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EXTRACTION AND IDENTIFICATION OF AROMA COMPOUNDS IN THE PEEL AND THE PULP OF THE MANGOES AT DIFFERENT STORAGE CONDITIONS

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SCHOOL OF FOOD SCIENCE AND NUTRITION
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DECLARATION

I hereby declare that the material in this thesis is my own except for the quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

In this study, extraction of volatile compounds from the peel and pulp of three species of fully ripe mangoes (*Mangifera odorata*, *Mangifera laurina* and *Mangifera rubropetalata*) was undertaken through headspace-solid phase microextraction technique using SPME device with 65 µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber. Identification of volatile compounds was achieved in a system of high resolution gas chromatograph coupled with mass spectrometer. A total of 110 volatile compounds were identified in the peel and pulp of all the three species of mango. The peel contained more volatile compounds compared to the pulp and therefore a significant difference exists between them. Among the three species studied, mangga Wani (*M. odorata*) had the highest number of volatile compounds followed by mangga Ayer (*M. laurina*) and Mempelam (*M. rubropetalata*) respectively. The major compounds identified in all the three species were hexenal, 2-hexenal, 3-hexenal, 3-carene, (+)-4-carene, biocyclo [4.1.0] hept-2-ene, 3,7,-trimethyl-, cyclohexene 1-methyl-4-(1-methylidene), pentanoic acid, D-limonene, 1,3,6-octatriene, caryophyllene and 1,4,7-cycloundecatriene. The effect of storage conditions on the volatile compounds was also investigated in this study. Two third of the mangoes from each species were stored at 10°C and -20°C for one week whereas rest of the samples were extracted at the fresh state. The peel and the pulp of the mangoes were separated and introduced into polyethylene bags prior storage. Fresh mangoes had the highest number of volatile compounds followed by frozen mango at -20°C and finally chilled mango at 10°C. Freezing increased slightly the composition of major compounds as listed above in all the three species and the volatiles obtained for mango stored in this manner provided very close agreement with those for the ideal, fully ripe mango. Chilling has resulted in considerable loss of volatile compounds and the effect was lesser in the peel compared to pulp of mangoes.

ABSTRAK

PENGEKSTRAKAN DAN PENGENALPASTIAN SEBATIAN BERAROMA DI DALAM KULIT DAN ISI BUAH MANGGA PADA KAEDAH PENSTORAN YANG BERBEZA

Di dalam kajian ini, pengekstrakan sebatian meruap daripada kulit dan isi tiga spesies buah mangga yang telah masak (Mangifera odorata, Mangifera laurina dan Mangifera rubropetalata) telah dijalankan dengan menggunakan kaedah mikropengekstrakan fasa pepejal. Kaedah ini menggunakan fiber Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) yang berukuran 65 µm. Identiti sebatian meruap diperolehi melalui gas kromatograpi yang dipasang dengan spektrometer jisim. Sebanyak 110 sebatian meruap telah dikenalpasti di dalam kulit dan isi ketiga-tiga spesies buah mangga. Kulit buah mangga mengandungi lebih banyak sebatian meruap berbanding isinya dan oleh sebab itu, satu perbezaan yang ketara diperolehi di antara kedua-dua bahagian buah itu. Di antara tiga spesies buah mangga yang dikaji, mangga wani (M. odorata) mengandungi bilangan sebatian meruap yang tertinggi dikuti dengan mangga ayer (M. laurina) dan mempelam (M. rubropetalata). Sebatian utama yang dikenalpasti di dalam ketiga-tiga spesies buah mangga adalah hexenal, 2-hexenal, 3-hexenal, 3-carene, (+)-4-carene, biocyclo [4.1.0] hept-2-ene, 3,7,-trimethyl-, cyclohexene 1-methyl-4-(1-methylidene), asid pentanoik, D-limonene, 1,3,6-octatriene, caryophyllene dan 1,4,7-cycloundecatriene. Kesan kaedah penstoran ke atas sebatian meruap juga disiasat di dalam kajian ini. Dua pertiga buah mangga daripada setiap spesies disimpan pada 10°C dan -20°C selama seminggu sementara sampel yang lain diekstrak pada keadaan segar. Kulit dan isi buah mangga diasingkan dan dimasukkan ke dalam beg polietilena sebelum distor. Buah mangga yang segar mengandungi bilangan sebatian meruap yang tertinggi dikuti dengan mangga yang disejukbeku pada -20°C dan mangga yang disejukkan pada 10°C. Penyejukbekuan meningkatkan sedikit komposisi sebatian utama yang disenaraikan di atas di dalam ketiga-tiga spesies dan sebatian yang diperolehi melalui buah yang distor dengan kaedah ini adalah hampir sama dengan buah mangga yang telah masak. Penyejukan pula telah mengurangkan bilangan sebatian meruap dan kesannya adalah kurang pada kulit buah mangga berbanding dengan isinya.

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LIST OF ABBREVIATIONS

min	minutes
CoA	Coenzyme A
LOX	Lipooxygenase
GC-MS	Gas chromatograph-mass spectrometer
SPME	Solid phase microextraction
PDMS	Polydimethylsiloxane
HS-SPME	Headspace-solid phase microextraction
DVB	Divinylbenzene
ppb	parts per billion
FSI	Fruit softening index

LIST OF SYMBOLS AND UNITS

g	gram
ml	mililiter
mg	miligram
cm	centimeter
m	meter
kJ	kiloJoule
µm	micrometer
mm	milimeter
°C	Degree Celcius
%	Percentage
°Brix	Degree Brix

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Aroma compounds contribute heavily to the overall sensory quality of fruits and vegetables (Barberan & Robins, 1997). Aroma is also a property of food which strongly influences the acceptance by consumers (Morton & MacLeod, 1990). It is a complex mixture of a large number of volatile compounds whose composition is specific to species and often to variety (Barberan & Robins, 1997). Aroma compounds are present in raw foods in free volatile form and also as non-volatile precursors such as substituted cystein sulfoxides, thioglycosides, glycosides, carotenoids and cinnamic acid derivatives (Solís-Solís *et al.*, 2007).

The volatile compounds that are involved in the fruit flavor are produced through metabolic pathways during ripening, harvest, post-harvest and storage, and depend on many factors related to species, variety and type of technological treatments (Torres *et al.*, 2007). There are considerable differences in volatile compounds which occur between varieties of mangoes grown in the same country to the same stage of ripeness (Andrade *et al.*, 2000).There are three types of biochemical pathways that involve the metabolism of three main classes of compounds in relation to aroma biogeneration. The three classes of compounds are fatty acids, amino acids and carbohydrate (Barberan & Robins, 1997).

Volatile compounds formed from lipids include several pathways such as α- and β-oxidation and oxidation via lipoxygenase enzymes. Amino acid metabolism generates aromatic, aliphatic and branched chain alcohols, acids, carbonyls and esters that are important to the flavor of fruit (Reineccius, 2006). There are only a few classes of aroma components in fruits and vegetables that originate directly from carbohydrate metabolism. The aroma compounds in fruits can be summarized into five groups namely hydrocarbons, esters, aldehydes and ketones, alcohols and acids and miscellaneous compounds (Morton & MacLeod, 1990). The volatile compounds of mangoes are also classified according to the groups stated above.

The mango, *Mangifera indica* L, is the most popular and the choicest fruit of the tropics (Nagy & Shaw, 1980). It is one of the most widely sold tropical fruits in Europe and its commercialization, as fresh cut fruit, is gaining importance in the market (Torres *et al.*, 2007). Mango is an evergreen tree that originated in the Indo-Malaysian region. It has been cultivated in India for over 4000 years (Morton & MacLeod, 1990). The mango spread throughout South-East Asia about 1500 years ago and to the east coast of Africa about 1000 years ago.

Mango is cultivated for the fruit which can be eaten in three distinct ways, depending largely on the cultivar: unripe (mature green, very popular in Thailand and the Philippines), ripe (common way to enjoy mango throughout the world) and processed (at various stages of maturity) (Verheij & Coronel, 1992). There are 17 species of *Mangifera* that can be found in Sabah itself (Hasnah & Mamot, 2004). The storage life of mango depends on the stage of maturity at which the fruit is harvested. Mango fruits are generally harvested at physiologically matured stage to get optimum fruit quality. Immature fruits display erratic ripening behavior and may not develop full flavor and aroma. Measuring maturity is of paramount importance to harvest fruit to have good post-harvest quality (Jha *et al.*, 2004).

Solid-phase microextraction (SPME) is rapidly emerging as a robust technique for the rapid, solventless extraction or preconcentration of volatile and semivolatile organic compounds in a variety of scientific disciplines (Pomeranz & Meloan, 2000). Pawliszyn's group was the first to develop the SPME method, and they applied it in environmental analysis. Since then, it has become a widely used technique for the analysis of volatiles in foods (Reineccius, 2006). The SPME technique has the following advantages: low cost, short time of extraction, simplicity, high selectivity and sensibility. The SPME consists of a fused-silica fiber, coated with polymeric stationary phase introduced into a liquid or gas sample.

The method involves two processes: the partitioning of the analytes between the coating and the sample and the thermal desorption of the analytes into gas chromatograph (Solís-Solís *et al.*, 2007). In gas chromatograph, the mobile phase is a gas that flows through the stationary phase, which could be a solid or a liquid coated onto a solid matrix. The separation of compounds is determined by the relative rate of reversible absorption or volatilization of the solutes into and out of the stationary phase (Fisher & Scott, 1997). Since the SPME technique requires no solvents and can be performed without heating the sample, the formation of chemical artifacts is greatly reduced, if not completely eliminated (Pomeranz & Meloan, 2000).

1.2 Objectives of the study

The objectives of the study are:

1) To determine and compare the difference in the presence of volatile compounds between the peel and the pulp of the fresh mangoes by using headspace-solid phase microextraction.

2) To investigate the effect of storage conditions (10°C and -20°C) on the volatile compounds in the mangoes.

3) To recommend the storage condition for the peel and pulp of the mangoes that gives the greatest retention of volatile compounds.

1.3 Scope of the study

This study will focus on the isolation of aroma compounds in three species of mangoes (*Mangifera odorata*, *Mangifera laurina* and *Mangifera rubropetalata*) by headspace-solid phase microextraction technique. The effect of storage conditions especially regarding volatile components will be investigated in two storage methods. The presence of volatile compounds in the peel and the pulp of the mangoes will also be determined by using GC-MS.

1.4 Benefits of the study

1) Give a clear distinction of the volatile compound content among three species of mango.

2) To make recommendations regarding storage conditions that gives the greatest retention of volatile compounds.

3) To study the losses/retention of volatile compounds in mango after storage.

4) To do comparison to know which part of the fruit (peel/pulp) has higher volatile compound.

CHAPTER 2

LITERATURE REVIEW

2.1 Aroma compounds

The aroma is one of the most significant and decisive parameters of quality in the election of a product. Aroma compounds are present in raw foods in free volatile form and also as non-volatile precursors such as substituted cystein sulfoxides, thioglycosides, glycosides, carotenoids and cinnamic acid derivatives (Solís-Solís *et al.*, 2007). The sugar moieties of glycosidically-bound aroma volatiles, which have been reported in the mango are α- terpenyl-β-D-glucopyranosides, α-terpenyl-6-o-rutinosides and α-terpenyl-6-o-(α-L-arabinofuranosyl)-β-D-glucopyranosides. Aroma compounds (aglycones) can be released from glycosidically-bound compounds by enzymatic or chemical reactions during maturation, storage, industrial pretreatment/processing (Lalel *et al.*, 2003).

Fruit aroma is the result of a special assortment and mixture of different metabolites. While sugars and acids contribute to sweetness and tartness, aroma is derived from combinations of volatile molecules. The different proportions of the volatile components and the presence or absence of trace components often determine aroma properties. The production of aroma compounds is highly influenced by storage time and temperature. Individual aroma compounds will be affected differently (Zavala *et al.*, 2004). Most aroma compounds arise as a result of degradation reactions. In fruits, the plant cell walls soften and internal organization is lost during ripening. This loss of organization permits enzymes normally associated with growth to attack various substrates normally not available to the enzymes. Enzymes typically involved in synthetic processes are involved in degradation reactions. This attack results in the formation of a host of low molecular weight products (volatile aroma compounds) many of which have significant sensory properties (Reineccius, 2006).

2.1.1 Biogenesis of fruit aroma

The typical flavour (aroma compounds) of fruits (e.g. bananas, peaches, pears and cherries) is not present during early fruit formation but develops entirely during a rather brief ripening period. This flavor development period, or ripening, occurs during the climacteric rise in respiration (Reineccius, 2006). Fruits are considered climacteric if their ripening stage is triggered by increased levels of ethylene. In climacteric fruits, ripening has also been shown to be triggered by vapors of acetic, propanoic and butanoic acids, probably by enzymatic transformation of these acids into ethylene (Fisher & Scott, 1997). During this period, metabolism of the fruit changes to catabolism, and flavor formation begins. Minute quantities of lipids, carbohydrates, proteins and amino acids are enzymatically converted to simple sugars or acids and volatile compounds. The rate of flavor formation reaches a maximum during the postclimacteric ripening phase (Reineccius, 2006).

a. Aroma compounds from fatty acid metabolism

Fatty acids seem to be the major precursors of volatile compounds responsible for the aroma of most plant products. They are catabolized through two main oxidative pathways which is β-oxidation and the lipoxygenase (LOX) pathway. β-oxidation is the main metabolic pathway producing primary aroma in fruits, whereas the LOX pathway may account for the widest variety of aroma compounds from fatty acids in distrupted plant tissues (Barberan & Robins, 1997).

The formation of flavors via β-oxidation is exemplified by considering flavor formation in pears. The decadienoate esters are generally considered carriers of the flavor of pear. These esters are formed via β-oxidation of linoleic acid. Linoleic acid is metabolized, two carbons at a time, to shorter chain: CoA derivatives that react with alcohols to yield esters. The widest variety of flavor compounds formed from lipids arises via lipoxygenase activity. Many of the aliphatic esters, alcohols, acids and carbonyls found in fruit are derived from the oxidative degradation of linoleic and linolenic acids (Reineccius, 2006). Another metabolic pathway that leads to aroma formation from fatty acids is the one resulting in lactones. Lactones are important aroma components in fruits such as peach, apricot, coconut, mango or strawberry (Barberan & Robins, 1997).

b. Aroma compounds from amino acid metabolism

Amino acids also represent an important source of volatile compounds contributing to the aroma of fruits and vegetables. They can act as direct precursors in which they are metabolized to produce aroma compounds or as indirect precursors in which they are transformed through different metabolic pathways into new amino acids derived compounds that give rise to volatile aroma compounds after enzymatic transformation upon cell distruption. The metabolism of amino acids acting as direct precursors generates alcohols, carbonyls, acids and esters, either aliphatic, branched or aromatic. These compounds contribute to, and in some cases determine the primary aroma of many fruits (Barberan & Robins, 1997).

c. Aroma compounds from carbohydrate metabolism

Only a few classes of aroma components in fruits originate directly from carbohydrate metabolism. Among them, terpenes, considered to be formed from either carbohydrates or lipids, are biosynthesized through the isoprenoid pathway, while furanones seems to be produced from intermediates of the pentose phosphate cycle. Terpenes of fruit aromas, mainly monoterpenes, are a class of volatile compounds that constitute the characteristics components of many essential oils, and contribute to the aroma of many fruits. Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) and its methylether derivative mesifurane (2,5-dimethyl-4-methoxy-3(2H)-furanone) are important aroma compounds which have been identified in many fruits. Furaneol could be biosynthesized from an intermediate of pentose phosphate cycle, in which fructose-6-phosphate being the best precursor candidate (Barberan & Robins, 1997).

2.2 Mango

The mango (*Mangifera indica* L.) is the most important seasonal tropical fruit (Verheij & Coronel, 1992). It is a highly prized fruit due to its attractive flavour, delicious taste and high nutritional value (Lalel *et al.*, 2003). The genus *Mangifera* is included in the Anacardiaceae family and consists of 70 species. There are 25 species of *Mangifera* in Malaysia and *Mangifera indica* is the most important species from the aspect of economy (Hasnah & Mamot, 2004).

Mango was thought to have originated in India, but studies (Counc. Sci. and Ind. Res. 1962) indicate that it probably originated in the Assam-Burma-Thailand region. It is one of the most important commercial crops of the world with India's production being the largest. It is also grown in the Philippines, Indonesia, Java, Thailand, Burma, Malaysia, Sri Lanka and Northeast Australia and is quite popular in Egypt, Israel, South Africa, Hawaii and the West Indies (Nagy & Shaw, 1980). Mango can grow from sea level to 1400m altitude. Limiting factor is occurrence of frost. It does well within a temperature range of 24-27°C but endures up to 48°C. It can grow in areas with average rainfall of 25cm only with irrigation but can withstand rainfall up to 250cm. However, high humidity or rains during flowering is very harmful (Samaddar, 2001).

There are hundreds of mango cultivars grown all over the world. However, all of them are not cultivated on a commercial scale and most of them are localized in a particular area. The nomenclature of mango cultivars has been complicated due to the existence of synonyms. There are two distinct races of mango: monoembryonic and polyembryonic. In the monoembryonic, the seed contains only one embryo produced by sexual process which produces a single plant. In the polyembryonic mango, the seed

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