# Highly selective molecularly imprinted polymer (MIP) based sensor for fruit maturity determination

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My parents, my wife, kids and newborn baby.
This is for you.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AET | Aminoethanethiol |
| AFM | Atomic Force Microscopy |
| AIBN | Azobisisobutyronitrile |
| ANOVA | Analysis of variance |
| APTS | Trimethoxysilane |
| BAW | Bulk Acoustic Wave |
| CAR/PDMS | Carboxen/poly(dimethylsiloxane) |
| CDC | Capacitance Digital Converter |
| CP | Conducting Polymer |
| CPU | Central Processing Units |
| CW/DVB | Carbowax/divinylbenzene |
| DAQ | Data Acquisition Card |
| DVB/CAR/PDMS | Divinylbenzene/Carboxen/poly(dimethylsiloxane) |
| EDX | Energy-dispersive X-ray Spectroscopy |
| EEPROM | Electrically Erasable Programmable Read-Only Memory |
| EGDMA | Ethylene Glycol Dimethacrylate |
| E-nose | Electronic noses |
| FETs | Field Effect Transistors |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GUI | Graphical User Interface |
| HMI | Human Machine Interface |
| I/O | Input/Output |
| $I^2C$ | Inter Integrated Circuit |
| IDC | Interdigitated Capacitive |

| | |
|---|---|
| IDE | Interdigitated Electrode |
| LCD | Liquid Crystal Display |
| LSD | Least Significant Difference |
| m/z | Mass/Charge Ratio |
| MAAP | Methacryl-amidoantipyrine |
| MFC | Mass Flow Controllers |
| MIP | Molecularly Imprinted Polymer |
| MMA | Methacrylic Acid |
| MOS | Metal Oxide Semiconductor |
| MOSFET | Metal Oxide Semiconductor Field Effect Transistor |
| MS | Mass Spectrometer |
| NIP | Non-Imprinted Polymer |
| NIST | National Institute of Standards and Technology |
| PA | Polyacrylate |
| Pani | Polyaniline |
| PCA | Principal Component Analysis |
| PCs | Principal Components |
| PET | Polyethylene Terephthalate |
| PLC | Programmable Logic Controller |
| PPM | Part Per Million |
| PPy | Polypyrrole |
| PTFE | Polytetrafluoroethylene |
| PTh | Polythiophene |
| QCM | Quartz Crystal Microbalance |
| QMB | Quartz Microbalance |
| RAM | Read Access Memory |

| | |
|---|---|
| $R_o$ | Figaro 2620 Sensor resistance in 300ppm of ethanol |
| $R_s$ | Figaro 2620 Sensor resistance in displayed gases at various concentrations |
| RSD | Relative Standard Deviation |
| RT | Retention time |
| SAW | Surface Acoustic Wave |
| SCCM | Standard Cubic Centimeters per Minute |
| SCL | Serial Clock Line |
| SDA | Serial Data Line |
| SIFI | Selected Ion Full Ion |
| SIR | Single Ion Recording |
| SPI | Serial Peripheral Interface |
| SPME | Solid Phase Micro Extraction |
| SPR | Surface Plasmon Resonance |
| TEOS | Tetraethoxy-silane |
| THF | Tetrahydrofuran |
| TIC | Total Ion Chromatograph |
| TSM | Thickness-Shear Mode |
| TSS | Total Soluble Sugars |
| UART | Universal Asynchronous Receiver Transmitter |
| UV | Ultra Violet |
| VOC | Volatile Organic Compounds |
| $\Delta C$ | Capacitance Change |
| $\Delta$Hz. | Frequency Change |
| $\Delta E$ | Energy Difference |

**Sensor Molekul Bercetak Polimer (MIP) Yang Sangat Selektif Untuk Menentukan Kematangan Buah**


## ABSTRAK

Kematangan buah boleh ditentukan dengan menggunakan alat olfaktometri buatan seperti sistem hidung elektronik.Walaubagaimanapun, prestasi sistem hidung elektronik terhad disebabkan beberapa isu pada kebolehan gas sensor yang sedia ada seperti tahap selektif yang rendah dan memerlukan suhu operasi yang tinggi. Salah satu alternatif penyelesaian kepada kekurangan gas sensor yang digunakan oleh hidung elektronik ialah dengan menggunakan sensor molekul bercetak polimer. Tesis ini akan membincangkan tentang pembangunan sensor polimer molekul bercetak (MIP) yang sangat selektif dan mampu untuk mengikat dengan ruapan mangga. Penentuan kematangan buah-buahan sentiasa menjadi aspek yang sangat penting dalam bidang pertanian terutamanya dalam penggredan kualiti buah-buahan.Melalui kajian kromatografi gas jisim spektrofotometer (GCMS), zat ruapan mangga (α-pinene, terpinolene dan γ-terpinene) telah berjaya dikenal pasti sebagai penanda kematangan. Kematangan buah mangga yang berbeza juga telah didapati akan mengeluarkan corak ruapan yang tertentu. Dari perisian pemodelan Hyperchem, nisbah optimum MIP (templat: MAA:EDGMA) untuk α-Pinene dan γ-terpinene didapati adalah 1: 5: 20, manakala bagi terpinolene, nisbah optimumnya ialah 1: 3: 20. Dengan menggunakan maklumat penanda kimia ini, sensor MIP akan dibangunkan untuk setiap ruapan mangga yang dipilih dan diintegrasikan bersama elektrod interdigit (IDE) dan Kuarza Kristal Timbangan (QCM) sebagai transduser. Sensor MIP adalah sangat selektif dalam membezakan zat meruap termasuk isomer.Tambahan pula, sensor MIP juga adalah sangat sensitif untuk mengesan penanda kimia kepekatan dengan serendah 1.7 ppm. Berbanding dengan sensor oksida logam semikonduktor (MOS), sensor MIP juga telah menunjukan respons/pemulihan dan kebolehulangan sensor yang sangat baik daripada sensor MOS. Sensor MIP juga telah didedahkan dengan pelbagai sampel buah mangga serta buah mangga yang berbeza peringkat kematangan dan telah berjaya bertindak balas terhadap ruapan sasarannya. Sistem MIP sensor juga kemudian telah dibangunkan bagi memantau secara nyata pelepasan ruapan mangga dan telah juga berjaya menentukan kematangan buah mangga Harumanis dengan keputusan yang baik. Dalam kajian ini, pendekatan menggunakan MIP sebagai satu elemen pengesanan samada sebagai sensor atau sistem deria telah berjaya dibangunkan. Oleh kerana sistem MIP ini adalah sangat selektif, ianya boleh dibangunkan dengan kos yang minimum dengan harapan menjadi suatu kaedah alternatif dalam menentukan kematangan buah tanpa memusnahkan sampel buah-buahan yang diuji. Bagi petani, ini mampu meningkatkan pemahaman mereka tentang kematangan buah dan seterusnya akan menyumbang kepada peningkatan pengeluaran kualiti buah.

**HIGHLY SELECTIVE MOLECULARLY IMPRINTED POLYMER (MIP) BASED SENSOR FOR FRUIT MATURITY DETERMINATION**

## ABSTRACT

Fruit maturity can be determined by using artificial olfactory equipment such as electronic nose system. However, the electronic nose system performance is limited due to several issues on existing gas sensor capability such as low selectivity and high temperature operation. An alternative is to use molecularly imprinted polymers (MIP) based sensors. This thesis discussed about the development of MIP sensors that are highly selective and able to bind with mango volatiles. Detection fruit maturity level has always been a very important aspect of final fruit quality grading in agriculture. From gas chromatography mass spectrophotometer (GCMS) studies, mango volatiles (α-pinene, terpinolene and γ-terpinene) were identified as maturity marker. It was found mangoes different maturity level will emit specific maturity marker pattern. By using Hyperchem Modelling, MIP optimum ratio (template: MAA:EDGMA) for α-pinene and γ-terpinene were found to be at 1:5:20 , while for terpinolene, the MIP optimum ratio was 1:3:20. Utilizing this information, the MIP sensor was first developed per the selected maturity marker. It was then integrated with Interdigitated Electrode (IDE) and Quartz Crystal Microbalance (QCM) as transducer. MIP sensor was highly selective in discriminating any non-target volatiles including isomers. Furthermore, the MIP sensor was highly sensitive to detect chemical marker as low as 1.7 ppm concentration. When compared to a metal oxide semiconductor (MOS) sensor, it was observed that the MIP sensor also offers excellent sensor response/recovery and repeatability than the MOS sensor. The MIP sensor was also exposed real time to mango with different maturity stages where the sensor response pattern responded towards its target analyte at various maturity phases. A multi sensor based MIP system was also developed to provide real-time monitoring of mango volatiles emission and has successfully determined Harumanis mango maturity with good scores. Since the MIP olfactory system is highly selective, low power and can be developed with minimum cost, this can be an alternative to provide an effective and non-destructive method for fruit maturity determination. For farmers, improving their understanding about the fruit maturity would certainly contribute to an increase of fruit production and quality.

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

One of the most important technological aspects in the agriculture industry is determining fruit maturity and its time to harvest. This is important because the fruits that are harvested has important effects on the consumer satisfaction level. Having the situation of not knowing when the fruit has reached their preferred state of ripeness can be frustrating for consumers. At the end, this can create some barrier for consumers to purchase. The fruits harvested either in over ripe or unripe, would have a major effect on the shelf life. If the fruit is harvested over ripe, then it would likely to become soft quickly, wrinkled and shorter shelf life. On the other hand, if the harvest was going through an immature process, the fruits will tend to be in an unfavorable taste and lack of flavor. Moreover, valuable nutritions such as vitamins will tend to degrade and hence downgrading the overall fruits quality. For premium fruits that are intended for international market, this could be financially damageable as lower consumer satisfaction would eventually led to lower sales and profit.

The term mature describes the stage at harvest that will ensure that the fruit's quality will meet or exceed the minimum level which is acceptable to the consumer at the time it is consumed (Reid, 2002). During harvest, identifying the maturity level is critical to the development of good flavor quality in the fruit when fully ripe (Kader, 2008). Thus, it is important to have effective methods of determining fruit maturity. For most fruits, this could be challenging as the inner and outer properties of the fruit would still continue to change even after harvesting.

Mango (*Mangifera indica* L.) is one of the most popular fruit of the tropics. It is reported that there have been an increase of worldwide mango import demand of 97,623 metric tons in 1996 to 826,584 metric tons in 2005 (Evans, 2008). Growing worldwide interest in mango has also been due to the increased knowledge of the health benefits derived from mango consumption. Apart from having high levels of vitamin C and fibers, mangoes are known to have certain antioxidants, which are believed to be helpful against colon, breast, leukemia and prostate cancers. In Malaysia, the mango industry has grown remarkably over the past years (Figure 1.1).



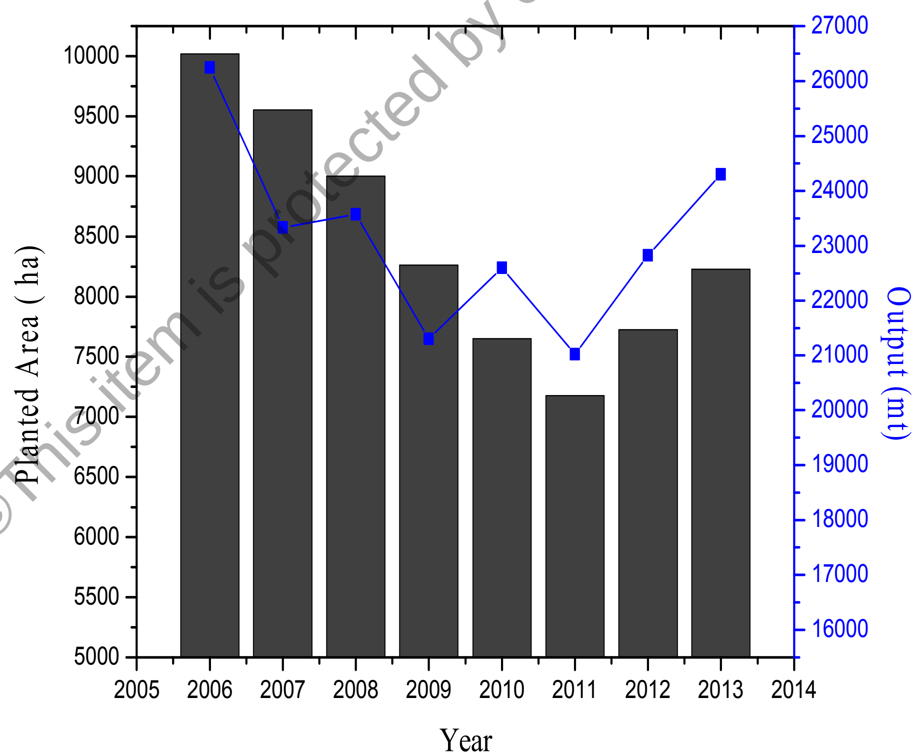


Figure 1.1 Production of Mango in Malaysia from 2006 to 2013
(National Mango Convention, 2013)

In Perlis for example, it played an important role in revitalizing the rural economy. In the third National Agricultural policy (NAP3), mango was identified as among 15 types of fruit to be developed as an export crop. Harumanis deemed as the 'king of mangoes' and also known as seasonal fruit that can only be available from April to May. Since the production is only available for short period of time, this has transformed Harumanis as a rare delicacy with premium price. The price of Harumanis can go up to RM35 per kilo, but this has not stopped people from buying boxes of this succulent, tropical fruit. According to Harian Metro news on 5 May 2015, 3,200 metric tons of Harumanis are expected to be harvested in 2015, in comparison to just 500 metric tons only in 2012.

Harvesting mango has always been a delicate task as this type of fruit will continue to ripe even after harvested. It is important to harvest the crop in the right maturity stage and make it available to the market in the best possible condition. As such, there is a need to find an effective, objective and non-destructive method for the evaluation and prediction of the quality attributes of mangoes during maturation. This will certainly help the farmers to improve their understanding about the fruit maturity as well as to increase the fruit production.



## 1.2 Maturity Determination

Improving the selection of fruit maturity has always been a subject of interest for farmers, vendors and even consumers. As fruit production increased tremendously in the past decades, there has always been continuous demand to improve the conventional method. This is important as the conventional method is usually destructive and labor intensive. Furthermore, everyone may have different perception about the ideal ripeness of