT OF MOLECULARLY IMPRINTED
POLYMER (MIP) BASED SENSOR ARRAY FOR
THE DETECTION OF MANGO VOLATILES

by

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LIST OF ABBREVIATIONS

ΔC	-	Capacitance shift
ΔE	-	Binding energy
ΔF	-	Frequency shift
AET	-	2-aminoethanethiol hydrochloride
AIBN	-	Azobisisobutyronile
AM1	-	Austin method 1
COOH group	-	Carboxyl group
E-nose	-	Electronic nose
EGDMA	-	Ethylene glycol dimethacrylate acid
FTIR	-	Fourier transform infrared spectroscopy
GC	-	Gas chromatography
GCMS	-	Gas chromatography mass spectrophotometer
H-bond	-	Hydrogen bond
IDE	-	Interdigitated electrode
L-MIP	-	Molecularly Imprinted Polymer for limonene
LO-MIP	-	Molecularly Imprinted Polymer for limonene oxide
MAA	-	Methacrylic acid
MeCN	-	Acetonitrile

MIP	-	Molecularly imprinted polymer
MIP-IDE	-	Molecularly imprinted polymer for interdigitated electrode
MIP-QCM	-	Molecularly imprinted polymer for quartz crystal microbalance
MRI	-	Magnetic resonance imaging
MS	-	Mass spectrophotometer
N ₂	-	Nitrogen gas
NIP	-	Non-imprinted polymer
NIR	-	Near infrared
O-H group	-	Hydroxyl group
P-MIP	-	Molecularly Imprinted Polymer for pinene
PET	-	Polyethylene terephthalate
PM3	-	Parameterization method 3
QCM	-	Quartz crystal microbalance
RT	-	Retention time
SAM	-	Self assembly monolayer
SPME	-	Solid phase micro extraction
TA	-	Titration acidity
TSS	-	Total soluble solid
UV	-	Ultra violet

PEMBANGUNAN BERASASKAN TATA SUSUNAN SENSOR MOLEKUL BERCETAK POLIMER (MIP) UNTUK MENGESAN ZAT MERUAP MANGGA

ABSTRAK

Tesis ini membincangkan tentang pembangunan berasaskan tata susunan sensor molekul bercetak polimer (MIP) untuk mengesan kehadiran zat meruap daripada mangga Harumanis. Kebiasaannya, fasa kematangan buah mangga dikesan melalui bau, tekstur, dan masa pemungutan buah. Namun, kaedah yang digunapakai ini bukanlah satu parameter yang saintifik untuk mengesan kematangan buah. Fasa matang dan masak buah dikesan melalui kehadiran zat meruap yang menjadi penanda dengan menggunakan alatan makmal seperti *solid phase micro-extraction (SPME)*, *crude extraction* dan *liquid-liquid extraction*. Peralatan makmal berskala besar ini tidak praktikal kerana tidak dapat digunakan diluar kawasan makmal. Kajian telah dilakukan dengan menggunakan kromatografi gas jisimspektrometer (GCMS) dan mendapati mangga Harumanis mengeluarkan beberapa zat meruap semasa fasa kematangan buah itu. Dengan menggunakan penanda kimia ini (zat meruap yang dikeluarkan oleh mangga), sensor MIP telah dihasilkan di atas platform elektrod interdigit (IDE) dan Kuarza Kristal Timbangan Mikro (QCM) untuk menguji prestasi kedua-dua jenis sensor itu. Di samping itu, reka bentuk model telah diimplementasi untuk menyerupai sifat MIP melalui permodelan molekul dan pengiraan termodinamik menggunakan perisian komputer *HyperChem 8.0*. Permodelan molekul dengan teknik semi-empirik AM1 (Austin Method 1) telah digunakan untuk mencari nisbah optimum kompleks templet molekul dan monomer berfungsi methacrylic acid (MAA). Berdasarkan ikatan tenaga (ΔE) yang diperolehi daripada permodelan, nisbah 5 untuk MAA terhadap templet molekul α -pinene dan γ -terpinene manakala nisbah 3 untuk MAA terhadap templet molekul terpinolene dipercayai mempunyai keupayaan mengikat yang baik semasa sintesis MIP. Tindak balas sensor QCM dan IDE didapati mempunyai pemilihan yang konsisten bergantung kepada platform yang digunakan iaitu kuarza atau PET (*polyethylene terephthalate*). Kajian masa sebenar telah dijalankan dan mendapati sensor MIP menghasilkan pola yang ketara apabila didedahkan dengan pelbagai fasa kematangan mangga Harumanis. Pola output sensor

telah menunjukkan bahawa sensor MIP memberikan tindak balas terhadap analit sasaran dan mampu mengelaskan mangga pada pelbagai fasa kematangan. Dalam kajian ini, sensor MIP telah dihasilkan dengan jayanya manakala tindak balas terhadap pemilihan telah dibuktikan secara eksperimen dan masa sebenar zat meruap yang dihasilkan oleh mangga.

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DEVELOPMENT OF MOLECULARLY IMPRINTED POLYMER (MIP) BASED SENSOR ARRAY FOR THE DETECTION OF MANGO VOLATILES

ABSTRACT

This thesis discussed the development of molecularly imprinted polymers (MIP) based sensor array for the detection of Harumanis mango volatiles. Conventionally, the detection of mangoes maturity are based on human smell, texture, and harvesting time. Unfortunately, these methods are not a quantifiable parameter to gauge the maturity. Maturity and ripeness were detected by the emission of volatiles as a marker using analytical equipment such as solid phase micro-extraction (SPME), crude extraction and liquid-liquid extraction. The analytical equipment is not practical because it cannot be operated outside the laboratory area. From gas chromatography mass spectrophotometer (GCMS) studies, Harumanis mangoes were found to emit certain volatiles during each different stage of maturity for example monoterpenes hydrocarbon which are known as marker component for mango maturity. Utilizing this chemical marker form GCMS data, the MIP sensor was developed on Interdigitated Electrode (IDE) and Quartz Crystal Microbalance (QCM) platforms where the sensors performances were tested. Computational simulation was implemented to simulate the MIP properties through molecular modeling and thermodynamic calculations using *HyperChem 8.0* software. The molecular modeling with the use of semi-empirical method of AM1 (Austin Method 1) was used to find the optimum ratio of complex template and functional monomer methacrylic acid (MAA). Based on the binding energy (ΔE) obtained from the modelling, ratio 5 of MAA over template α -pinene and γ -terpinene and ratio 3 of MAA over terpinolene have good binding capabilities during polymeric synthesis. The sensors responses on QCM and IDE were found to have consistent selectivity regardless of the platform used which is quartz crystal or PET (Polyethylene terephthlate). The MIP sensor was also exposed real time to Harumanis mango where the response pattern indicated that the sensor responded towards its target analyte and able to clarify fruits at various maturity phases. In this research, MIP sensor was successfully developed and the selectivity response was verified with experimental and real time monitoring of volatile released by the mango.

CHAPTER 1

INTRODUCTION

1.1 Background

Mango or known as *Mangifera Indica* L., originated in Southeast Asia is one of the oldest of cultivated fruits. It is also labeled as “apple of the tropics” and one of the most popular tropical fruits worldwide. Mango fruits are usually eaten ripe when the pulp is soft and tastes sweet. Some people prefer to eat mango fruits unripe when the pulp is harder with a more sour taste. All these criteria make the demand for fresh mango in U.S.A continues to grow. According to USA National Mango Board’s 2011 reported on mango attitude and usage survey, the incidence of eating mango increased since 2007 from 67% to 78%, with out-of-home consumption and liking mango flavor at high levels. Mango fruits are rich in nutritional contents such as carbohydrates, amino acids, fatty acids, organic acid, protein, minerals and vitamins. Mangoes are initially acidic, astringent and rich in ascorbic acid (vitamin C) during ripening period. In ripe mangoes, the levels of vitamin C are moderate, but fairly rich in provitamin A and vitamin B₁ and B₂ (Litz and Mukherjee, 2009).

It is important for every harvesting fruit to have an effective method of determining the maturity of mango. This is because the maturity level at harvest is essential to determine good flavor quality in the fruit when fully ripe. Normally, mangoes are harvested based on the change of skin color. Unfortunately, the appearance of red color on the skin is not a reliable index of maturity. Therefore, it is important to know the stage of maturity in order to determine the harvest time of the fruits since fruits which are harvested at an immature stage will not satisfy the consumers (Slaughter, 2009).

The measurement of mango's maturity level is a crucial part in determining its optimum harvest time. The maturity markers for mango include changes in biochemical, volatiles (aroma) and physical characteristic. There are specific instrument and equipment used to measure the maturity markers depending on their purpose. For example, volatiles are identified using GC (Gas Chromatography) by Lalel et. al, (2003), GCMS (Gas Chromatography Mass Spectrophotometer) (Lebrun et. al, 2008), SPME (Solid Phase Micro Extraction) (Lalel et. al, 2003), e-nose (Electronic nose) performed by Lebrun et. al, (2008) and Li et. al, (2009), solvent extraction and crude extraction (Lai et. al, 2005). For biochemical change, acidity is measured by using a pH meter and titrable acidity, while total soluble sugar (TSS) is measured by a refractometer (Lebrun et, al, 2008). The physical characteristic changes are usually measured using an optical instrument such as Infrared and Magnetic Resonance Imaging (MRI) by Slaughter (2009) and Near Infrared (NIR) by (Guthrie and Walsh, 1997; Schmilovitch 2000; Mahayothee 2004; Saranwong 2004& 2005; Sivakumar 2006 and Subedi et. al, 2007).

In this research, the mango volatiles were chosen as chemical markers. MIPs are then designed and used to adsorb these volatiles (Kikuchi et. al, 2006 & Iqbal et. al, 2010), hence acting as sensing elements. They are used to determine the maturity levels of the mango. The technique of MIP was built upon a simple concept of molding a recognition “lock” around a molecular “key”. This “lock and key” concept attributes allow MIPs to be highly selective. Generally, MIP consists of template or known as imprinted molecule, functional monomer and cross-linker. These molecules were pre-arranged by self-assembly monolayer and polymerized under thermal or UV conditions to form a rigid polymer (Sontimuang et al, 2011). The resultant polymer undergoes an extraction process to remove the template molecule, leaving the cavity that is selective only for their template.

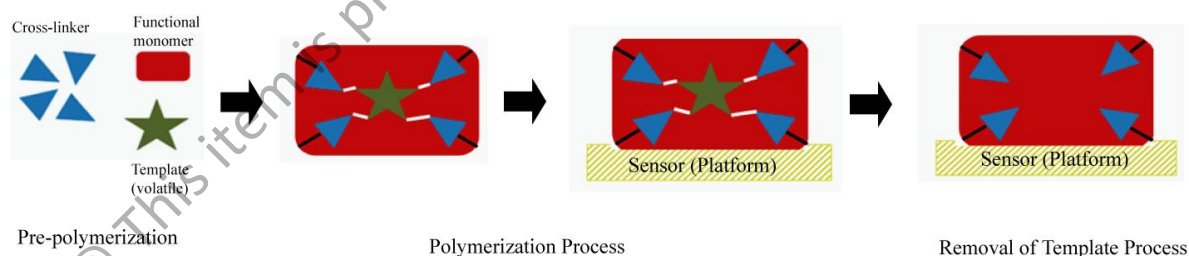


Figure 1.1: Simplified diagram illustrating the MIP process

A molecular design approach was used by understanding the chemicals interaction between sensing materials and other materials to obtain the optimum condition to synthesize the MIP. The usefulness of computational aids in the development of MIP has emerged for several years and has been widely used until now. In this project, computational approach was introduced and implemented in order to design the MIP. This is an advantage since it could eliminate time-consuming experiments (Piletsky et. al, 2002).

There are various synthesizing procedures for MIP depending on their final products and applications. Two sensor platforms were used to test the MIP-based sensing concept, namely QCM and IDE on a PET (polyethylene terephthalate) substrate. The quartz crystal microbalance (QCM) is widely used as sensor since it is highly sensitive and able to provide mass resolutions in the range of 1ng (Dickert et. al, 1998). The earliest application of MIP-QCM sensor done by Maliesta (1997) was a glucose monitoring system. To date, no research on PET substrate found especially for MIP purpose. Therefore, in this research, the MIPs were synthesized on two different sensor platforms; quartz crystal and PET substrate.

1.2 Problem Statements

1.2.1 Subjective conventional method of determining mango fruit ripeness

Traditionally, the determination of fruits ripeness was performed through the conventional harvesting method. The conventional method used are based on human smell, texture and harvesting time (Christensen, 1983 and Povey, 1989). These methods have many drawbacks in term of subjectivity and objectivity. In some cases, immature harvesting are done for export markets, but the subsequently ripen has resulted of poor quality due to fruit disorders. Maturity stage during harvesting mango is an important parameter that determines the quality of mango. Maturity and ripeness were measured by the change in biochemical compound, aroma (volatiles) and physical characteristic. Most biochemical and physical experiments on mango required a destructive test. This research focused on volatiles organic compounds as the chemical markers for maturity and ripeness of mango.

1.2.2 Expensive analytical equipment used to determine volatiles release by mango

Volatiles released by mango were determined using analytical equipment. Around 1958, molecule terpenes were explored by using a technique such as low-temperature chromatography by Clements (1958). Other analytical equipments used to extract volatiles are SPME, GC, GCMS, crude extraction, liquid-liquid extractions and etc. Most of these volatile extraction and identification methods involve large quantity of chemical and also expensive equipments. In addition, only trained personnel are

authorized to handle this type of equipment. In fact, it is not practical as it cannot be operated outside the laboratory area.

1.2.3 Selectivity of sensor

Recently, the artificial olfaction device such as electronic nose (E-nose) was used to characterize the volatile profiles of ripening fruits (Molto et. al 1999; Brezmes et. al, 2000; Natale et. al, 2001 and Du et. al, 2010). Li et. al, (2009) developed volatile compounds detection system using an e-nose which is zNoseTM. 18 metal oxide sensors (MOS) based E-nose (FOX 4000, France) along with GC for the classification maturity of “*Cogshall*’ mango was conducted during this research (Li et. al, 2009). Compared with olfactory receptors from invertebrate animals such as fruit flies, the sensors used in E-noses are less independent and are more narrowly tuned to certain VOCs (Berna et. al, 2009). Even though pattern recognition and correction algorithms can be applied, major limitation of currently available MOS sensors remains towards their independence and selectivity.

1.3 Research Objectives

The research objectives of this study are as follows:

- a) To identify the volatiles release by Harumanis mangoes as a chemical markers from mature to ripe phase.
- b) To select an optimum ratio of functional monomer and template by optimizing complex molecules using *HyperChem* software.
- c) To synthesize MIP based on the resultant mole ratio of functional monomer to the template from (b) on two different sensor platforms (IDE and QCM)
- d) To evaluate the response of MIP sensor on QCM and IDE when the array is exposed to the target analytes and Harumanis mangoes.

1.4 Research Scopes

- 1) Identify the major volatiles emanated from mature and ripe mango.

This area is focused on the volatiles released by Harumanis mango starting from Week 7 to Week 10 using GCMS. The data obtained from GCMS were compared with previous related work by other researchers. The resultant volatiles were further synthesized as templates for the MIP.

- 2) Applying computational design to find the optimum condition during the pre-arrangement stage of MIP.

HyperChem 8.0 is used to calculate the binding energy between template and functional monomer; methacrylic acid (MAA). Three mango volatiles obtained from GCMS result in (I) and general studies from certain mango (Lalel et. al, 2003 and Lebrun et.al, 2008) were used as templates. Binding energy value from optimization and calculation of energies determines the optimum mole ratio for functional monomers to be synthesized with the template.

- 3) Synthesize the MIP and NIP (control) on two different platforms:

MIP and NIP were synthesized on two different platforms which are thermoplastic polymer PET and quartz crystal.

- 4) Rebinding test to measure the sensor response.

The array of sensors was exposed to the target and non-target analyte to measure the response and the ability to distinguish between target and non-target analyte. Online monitoring test were performed where the array of sensors was exposed to the real sample (Harumanis mango).

The following assumptions are made during this research:

- 1) Stable room temperature, pressure and all other unmentioned parameters during testing.
- 2) Humidity (55%±)
- 3) MIP and NIP have the same thickness (spin coating at 2500rpm for 1 minute)

1.5 Thesis Outline

Chapter 2 presented the maturity indices for mango to determine their ripeness stage and a clear differentiation between the term “mature” and “ripeness”. All maturity indices that lead to the mango ripeness were clearly presented here. Among all the maturity indices, volatiles released by mango were chosen to be studied thoroughly due to the ability to be imprinted as a template for Molecular Imprinted Polymer (MIP). An insight view on MIPs nature, variety, applications, and previous work done by researchers is also described in this chapter.

Chapter 3 focused on the methodology applied during this research. The method consists of finding volatiles from Harumanis mango using GCMS and calculation of binding energy using molecular modelling software, HyperChem. Then, MIP is synthesized based on the resultant mole ratio from binding energy study. Quartz crystal and IDE electrode were used as the sensing platform of the MIP. Finally, the performances of the MIP sensors were then tested by two different ways. The selectivity and sensitivity of the MIP sensor were tested by allowing 3 different volatiles to pass through the MIP sensors. Online monitoring experiment was done on 50 pieces Harumanis mangoes from week 7 to week 10 to measure the sensor performance on real sample.

Chapter 4 was divided into 3 sections. The first section discussed on the ratio study to clarify the molecular modelling experiment in Chapter 3. An array of MIP sensors were exposed to γ -terpinene vapour to study the desorption properties of each ratio of complex MAA-template. The second section presented the sensor response towards the target analytes. Both sensors (IDE and quartz crystal) were exposed to certain volatile to measure its response and the ability to distinguish non-target analyte. The modification structure of MIP molecules was discussed in this chapter by presenting FTIR data during polymerization, removal template and rebinding process. The third section discussed the sensors response on real sample (Harumanis mango). The data was analyzed based on 50 mangoes for mature and ripe stage (week 8 and week 10)

Chapter 5 concluded the entire research and recommendations for future works related to this research.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of mango ripening stage

Mango is a climacteric and among the world's most popular fruits. Mitcham E, reported that there is an increase of mango consumption per capita in the US estimated at 2.2 pounds per year in 2008. Mango fruit contains carotenes, chlorophyll, xanthophylls and anthocyanins. During the ripening process, the chloroplasts in the mango peel (peel colour is cultivar dependent) become chromoplasts which contain red and yellow pigments (Krishnamurthy and Subramanyam, 1970; Akamine and Goo, 1973; Salunke and Desai, 1984; Mitra and Baldwin, 1997). The ripening color of mango fruits is different for each cultivar, for example *Carabao*, *Manila*, *Mulgoa* and *Arumanis (Harummanis)* are greenish–yellow during ripening while *Dashehari* and *Alphonso* are yellow. *Haden*, *Keitt* and *Tommy Atkins* show red blush color during ripening (Litz, 2009). The red blush color is due to the presence of anthocyanins (Lizada, 1991).

Reid (2002) describes the term 'mature' from the Webster's dictionary definition which is "*having completed natural growth and development*" and further elaborates the term mature which describes the stage at harvest that will ensure the fruit's quality exceed and meet the minimum level acceptable to the consumer at the time it is consumed. The term 'ripe' was described as "*having attained a final or desired state*" by Webster's