ANALYSIS OF AROMA ACTIVE COMPOUNDS OF SMOKE AND LIQUID SMOKE FROM *TIAN OP*

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN FOOD SCIENCE (INTERNATIONAL PROGRAM)

FACULTY OF AGRO-INDUSTRY

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2010

KMITL-2010-AI-D-051-067

Thesis	Analysis of aroma-active compounds of smoke and liquid smoke from *Tian Op*
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Student ID.	47063102
Degree	Doctor of Philosophy (Food Science)
Program	Food Science (International program)
Year	2010
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ABSTRACT

Tian Op, a traditional Thai scented candle, is used for the smoking and flavoring of sweets, cakes and other desserts for the purpose of adding a unique aroma to the final product. Gas chromatography-olfactometry (GCO), aroma extract dilution analysis (AEDA) and G-MS were applied to identify the potent odorants in two types of Thai desserts *(Num Dok Mai* and *Gleep Lum Duan)*. The volatile constituents of these two desserts could be separated into 10 chemical groups. The most abundant groups in *Num Dok Mai* (water-based) and *Gleep Lum Duan* (oil-based) were alkenes followed by acids, aldehydes, alkanes, ketones and alcohols. The volatile compounds were present at higher levels in *Gleep Lum Duan* than in *Num Dok Mai*. Predominant odorants in the two desserts were vinyl ketones (C_5-C_9), *n*-aldehydes (C_5-C_{11}), (*E*)-2-unsaturated aldehydes (C_8-C_{11}) and ω-1-unsaturated aldehydes (C_8 and C_9).

Separate studies related to the isolation of volatile compounds of *Tian Op* smoke found that propylene glycol was a superior trapped solvent than glycerol or water. Aroma-active compounds of *Tian Op* smoke were evaluated by GCO, AEDA and GC-MS. Octanal and decanal (sweet honey/ floral-like) were the most intense aroma-active compounds in *Tian Op* smoke while other potent aroma-active compounds, nonanal (orange peel), undecanal (waxy floral citrus), heptanal (sweet fatty), dodecanal (sweet waxy) also found at low level. Log_3 FD-factors of decanal (4), Octanal (4), and nonanal (3) were similar between *Tian Op* smoke flavoring and the volatile compounds obtained by burning a mixture of 97% beeswax, 1% dry kaffir lime peel, 1% sandal wood, and 1% benzoin at 160°C for 4 hours. When burning temperature was above

160°C (160 to 200°C), greater amounts of esters, acids, alkanes and alkenes were produced while less amounts of aldehydes and ketones were formed. Mean difference score of flavor mimic, model 1 (heptanal, octanal, nonanal, decanal, undecanal and dodecanal) and model 9 (heptanal, octanal, nonanal, decanal and undecanal) received mean difference scores of 0.07 and 0.04, which meant that the two model mixtures had nearly the same aroma as *Tian Op* smoke. The result showed that dodecanal was not an important aroma-active compound for *Tian Op* liquid smoke production.

ACKNOWLEDGEMENTS

It is my pleasure to thank my research advisor, Assoc. Prof. Dr. Kittiphong Huangrak, and committee members, Assoc. Prof. Dr. Wanna Tungjaroenchai, Assoc. Prof. Dr. Ratiporn Haruenkit, and Asst. Prof. Dr. Porjai Thamakorn, for their encouragement and support throughout the period of my research.

I am most grateful to Professor Dr. Keith R. Cadwallader, my committee member from University of Illinois at Urbana-Champaign, for his invaluable help and guidance. The success of my dissertation would not have been possible without his kind support, and remarkable patience.

Finally, I offer my regards and blessings to all of the graduate students of Faculty Agro-Industry at King Mongkut's Institute of Technology Ladkrabang, and members of Flavor Lab at Kasetsart University for their help, friendship and cooperation.

Wanwarang Watcharananun

March 2010

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CHAPTER 1

INTRODUCTION

The unique characteristics of Thai desserts are their flavor and appearance. Jasmine flower (*Jusminum adenophyllum*), Pandanus leaf (*Pandanus amaryllifolius* Roxb.) and *Tian Op* are widely used to impart and enhance the delicate and feathery odor of many Thai desserts. *Tian Op* is well known as a type of traditional scented candle in Thailand and has long been used for fumigation or smoking of desserts. The main ingredients of *Tian Op* are beeswax, benzoin (*Styrax benzoin* Dryand), kaffir lime peel (*Citrus hystrix* DC.), sandalwood (*Santalum album*), and borneo camphor (*Pogostemon calslin* Benth). Each ingredient is thought to impart different aromas to the smoke. For example, dry kaffir lime peel provides a sweet/citrus/lime peel like aroma, benzoin a sweet aroma like resin and sandal wood a woody/resinous aroma. Typically, the dessert is smoked in closed container 30-60 minutes. This process is repeated 3-5 times depending on type and quantity of desserts. The smoke absorbing ability of each dessert depends on it oil content since oil can trap volatile compounds in smoke better than water or other hydrophilic materials. From this reason, a shorter fumigation time is required for high fat containing desserts.

The traditional way to smoke Thai dessert has some disadvantages. For instance, the shelf life of the dessert will be shortened by bacteria growth during the smoking period, the intensity of flavor is not consistence in every piece of dessert, and the burned carbon dust from the candle may stick on the dessert surface. For the food industry, large scale production cannot accommodate these traditional problems. A potential solution to these problems is the use of liquid smoke, which could allow for better control of the intensity of the aroma. It's use may not only reduce smoking time and increase ease of use, but also allow for better consistency in the characteristic and intensity of the flavor.

The goals of the present research was 1) to determine the characteristic aroma components of two types of desserts produced using the traditional *Tain Op* smoking process and 2) to develop a natural liquid smoke flavoring which could replace the *Tian Op* smoking process for the large scale production of Thai traditional desserts.

Specific objectives of this research were:

1. To determine the key odorants of the *Tian Op* smoked deserts *Num Dok Mai* and *Gleep Lum Duan*,

2. To determine the aroma compounds of *Tian Op* smoke

3. To determine optimum trapping solvent for *Tian Op* volatile constituents.

4. To study on optimum burning conditions, such as the temperature and time for producing liquid smoke from *Tian Op*

5. To mimic *Tian Op* smoke odor using standard flavor compound solutions.

CHAPTER 2

LITERATURE REVIEWS

2.1 *Tian Op*

Tian Op is a scented candle using for fumigation and smoking. It is composed mainly of beeswax but may also contain some minor ingredients such as benzoin (*Styrax benzoin* Dryand), kaffir lime peel (*Citrus hystrix* DC.), sandalwood (*Santalum album*), and borneo camphor (*Pogostemon calslin* Benth). These components may have an effect on the aroma of *Tian Op*.

2.1.1. Beeswax

Beeswax is an abdominal secretion of bees (*Apis mellifera*) used to form the hive cells. Normal beeswax is yellow to orange in color, soft when warmed to about 32°C and melts at about 62 to 66°C. It is insoluble in water, partly soluble in some solvents and miscible with fats, oils and other waxes. Beeswax is soluble in certain solvents, e.g. ethers, carbon tetrachloride, benzene, acetone, gasoline and trichloroethylene. It consists of more than 300 individual components. Presence of over 100 volatile constituents have been reported in bees wax (Tulloch, 1980). The characteristic aroma of beeswax is a result of at least 48 compounds. The chemical compositions of beeswax is 14% hydrocarbons, 35% monoesters, 14% diesters, 3% triester, 4% hydroxy monoesters, 8% hydroxy polyester, 1% acid esters, 2% acid polyesters, 12% free acids, 1% free alcohol and 6% unidentified compounds (Tulloch, 1980). Beeswax is brittle at low temperature, but at higher temperatures, e.g. when cast, is plastic or malleable. It is especially malleable or plastic when extruded or deformed in other ways, because pressure disrupts the molecular structure. The hydroxyl acids of beeswax are responsible for its special properties (Tolloch, 1980) and esters and acid esters derived from hydroxyl acids and diols contribute to the plasticity of beeswax (Tulloch, 1980). Wax consisting mainly of monoesters or of diesters based on the hydroxyacids or diols (which occur in carnauba wax) is much harder and the melting point appreciably higher (as in the case of carnauba wax).

Volatiles in molten beeswax at 65°C were determined by Ferber and Nursten (1977). It was found that ρ-cymene (oxidized citrus oil), limonene (sweet citrus), *cis*-linalool oxide (floral-minty odor), *trans*-linalool oxide (floral-minty odor), α-terpineol (woody-lilac notes), guaiacol (sweet smoke), phenol (phenolic medical), cresol (phenolic medical), octanal

(sweet honey), nonanal (fatty-floral), decanal (waxy orange) and benzaldehyde (bitter almond oil) were the major volatile compounds.

2.1.2. Benzoin

Most benzoin is known as *Kumyan* in Thailand and is imported from Lao PDR. Benzoin (*Styrax benzoin Dryand*) is the resin from styrax trees (*Styrax styracaceae*). It consists of hard, usually cream-colored/pale orange pieces, which if broken reveals a milky white color. Benzoin is pale in color when freshly collected but darkens gradually during storage to a sandy-orange color. The chemical compositions of benzoin are coniferyl cinnamate, sumaresinolic acid, benzoic acid, cinnamic acid, stylene, vanillin and benzaldehyde (Lawless, 1995).

2.1.3. Sandalwood

Sandalwood is a name for several fragrant tropical woods, especially for *Santalum album*. Distilled oil from the wood is used extensively as a perfume and also had a place in medicine. Chemical compounds of sandalwood are phenol, cyclotene, guaiacol, methylguaiacol, syringol, santalols, sesquiterpene hydrocarbons, santene, teresantol, borneol, santalone and tricycloekasantalal (Duke, 1985).

2.1.4 Kaffir lime peel

The kaffir lime (*Citrus hystrix* DC.), also known as *Makrut* in Thailand, is a Southeast Asian citrus plant with very pungent leaves. The green lime fruits are distinguished by their bumpy exterior. The characteristic odor of kaffir lime peel is fruity, tangy and zesty. The volatile oils in the kaffir lime peel are composed of 2.5% α-pinene, 0.2% camphene, 30.6% β-pinene, 22.6% sabinene, 1.4% myrcene, 29.2% limonene, 1.3% cineol, 0.1% γ-terpinene, 0.1% ρ-cymene, 0.1% terpinolene, 0.6% trans-sabinene hydrate, 4.2% citronellal, 0.6% copanene, 0.5% linallol, 0.5% β-cubebene, 4.2% terpinen-4-ol, 0.3% caryophyllene, 0.2% citronellyl acetate, 0.2% α-terpineol, 0.1% geranial, 0.4% citronellol, 0.3% δ-cadinene, 0.1% geraniol, 0.1% nerolidol and 0.3% elemol (Lawrence et al.,1971)

Pudil et al.,(1998) analyzed the change in the composition of the volatile compounds from the peel of Indonesian kaffir lime (*Citrus hystrix* DC) oil during autoxidation at 40°C. They found that the citronella in the oil was reduced by autoxidation while linalool oxide was formed.

2.2 Liquid smoke

Today, consumer have concerns about health and well-being. These have driven the trend toward natural food flavors and ingredients. Many traditional ingredients, derived from naturally occurring substances, obviously meet these requirements. There are several methods for the production of natural ingredients e.g. liquid smoke can be produced from bagasse and coconut husk by fractional burning and trapping in the suitable solvent and used directly as a flavoring. Use of liquid smoke as flavor ingredients will meet consumer requirements on natural product as well as health benefits. For over 40 years, smoke flavorings have been widely used as commercial flavorings for addition to a variety of foods, such as meat, fish, dairy (cheese), nuts and snack products, to mention only some examples. Their application is an alternative to traditional smoking. The main function is to flavor foods, giving them a unique "smoky touch", highly attractive to the consumer. As compared to smoking, application of smoke flavorings offers several advantages in product safety (absence of carcinogenic aromatic hydrocarbons), processing economy and convenience as well as environment protection (Borys, 2001)

Smoke flavorings are considered as additives of natural origin. They are obtained from wood as products of its thermal decomposition in controlled physical conditions (temperature, oxygen access) followed by formation of two-phases (water and tar) of smoke condensate and their further processing (Daun,1972). Most commercially available smoke flavorings are made from the water phase of smoke condensate; however it has been found that tar phase is also a rich source of sensory active volatiles and might be used for smoke flavorings manufacturing.

In the Polish Meat and Fat Research Institute, a number of methods of smoke flavoring processing from the tar phase have been developed. The most important are the methods based on extraction and distillation of the tar and other one, applying two-stage distillation only; both methods were patented. Various woods were used as raw material for smoke development and its effect on smoke flavorings composition was analyzed (Borys, 2001). The volatile compounds of smoke produced from different woods are different. The volatile profiles of smoke originating from woods high in hemicellulose contain appreciable levels of furans and aliphatic acids. Volatile profiles of smoke from woods high cellulose contain high levels of hydroperoxides, carbonyls and carboxyls. Meanwhile the volatile profiles of smoke from woods high lignin contain ferulic acid, sinapic acid, guaiacyl lignin and phenyl propane at high levels (Borys, 2001).

As various woods differ in chemical composition (cellulose, hemicellulose and lignin content and ratio to each other) and conditions of thermal degradation as well as further

processing methods may vary, chemical composition of smoke flavoring is naturally variable. Several authors studied the effect of wood and temperature of thermal degradation on the amount and chemical composition of smoke preparation or its fraction (Chen and Maga, 1993). The three temperature zones involved in the breakdown process of chemical compounds of wood from burning are 200-260^{o}C (hemicellulose breakdown), 260-310^{o}C (cellulose breakdown), and 310-500^{o}C (lignin breakdown) (Chen and Maga, 1993). Much less information is available on the effect of processing method of smoke condensate on the sensory characteristics of smoke flavoring.

The variability of raw material and multi-stage processing of smoke flavoring preparations is responsible for the complexity of their chemical compositions. Smoke flavoring is a mixture of numerous volatiles and some non-volatiles of various structure, reactivity and sensory activity. Among them, phenol, syringol, guaiacol and their derivatives, as well as carbonyls and some other compounds (as catechol and naphthalene derivatives), are represented (Borys, 1998). All volatiles might be considered as potential contributors to smoke-curing aroma and flavor. However, the question which of them and in which combination to each other contributes to the typical, palatable smoke-cured sensory characteristics remains still not clear. Numerous studies have addressed this matter over the last four decades and have still not provided a definite answer (Fiddler et al., 1970).

The processing factors that affect the aroma compounds in liquid smoke from wood are (Chen and Maga, 1993):

2.2.1 Temperature and time effect

Attaviroj (2003) studied liquid smoke production from bagasse and coconut husk. The results showed that the temperature of heating had an effect on the quality of the liquid smoke produced. At higher temperatures (200 to 350^{o}C) higher quantities of phenolics, carbonyls, furans, polycyclic aromatic hydrocarbons and acidity were produced in the liquid smoke.

Tulloch (1980) studied the effects of heating temperature on the chemical compounds in beeswax. The chemical compounds of pure beeswax were changed significantly by prolonged or excessive heating. Longer heating or higher temperatures led to greater degradation and loss of hydrocarbons and these changes also influenced the physical characteristics of the wax. Excessive heating during rendering or further processing changed the wax structurally and

altered the beneficial characteristics of the aromatic and volatile compounds and also many of its minor compounds.

Several investigators observed a marked dominance of C_7-C_{13} aldehydes in the volatile products of low temperature oxidation. At the higher temperatures, however, acid and ester group were the major volatile form (Parliment et al., 1989). The aldehydes are typical scission products of palmitate hydroperoxides. Kimoto and Gaddis (1969) speculated that the carbon-carbon bond (Type A) is the most vulnerable to cleavage under moderate conditions of autoxidation, while scission at the carbon-oxygen linkage (Type B) is favored under stress such as heat (Figure 2.1).

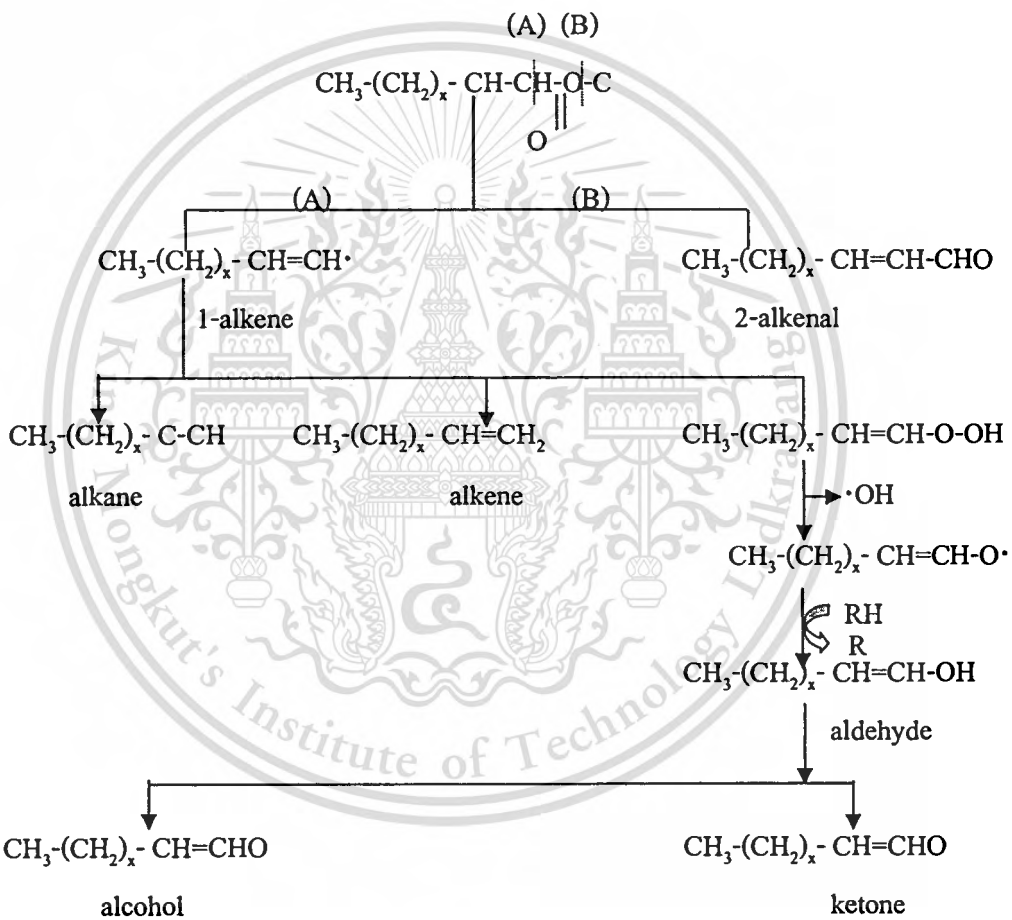

Figure 2.1 Formation of volatiles from fatty acid via oxidative pathway at low temperature

Source: Kimoto and Gaddis (1969), (Pan et al., 2004)

The esters and acids might be produced by cleavage of the hydroperoxide of myricyl palmitate. Further oxidation produced the corresponding carboxylic acid which upon decarboxylation changed to an ester at temperatures higher than 160°C. The 8-alkoxy radical

might also break down to give the C_7 alkyl radical, which would yield C_8 ester and C_8-C_{15} acids or form a terminal hydroperoxide, and so on. Polymerization of myricyl palmitate, both intra- and intermolecular, was also a major reaction at high temperature oxidation. Combination of alkyl, alkoxy, and peroxy radicals made varieties of dimeric and polymeric compounds with C-O-C or C-O-O-C crosslinks.

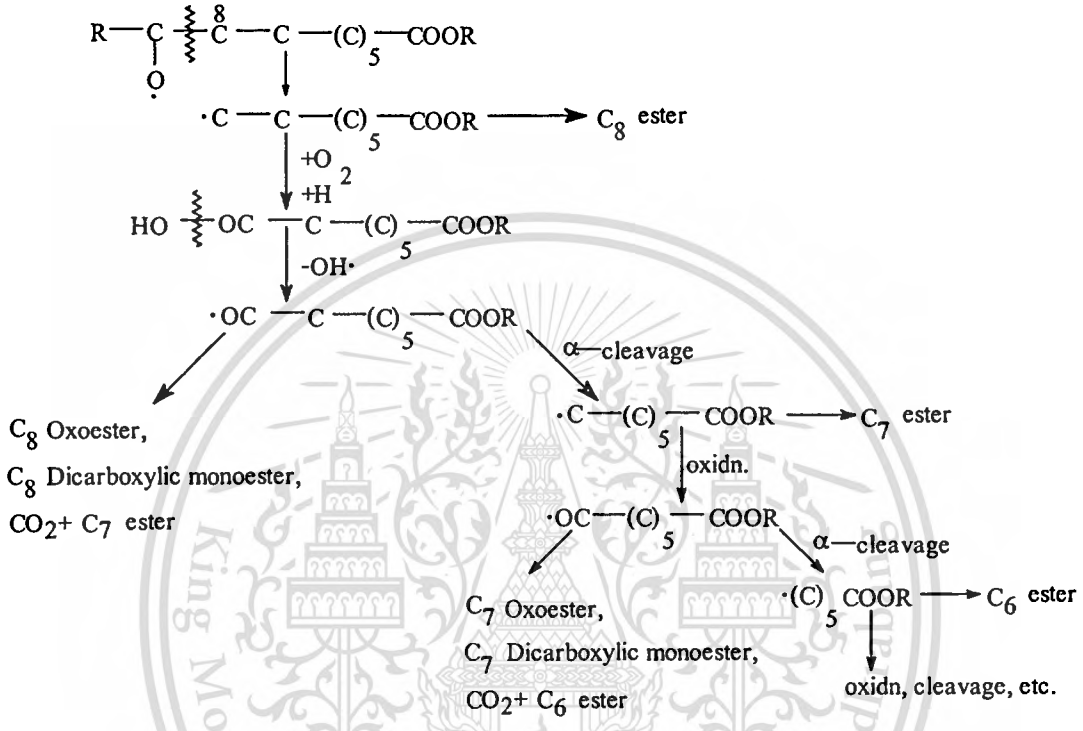

Figure 2.2 The formation of volatiles from fatty acid via oxidative pathway at high temperature

Source: Parliament et.al. (1989)

2.2.2 Trapping solvent

Attaviroj (2003) determined the optimum compositions of solvent solutions used to trap smoke from bagasse and coconut husk. It was found that a 5% propylene glycol solution was the suitable trapping medium because the quantities of phenolics, carbonyls, furans, polycyclic aromatic hydrocarbons and acidic compounds in trapping solvent were higher than when using glycerol or water.

Propylene glycol, also known by the systematic name of propane-1,2-diol, has two hydrophilic alcoholic hydroxyl groups (OH^-). It is a diol alcohol, and is usually a tasteless, odorless, and colorless clear oily liquid that is hygroscopic and miscible with water, acetone, and chloroform, manufactured by the hydration of propylene oxide. Propylene glycol is generally

recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) for use as a direct food additive under the conditions prescribed. It is approved by the U.S. FDA for certain indirect food additive uses. Propylene glycol has a wide range of practical applications such as antifreezes, coolants and aircraft deicing fluids, heat transfer and hydraulic fluids, solvents, food, flavors and fragrances, cosmetics and personal care products, pharmaceuticals, chemical intermediates, plasticizers, and thermoset plastic formulations (Greenwood and Earnshaw, 1997)

Glycerol (glycerin and glycerine) is a sugar alcohol with three hydrophilic alcoholic hydroxyl groups (OH^-) that are responsible for its solubility in water. Glycerol is a colorless, odorless, hygroscopic, and sweet-tasting viscous liquid. It is produced from dihydroxyacetone phosphate (DHAP) by the enzyme glycerol three-phosphate dehydrogenase in the cytoplasm of the eukaryotic cell during glycolysis. When referring to its function in living organisms, the term glycerol is preferred. Glycerol is an important component of triglycerides (i.e. fats and oils) and of phospholipids. It is a three-carbon substance that forms the backbone of fatty acids in fats (Greenwood and Earnshaw, 1997).

Dainius et al., (2006) developed a method of producing a liquid smoke from wood tar for use in food processing using propylene glycol as a trapping solvent. The liquid smoke product that was produced contained neither detectable amount of 3,4-benzopyrene nor those components of the heavy, essentially water insoluble material that settled out of an aqueous condensation of wood smoke. Most of which were soluble in propylene glycol.

2.3 Flavor compound analysis

2.3.1 Extraction methods

Aroma compounds must be isolated and concentrated to a suitable concentration prior to analysis by Gas Chromatography (GC). Essential oils and aqueous samples can be easily directly injected after dilution or concentration. For complex food samples, they must firstly be extracted. Extraction techniques for aroma in food are based on two different properties of their volatile constituents, their volatility and affinity toward non-polar solvents or polymers. Distillation and headspace trapping use volatility to selectively remove volatile components from food matrix. Solvent extractions and adsorptions polymers use their hydrophobicity for the same purpose (Etievant, 1996). The extraction method must be represented the original flavor present in the matrix and avoided the formation of any artifact during sample preparation (Chaintreau,

1999). Chaintreau (2001) classified the extraction methods into three main categories (Table 2.1). Some of the extraction methods that have been successfully used are described as following.

Table 2.1 Main extraction methods of aroma compounds

Solubility	Volatility	Miscellaneous
Soxhlet	Steam distillation	Simultaneous distillation-extraction (SDE)
Liquid-liquid extraction	Vacuum transfer	Distillation-membrane extraction
Supercritical fluid extraction (SFE)	Headspace (HS)	Simultaneous distillation- adsorption
Solid phase extraction (SPE)		
Solid phase micro-extraction (SPME)		

Source: Chaintreau (2001)

2.3.1.1 Distillation Methods

Several distillation techniques have been used to extract aroma components from foods. Distillation technique can change flavor of fresh food into flavor of cooked food, especially when raw food is studied this technique should be avoided (Etievant et al., 1996). Some of distillation techniques will be described as following:

a) Direct distillation is the technique in which sample and water are placed in a round-bottom flask and the flask is heated directly to carry over the steam distillable components. Problems can be encountered due to scorching of the sample if too much heat is applied and in addition bumping may occur (Parliment, 1997).

b) Indirect steam distillation: this technique is more advanced over the direct distillation techniques. It is more rapid and results in less decomposition of the sample since the sample is not heated directly. The steam may be generated in an external electrically heat system generator or in a round-bottom flask heated by a mantle. The steam and volatiles are condensed in a series of traps cooled with a succession of coolants ranging from ice water to dry ice/acetone or methanol (Parliment, 1997).

c) Simultaneous steam distillation extraction (SDE): this technique was once one of the most popular and valuable methods used for flavor analysis. SDE apparatus provides for the simultaneous condensation of the steam distillate and an immiscible organic solvent. Both liquids are continuously recycled and the steam distillable-solvent soluble compounds are transferred from the aqueous phase to the solvent. The advantages of this technique include the

followings: a single operation removes the volatile aromas and concentrates them, a small volume of solvents is required, reducing problems of artifact build up, as solvents are concentrated, recoveries of aroma compounds are generally high and the system may be operated under reduced pressure to reduce thermal decomposition because the system can be operated at lower temperature (Parliment, 1997).

d) The simultaneous steam distillation extraction method most commonly used today is some modification of the original Liken and Nickerson distillation head as shown in Figure 2.3A (Reineccius, 1998). Microapparatus for simultaneous steam distillation extraction are designed to concentrate volatiles into 1 ml of solvent, thus achieving a high concentration factor (Figure 2.3A and B). It allowed direct GC injection of the organic extract without a further concentration of the solvent, which might alter the proportions of individual aroma components (Chaintreau, 2001).

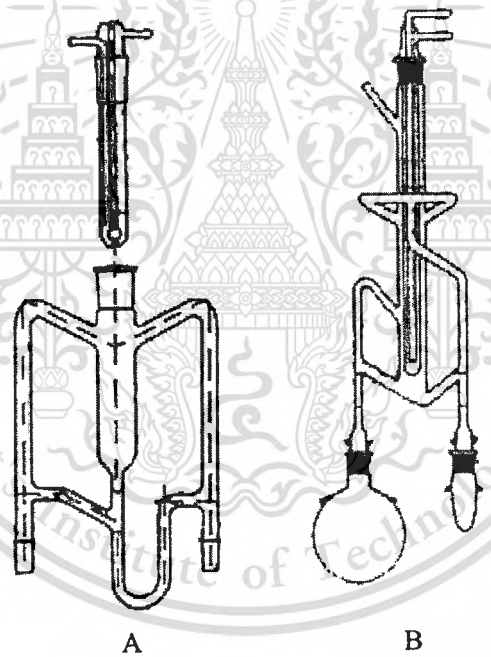

Figure 2.3 SDE apparatus. A: Likens and Nickerson's apparatus, B:Micro-SDE apparatus

Source: Chaintreau (2001)

e) Vacuum steam distillation is the simple apparatus of vacuum steam distillation (rotary evaporator). When the composition of the samples is concerned, the steam distillation may be operated under vacuum. In this case inert gas should be bled into the system to aid in agitation.

f) Steam distillation is among the oldest techniques used to separate volatile from non-volatile material. Although aroma extracts can be obtained very fast and simply by steam

distillation, however the elevated temperatures applied during distillation may lead to artifact formation. Based on the high vacuum distillation technique (Figure 2.4), which has some drawbacks (Engel et al., 1999) e.g. high boiling point aroma compounds, may partially condense inside the tubes before reaching the traps. However only diethyl ether and dichloromethane extracts can be used otherwise frozen solvent may close the tubes and traps, high concentrations of saturated fat may plug up the stopcock of the dropping funnel and the apparatus takes a lot of bench space and is fragile.

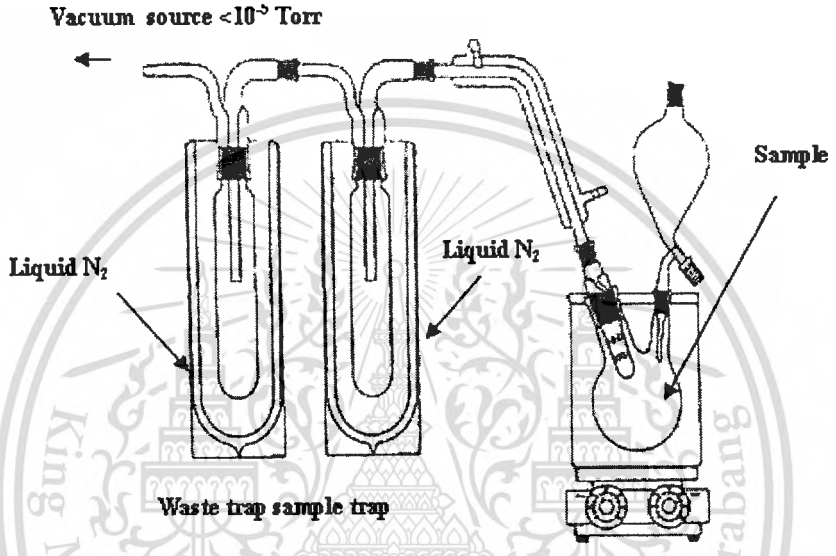

Figure 2.4 High vacuum distillation equipment

Source: Engel et al. (1999)

Engel et al., (1999) developed solvent assisted flavor evaporation (SAFE). It was a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices (Figure 2.5). This technique provided the fast and careful isolation of volatiles from either solvent extracts of foods, oil samples or fruit pulp. Besides its efficiency in aroma isolation, the use of the equipment saves time and reduces cost due to the stability and compact apparatus.

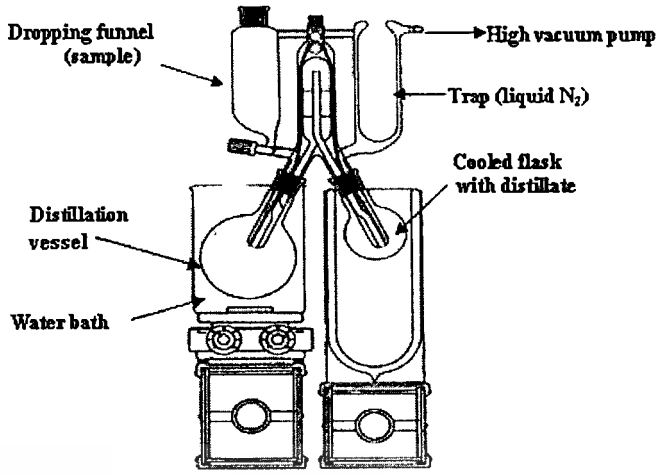

Figure 2.5 View of the assembled equipment for SAFE

Source: Engel et al. (1999)

2.3.1.2. Solvent extraction

Solvent extraction is a method to extract a solute from one liquid phase to another phase. The extraction solvent should be selected on the basis of selectivity of compounds of interest. The solvents most commonly used for extraction are diethyl ether, diethyl ether/pentane mixture, hydrocarbons, Freon and methylene chloride. Freon and methylene chloride have the advantage of being nonflammable. Non-polar solvents such as Freon, hydrocarbons should be used when the sample contains alcohol. Diethyl ether and methylene chloride are good general-purpose solvents. Methylene chloride is a satisfactory general purpose solvent, particularly for flavor compounds with an enolone structure (e.g. maltol and Furaneol) (Parliment, 1997). Saturated aqueous solution with salt will assist to complete the solvent extraction. Commonly used salts are sodium sulfate and sodium chloride which are added to salt out the organic compounds (Teranishi et al., 1971).

2.3.1.3. Headspace extraction

Headspace extraction is a method for monitoring the gaseous headspace above a liquid or solid in a sealed container and suitable for aroma analysis. It is a non-destructive technique of mild conditions and easy sample preparation. This technique is divided into three broad categories: static headspace, dynamic headspace and purge & trap. The fundamental principle of each technique is the same. Volatile analytes from a solid or liquid material are sampled from the atmosphere adjacent to the sample.

In static headspace techniques, a small sample of the atmosphere above the sample is injected directly onto a GC column. In dynamic headspace techniques, the organic volatile analytes from the headspace are first concentrated and then transferred to the GC. The term dynamic headspace is usually used when referring to the analysis of solid materials and the term purge and trap generally refers to the analysis of liquid samples by bubbling the purge gas through matrix (Wampler, 1997). The gas flow entrains the analytes on to adsorbent trap, where they remain until the trap is heated to desorb the analytes into the carrier gas stream (Hinshaw, 2000). Dynamic headspace extraction involves the adsorption of organic molecules swept by an inert gas on an organic polymer. There are many types of available polymers such as Tenax, Porapak, Chromosorb and charcoal. Among these polymers, Tenax is the most often chosen (Etievant et al., 1996). The trapping step may involve adsorption onto a high surface sorbent material or cold trapping by condensing or freezing (Wampler, 1997).

In cold trapping or cryofocusing, the carrier gas flow containing the broad sample band is directed through a cold trap where the analytes condense or are retarded. Next by the rapid heating up of the trap, the accumulated analytes are released, but the analytes are now in the form of sharp band, which is then carried by the carrier gas onto the column (Kolb and Ettre, 1997). A cold trap is maintained by liquid nitrogen or a solid CO_2 solvent slurry (Hartman et al., 1993).

In direct thermal desorption technique, the sample of interest is placed directly into the glass lined stainless steel desorption tube in place of adsorbent. The sample is then basically heated in a stream of inert carrying gas to gas volatile flavor, and the desorbed components are flushed into the GC injector in a one step analysis (Hartman et al., 1993).

2.3.1.4 Solid-phase micro-extraction (SPME)

Solid-phase micro-extraction (SPME) is an innovative, completely solvent free sample preparation method. It can be used to concentrate flavors and fragrances that significantly reduced the time and cost required for sample preparation. This technique can be used to concentrate volatile organic compounds from liquid samples by immersion SPME or headspace above a liquid or solid sample by headspace SPME. The analytes from sample are concentrated on fused silica fiber coated with a polymer film. The equilibrium distribution of analytes is established between the stationary phase (the microfibers) and the aqueous or gas phase (sample).

Once equilibrium has been established, the concentrated compounds are thermally desorbed in the injector of a gas chromatograph and transfer to the capillary column. SPME fibers of different polarities are available for extracting organic compounds from various sample matrices. These include PDMS (polydimethylsilosane), PA (polyacrylate), PDMS/DVB (polydimethylsilosane/divinyl-benzene), CW/DVB (carbowax/divinylbenzene), Carboxen-PDMS (carboxen-polydimethylsilosane) and DVB-carboxen (divinyl-benzene/carboxen) (Werkhoff et al., 2002). The accuracy of SPME for quantitative analysis is highly dependent on experimental conditions, sample matrix, analyte characteristics, the type of fibers and calibration techniques used (Hinshaw, 2000; Marsili, 2002).

2.3.2 Instruments for flavor analysis

2.3.2.1 Gas chromatography (GC)

Gas chromatography (GC) is a technique based on the separation of a mixture of volatiles components, following vaporization in an injection port and transfer into a narrow bore capillary column (typically, 0.25-0.32 mm i.d.). Separation is achieved by differential partition of the analytes between an inert carrier gas, such as helium, which continuously flows through the column and polymeric stationary phase (of selected polarity). The inside of the column is coated or chemically bound with the stationary phase. The column is housed in a temperature-controlled oven, enabling the separation to be isothermal or temperature programmed. Then, the analytes are detected sequentially as they elute from the column by a detector (Peppard, 1999). Only those materials that can be vaporized without decomposition are suitable for GC (Kitson et al., 1996).

2.3.2.2 Gas chromatography-mass spectrometry (GC-MS)

GC-MS is the combination of two powerful analytical techniques. The gas chromatograph separates the components of a mixture in time and the mass spectrometer provides information that aids in the structural identification of each component (Kitson et al., 1996). Flavor extracts are separated into their individual components by GC and a characteristic mass spectrum (fingerprint) is recorded as the component passes in turn into the mass spectrometer. Individual components of mixtures are identified by comparing their mass spectra (and GC retention times or indices) to those of authentic reference compounds. Different molecules can be broken up into fragments in a consistent and repeatable manner when subjected to high-energy

electron bombardment (typically 70 eV). This occurs within the ion source of a mass spectrometer operated in electron impact mode. The results of electrically charged fragments may then be separated according to their mass/charge (m/z) ratio; subsequent detection of these ions yields a mass spectrum characteristic of the original molecule, which is dependent on the type of compound involved. In addition, mass spectra may be searched against huge libraries of spectra, including commercial and in-house or proprietary libraries (Peppard, 1999).

2.3.2.3 Gas chromatography-olfactometry techniques

Gas chromatography-olfactometry (GCO) or gas chromatography sniffing is an analytical tool for flavor research. In GCO, the aroma effluents from sniffing port of gas chromatography column are evaluated by human nose. This is a sensitive and specific technique for aroma research since the human nose has an odor detection limit of about 10^{-19}moles (Reineccius, 1994). Theoretically, the maximum difference in sensitivity between the nose and flame ionization detector (FID) is close to 10^{7} times. This mean that up to 23 two-fold or 15 three-fold dilutions may yield a positive physiological detection in aroma extracts dilution analysis (AEDA) after the FID has stopped detecting any flavor constituent (Etievant et al., 1994). This makes gas GCO a very valuable and sensitive tool for detection of odor active volatiles (Mistry et al., 1997). Marsili (2002) stated that aroma can be detected even when no observable peak occurs in the chromatogram because the sense of smell is a far more sensitive detector than any available gas chromatography detector. Gas chromatography-olfactometry technique helps to detect potent odorants without knowing their chemical structures, which might be overlooked by the odor activity value (OAV) concept if the sensory aspect is not considered from the very beginning of the analysis (Blank, 1997). Because of the hot gas chromatography effluent, it must be combined with humidifier air to reduce nasal dehydration and the discomfort associated with sniffing (Acree, 1993). A diagram of an olfactometry detector is shown in Figure 2.6. Several techniques have been developed to objectify GCO data and to estimate the sensory contribution of single aroma components. But dilution techniques (Charm Analysis or Charm and aroma extract dilution analysis: AEDA) and time-intensity measurements (OSME) are the two main GCO methods (Blank, 1997). Dasilva et al. (1994) also supported that Charm Analysis and AEDA are most often cited in the flavor literature and the new technique, OSME is fairly often used as compared with traditional GCO techniques. All of these techniques have been used to identify odor active

compounds in food products in several scientific papers. Among these techniques of GCO, no real significant differences were observed (Mistry et al., 1997).

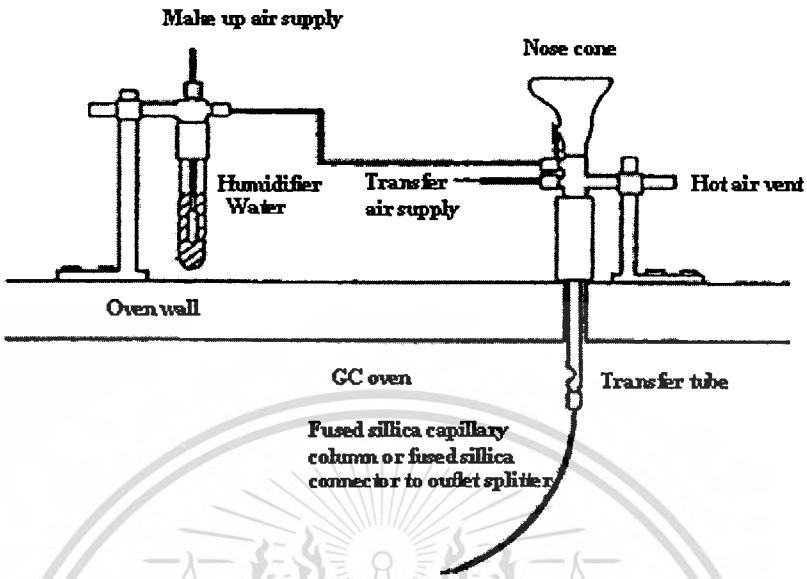

Figure 2.6 Olfactory detector

Source: Marsili (2002)

2.3.3 Aroma-active compound analysis

2.3.3.1 Aroma extracts dilution analysis (AEDA)

AEDA was developed by Grosch and coworkers from Deutsch Forschungsanstalt für Lebensmittelchemie, in Germany (Ullrich and Grosch, 1987). As a dilution technique, an extract is diluted, usually as a series of 1:2 or 1:3 dilutions, and each dilution is sniffed until no significant odor is detected. Several injections are required to reach a dilution of the aroma extract in which odorous regions are no longer detected (Van Ruth, 2001). This means that AEDA is based on the odor detection thresholds of the volatiles analyzed. In AEDA, the dilution factor (FD value) is simply the last dilution at which an odor active compound is detected (Van Ruth, 2001). Mistry et al., (1997) stated that the FD factor was a relative measurement and was proportional to the OAV of that compound in air. The results were usually presented as the logarithm of the factor of dilution (Log FD) versus the retention index or by listing the FD values (Blank et al., 1989). When the FD factors for the compounds are determined, an FD chromatogram is plotted. The FD chromatogram consists of the retention indices on the x-axis and the Log FD on the y-axis (Mistry et al., 1997). The chromatograms obtained from FID and olfactometry response are different. Aroma-active compounds usually do not correspond to the

major volatiles components in food. As shown in Figure 2.7, many importance odorants of white bread crust were not visible in a FID chromatogram. The method of AEDA has been used to determine the potent odor active compounds in many different food products, including: young Grenache wines (Marti et al., 2003), hen meat (Farkas et al., 1997), soy protein isolates (Boatright and Lei, 1999), essential oils (Kamath et al., 2001), buckwheat honey (Zhou et al., 2002) and French beans (Hinterholzer et al., 1998).

More recently, a new concept of AEDA using static headspace instead of extracts has been used. Dilution steps were made by injecting decreasing headspace volumes to evaluate the contribution of a compound to the whole composition (Van Ruth, 2001).

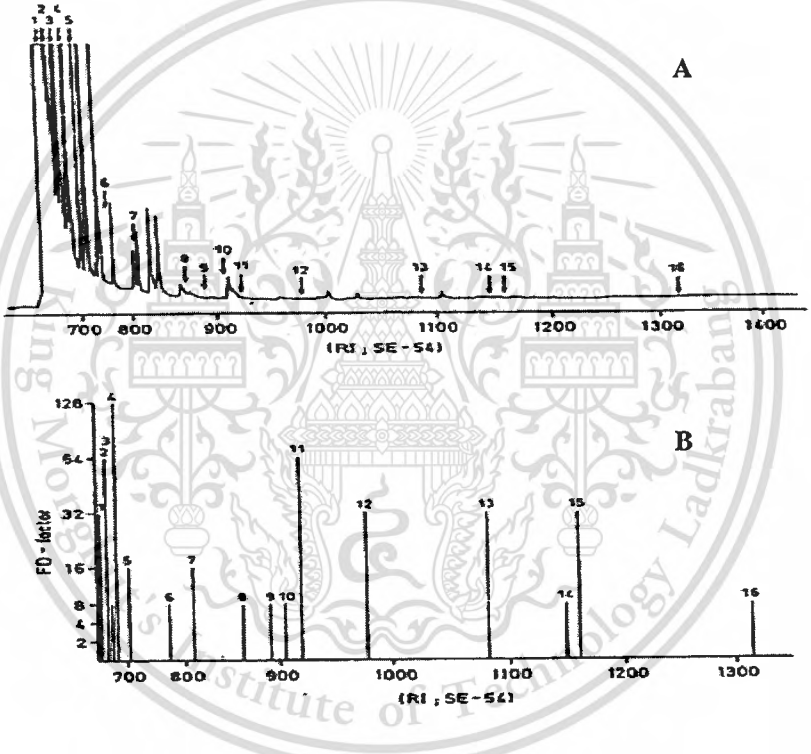

Figure 2.7 Gas chromatogram (A) and FD chromatogram (B) of the headspace volatiles of fresh white bread crust

Source: Blank (1997)

2.3.3.2 Combined hedonic aroma response measurement (CHARM)

CHARM Analysis is developed by Acree and coworkers (Acree, 1993). It is based on successive dilution and GCO analysis of a flavor extract, until the assessor no longer detects the odor at the sniffing port (Mistry et al., 1997). In CHARM Analysis, the dilutions are presented in randomized order to avoid bias introduced of the samples. The assessor points out

the beginning and ending of each particular odor perception (duration of the smell) with a sensorial descriptor. Times of the individual sniffs are combined and graphed to yield a chromatogram with peaks and quantified peak areas (CHARM values), which are used to quantify potency (Van Ruth, 2001). A CHARM value can be calculated according to the formula $c=d^{n-1}$ where *n* was the number of coincident responses and *d* is the dilution value (Blank, 1997; Van Ruth, 2001). CHARM response chromatogram was plotted between CHARM values and retention times or retention indexes. The chromatogram gave a profile of the odor activity of the sample (Gaffney et al., 1996). CHARM Analysis has been frequently used for determination of potency of odor active compounds in foods, such as orange juice (Marin et al., 1992), *Citrus sinensis,* peel oil (Gaffney et al., 1996), grapes (Braell et al., 1986) and lime oil (Chisholm et al., 2003).

A compared CHARM response chromatogram produced by GCO and a chromatogram produced by GC-FID are shown in Figure 2.8. In this figure, the horizontal axis, retention index (RI), is the same in both chromatograms but the vertical axis is different. FID response is a measure of the relative mass; its chromatogram peak indicates the amount of volatile component present. In GCO, the dilution value is the number of dilutions. The sample must undergo to reach a subject's threshold; peak area is CHARM value (proportional to odor activity units in air) that equals to the mass of the odorants in the sample divided by detection threshold for the odor and the subject (Gaffney et al., 1996). In CHARM analysis, times of the individual sniffs are combined and graphed to yield a chromatogram complete with peaks and quantified peak areas, which are used to quantify potency. The mathematics involved in CHARM analysis yield peaks with areas that are proportional to the amount of odorant in the extract and retention index that are reproducible.

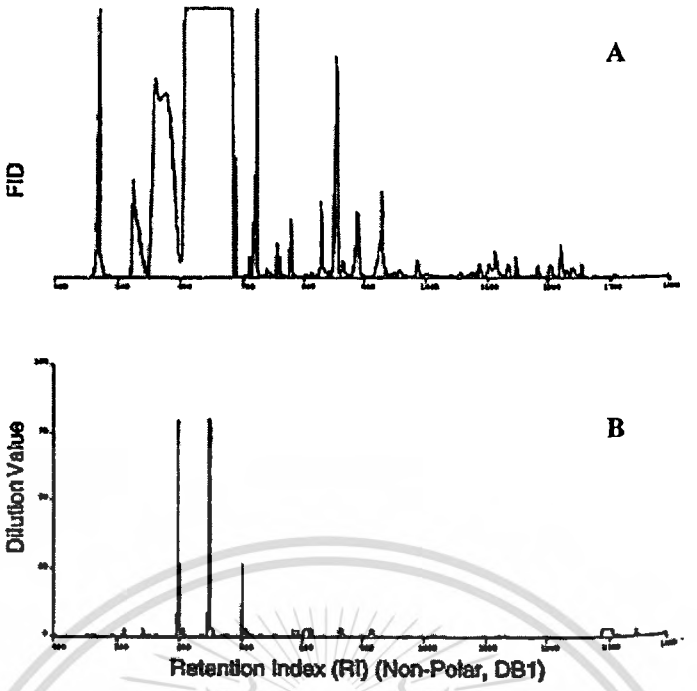

Figure 2.8 ID chromatogram (A) and CHARM response chromatogram (B) produced of the Valencia orange oil

Source: Gaffney et al. (1996)

2.3.3.3 OSME

OSME, a time-intensity method, was developed by McDaniel and coworkers at Oregon State University. It is derived from a Greek word meaning smell (Mistry et al., 1997). It is a quantitative bioassay method used to measure the perceived odor intensity of a compound detected by GCO. This method is different from CHARM Analysis and AEDA techniques in that OSME is not based on odor detection threshold (Miranda-Lopez et al., 1992). OSME combines the modern concepts of sensory descriptive analysis with novel techniques of computerized data collection and applies time-intensity approach of evaluating the odor significance of compounds in the Gas Chromatography effluent. With OSME, trained subjects sniff the Gas Chromatography effluent mixed with humidified air and directly record the odor intensity and duration time of each odor active compound detected at the sniff port and described its odor quality in the entire range of a sixteen-point intensity scale (0 = none, 15 = extreme). The plot of the retention time versus odor intensity, called Osmegram, provides a graphical representation of the compound's odor significance in the flavor extract; higher peaks suggest greatest importance (Figure 2.9).

Currently, the time-intensity methods have not been used very frequently for GCO, because methodological aspects should receive more attention before the value of this

technique can be fully evaluated (Van Ruth, 2001). OSME has been used to characterize the aroma compounds in orange juice (Bazemore et al., 1999), Pinot Noir wine from grape (Miranda-Lopez et al., 1992), sheep meat (Braggins, 1996), cashew apple nectar (Valim et al., 2003) and freshly prepared essential oils prepared by steam distillation (Kamath et al., 2001).

In conclusion, AEDA and CHARM Analysis are the dilution techniques and are based on the odor detection threshold concept. They are relatively simple sensory procedures in which the assessor indicates only whether or not an odor can be perceived. Numerous injections are required for both AEDA and CHARM Analysis. AEDA is easier to conduct than CHARM Analysis because no computer software is required but the continuous signal recording over the whole GC run duration is lost. OSME is the time-intensity method and odor intensities of peaks eluting from the Gas Chromatography column are evaluated. It requires only the injection of the aroma extract at one level of dilution and time dependant signal is continuous record by computer. However, it requires a significant amount of time to initially train the panelist (Chaintreau, 2002).

GCO is time intensive and typically uses 1-2 sniffers who must be pre-screened for sensitivity and specific anosmias. The olfactometry sensitivity of experimental error and variation can be reduced by closely monitoring in sample preparation, room temperature, sample temperature, time of the day, duration of analysis, repetition of analysis, repeated standardization of sniffers and use of a standard lexicon (Friedrich and Acree, 2000).

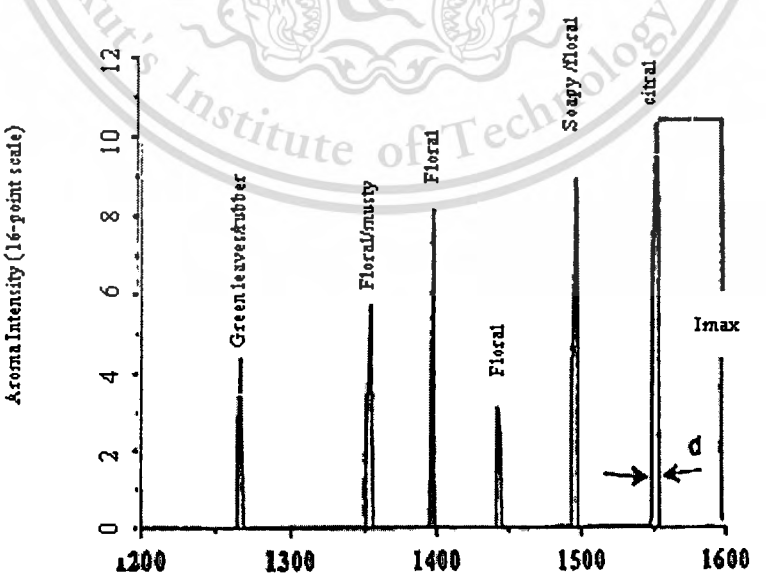

Figure 2.9 OSME equipment and Osmegram

Source: Miranda-Lopez et al. (1992)

2.3.4 Descriptive analysis

Descriptive Analysis is a consensus technique where the descriptions for the product and the product evaluation are achieved by reaching agreement among the panel members. In descriptive analysis, consideration of the overall flavor and the individual detectable flavor components of a food system are done. The profile describes the overall flavor and the flavor notes and also estimates the intensity and amplitude of these descriptors. The technique provides a tabulation of the perceptive flavors, and their intensities, order of perception, aftertastes, and overall impression. If the panelists are appropriately trained, this tabulation is reproducible. Using standardized techniques of preparation, presentation and evaluation, the four to six judges are trained to precisely define the flavors of the product category during a 2- to 3-week program. The food samples are tasted and all perceived notes are recorded for aroma and flavor (Lawless and Heymann, 1998).

The fundamental relationship that exists between descriptive analysis and the analytical properties as determined by instruments is of interesting for its potential in development of new food products. For new product development guidelines, the descriptions of QDA can link with the potent chemical compound or analytical data from instruments and can offer food product scientists guidelines as to which properties should be emphasized in terms of product development decisions. In quality assurance control, the relative importance of data mentioned above can be useful in selecting those variables for which control is valuable from the standpoint of maintaining sensory quality. The principle advantage of QDA is that the scores have absolute meaning when the scale was used by all panelists in the same way. The disadvantage of the procedure is associated with the difficulties of panel development and maintenance. Training of panels is usually very times consuming. Panelists have to be exposed to the samples and understand the vocabulary chosen to describe the product. They are asked to grasp the underlying technical details of the product and they are expected to appreciate sensory perception. After all that they must also be extensively tuned to one another to ensure that all panelists are using the scales in the same way (Lawless and Heymann, 1998).

2.4 Aroma-impact compounds in food

The odor perception takes place in the upper part of the nasal cavity in the olfactory epithelium. Volatile aroma chemicals reach the receptors either the breath directly through the nose or indirectly via the retronasal cavity (Matheis, 1999) so that odor must be volatile. More

than 6,900 volatile compounds have been identified in foods and beverages and only few of them are responsible for the characteristic odor of a particular food (Mistry et al., 1997). Even though a food product contains hundreds of volatile compounds, most of them do not possess aroma activity at the concentrations found in the food. Major compounds do not always have high odor intensity in the food. For example, limonene which is the major component of orange peel oil has low aroma intensity, whereas linalool and octanal that are less than 1% have much higher intensity in orange aroma than limonene (Gaffney et al., 1996). This type of compound is known as a key odorant compound, aroma or character impact compound or odor active compound. McGorrin (2002) defined character impact compound for a particular flavor or aroma as a unique chemical substance which provides the principal sensory identity. Then the ultimate goal of flavor research is to identify and classify unique chemicals that contribute to the characteristic odor and flavor of foods or aroma-impact components.

The knowledge of aroma-impact components in food enables flavor duplication through nature-identical or biosynthetic pathways. Such knowledge facilitates better quality control of raw materials by screening of the appropriate analytical target compounds. Key aroma components can be used in formulation of a natural identical or artificial flavor and used to understand the changes in processing, formulation and the impact of packaging (Reineccius, 2000). Different food product or plant has different odor active compounds. Cadwallader et al., (1999) found that (*Z*)-3-hexenal which has green/cut-grass odor and an unknown odorant contributing rancid sour and old cut-grass had the greatest impact on the aroma of fresh-picked cilantro.

The compounds that are accounted for the key odorant or character impact compound in food should have high odor activity values. Odor activity value (OAV) is the ratio of the concentration of an aroma compound to its threshold in air, thereby indicating how much the actual concentration of a compound exceeds its sensory threshold (Mistry et al., 1997). Rouseff and Naim (2000) stated that if the ratio is greater than one the component should have aroma activity and if the ratio is less than one, no aroma activity should be perceived.

The threshold concentration is defined as the lowest concentration at which a chemical compound can be distinguished with certainty from a blank under standard condition (Bauer et al., 1997). In a recent study, aroma-impact components in food have been identified and determined by GCO techniques (Serot et al., 2001; Mcgorrin, 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials

3.1.1 Beeswax was purchased from Sayun Bee farm, Chiang Mai, Thailand. Its qualities are as described: yellowish color, saponification value 87-110 mgKOH/g, acid value 17-24 mgKOH/g, ester value 70-86 mgKOH/kg, melting point 62-65°C and the impurity was 0.1%.

3.1.2 Kaffir lime peel (*Citrus hystrix* DC) was purchased from Talad Thai market, Pathum Thani, Thailand. It was dried at 35°C in tray dryer for 36 hours, then milled and sieved through 0.25 mesh sieve and kept in a desiccator. The moisture content of ground dried kaffir lime peel was 12%.

3.1.3 Benzoin (*Styrax benzoin Dryand*) was purchased from Yaowarat District, China town in Bangkok, Thailand. It had been collected in Chiang Kwang, Laos PDR. The moisture content was 12% and it was kept in desiccator and freshly ground before use.

3.1.4 Sandalwood (*Santalum album*) was purchased from the Ministry of Public Health Thailand. It had been collected in Chumporn Province, Thailand. The wood was milled and sieved through 0.25 mesh sieve and kept in a desiccator. The moisture content of ground sandalwood was 11%.

3.1.5 Odorless water was produced by boiling distilled water until the remaining volume was 2/3 of the initial volume.

3.1.6 Jasmine rice was purchased from C.P food product Co., Ltd (Royal Umbrella brand).

3.1.7 Wheat flour was purchased from United flour mill public Co., Ltd. (all purpose flour, Kite brand)

3.1.8 Rice flour was purchased from Thai Wah Food Publish Co., Ltd. (New Grade brand)

3.1.9 Sugar was purchased from Wang Kanai group Co., Ltd. (Nakornrachaseema, Thailand).

3.1.10 Cotton was produced in Jana district, Songkhla.

3.1.11 Ylang-ylang flower from Kasetsart University, Bangkok.

3.2 Reagents

3.2.1 Propylene glycol (99.5%) from Italmar Co., Ltd. Thailand.

3.2.2 Glycerol (99.5%) from Italmar Co., Ltd, Thailand.

3.2.3 Hexanal (98%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.4 Heptanal (92%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.5 Octanal (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.6 Nonanal (95%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.7 Decanal (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.8 Undecanal (92%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.9 2-methyl-3-heptanone (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.10 Methyl benzoate (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.11 Benzyl benzoate (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.12 Rose oxide (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.13 Isoeugenol (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.2.14 Terpineol (99%) from Sigma-Aldrich Chemical Co., Ltd. USA

3.3 Equipments

3.3.1. Gas chromatography mass spectrometer (GC-MS) (Agilent 6890) and mass spectrometer detector (Agilent 5973), USA.

3.3.2 Gas Chromatography Olfactory (GCO) (Hewlett Packard 5890), USA equipped with Olfactometry (SGE), Australia

3.3.3 Polar Column DB-wax®, 15 m x 0.32 mm i.d.; 0.5 μm film; Restek, Bellefonte, PA and non polar column HP®-5MS, 15 m x 0.32 mm i.d.; 0.5 μm film (Restek).

3.3.4 Air Pump (XP-2000) from Xingxing Aquariums Co., Ltd. Thailand

3.3.5 Sniffing Bottle (Poly Teflon) from Cabtec (Thailand) Co., Ltd. USA

3.4 Methods

3.4.1 Identification of predominant odorants in Thai desserts flavored by smoking with *Tian Op*

3.4.1.1 Sample preparation

***"Gleep Lum Duan"* (GLD) Preparation.** Wheat flour (60% of total weight) and sugar (10% of total weight) were sieved together. Palm oil (30% of total weight) was slowly added while mixing. After thoroughly mixing, the dough was cut to 1 cm^3 cubes and baked on a flat sheet in an oven at 120°C for 15 minutes. Three separate batches of GLD were prepared.

***"Num Dok Mai"* (NDM) Preparation.** Water (30% of total weight) and sugar (10% of total weight) were mixed and then heated until the sugar was completely dissolved. After cooling to room temperature, rice flour (60% of total weight) was added and the mixture was stirred until evenly distributed. The slurry was transferred to a glass bowl (20 cm diameter x 3 cm high) and steamed for 10 minutes. After steaming, the sample was cut to 1 cm^3 cubes. Three separate batches of NDM were prepared.

Preparation of *Tian Op* Candle. *Tian Op* candle was made from beeswax (24.25 g), benzoin (0.25 g), dried kaffir lime peel (0.25 g), sandalwood (0.25 g) and benzoin (0.25 g). Beeswax was heated by placing in the stainless steel pot until it was soft and pliable. The other ingredients were thoroughly blended with the softened beeswax then the mixture was rolled into a flat sheet (0.3 cm thick x 15 cm width). A cotton wick was placed on the sheet and then the candle was formed by rolling the sheet into a cylindrical shape. At least 1.5 cm of the wick was left exposed from each end of the candle. A *Tian Op* candle was then bent into a half circle shape.

Smoking Process. For smoking, 500 g of sample, in a single layer, was placed into a 1-liter cylindrical glass vessel along with a *Tian Op* candle. The candle was lightened, then the vessel was sealed with a glass cover and the smoking process was allowed to continue for 30 min. (Appendix A)

3.4.1.2 Analysis method

Isolation of volatile compounds. One hundred grams of either an untreated dessert (NDM or GLD) or a smoked dessert (S-NDM or S-GLD) plus 50 □L of an internal standard solution (500 □g/mL of 2-methyl-3-heptanone in methanol) and 1 mL of a butylated hydroxyl toluene solution (100 □g/mL of BHT in ether) were soaked for 1 hour in diethyl ether (50 mL) in a 500-mL glass bottle sealed with a PTFE-lined cap. The solvent layer was removed and the remaining mixture

was extracted two more times with 50 mL ether. The pooled solvent extract was evaporated to 50 mL using a Vigreux column in a 45°C water bath and then subjected to a high-vacuum distillation (5 x 10^{-5} Torr operating vacuum) with clean up step (Karagul-Yuceer et al., 2001) for 3 hours to remove the nonvolatile residue, the sample was kept at room temperature for the first 1.5 hour and then warmed to 50°C using a water bath. The resulting aroma extract was concentrated under a gentle stream of nitrogen gas to 10 mL, dried over 2 g of anhydrous sodium sulfate, and then further concentrated to 200 μL under a nitrogen gas stream.

Aroma extract dilution analysis (AEDA). AEDA was conducted to determine the relative potency of individual odorants according to the method described by Grosch (1993). Stepwise dilutions (1:3) were prepared with diethyl ether. Each dilution was kept in a 2 mL amber vial equipped with PTFE-lined screw cap at -70°C until analysis. The gas chromatography-olfactometry (GCO) system consisted of a 6890 GC equipped with a FID, an on-column injector and an olfactory detection port. Each aroma extract was injected by cool on-column mode (+3°C temperature tracking mode) into a polar capillary column or a non-polar column. At 250°C, column effluent was split 1:5 between FID and olfactory detection port using deactivated fused silica tubing. The GC oven temperature was programmed from 35°C to 225°C at a rate of 10°C/min with initial and final hold times of 5 and 15 min., respectively. Helium was used as a carrier gas at a constant flow rate of 2.2 mL/min (Zhou et al., 2002).

Gas chromatography-mass spectrometry (GC-MS). Each aroma extract (1 μL) was injected by cool on-column method (+3°C temperature tracking mode) into a 6890 GC/5973N MSD. Separations were performed using either a polar capillary column or a non-polar column. The oven temperature was programmed from 35°C to 225°C at a rate of 6°C/ min with an initial and final hold times of 5 and 10 min., respectively. Helium was used as carrier gas at a constant rate of 1.0 mL/min. The MSD conditions were as following: capillary direct interface temperature 280°C; ionization energy 70 eV; mass range 35 to 300 amu; electron multiplier voltage (Autotune + 200 V); scan rate 5.27 scans/s.

Compound identification. Compound identification was based on matching retention indices (on two different GC column phases) and mass spectra of unknown with those of authentic standards. A homologous series of *n*-alkanes was used for the determination of retention indices according to Van den Dool and Kratz (1963).

Quantitative analysis. The concentration of a selected odorant was based on its area response ratio [extracted ion chromatogram area of compound /extracted ion (m/z 128) chromatogram area of the internal standard (2-methyl-3-heptanone). Concentrations were corrected by use of MS response and recovery factors determined by spiking/recovery experiments using unsmoked NDM and GLD as sample matrices and by applying the same extraction and analysis methods described earlier. Determinations were preformed in triplicate. Concentrations were expressed in μg/kg (ppb) on a wet basis.

Determination of odor detection thresholds. ASTM procedure E679-91 (ASTM, 1992) was used to determined orthonasal odor detection thresholds in odor-free water for 2-methyl-(*E*)-2-pentenal, 7-octenal, 8-nonenal, and (*E*)-2-undecenal. Stock solutions were prepared in methanol. Aliquots of the stock solutions were dissolved in the water matrix and then presented to panelists in 125-mL Teflon squeeze bottles (Guadagni and Buttery, 1978). The twelve trained panels (2 males, 10 females, 25-33 years old), approximately trained for 50 hours, were given each concentration (1:3 dilution series) along with two matrix blanks containing the same volume of methanol used in preparing the sample solutions. A group of seven series was tested in ascending order. The individual best estimate threshold was calculated as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response using the criteria (ASTM, 1992). The group best estimate threshold (BET) was calculated as the geometric mean of the individual BETs.

Sensory descriptive analysis. Panelists were selected based on their ability to discriminate odor and flavor differences and to communicate their perceptions by use of duo-trio and ranking/rating tests (Meilgaard et al., 1991). The twelve trained panels selected terms and references to describe the aroma attributes of the unsmoked and smoked desserts (Appendix B) and learned to use an aroma intensity scale anchored by 0 = "none" on the left and 3 = "very" on the right, 0.5 unit increments as previously described (Czerny et al., 1999). For evaluation, samples were presented in PTFE bottles (150 ml) coded with 3-digit random numbers (Zhou et al., 2002). Samples were evaluated in duplicate. Differences among samples were evaluated by analysis of variance with means separation using SPSS software (SPSS Inc., Chicago, IL).

Sensory comparison of aroma models. Triangle difference test (Roessler et al., 1978; Meilgaard et al., 1991) was employed to determine if panelists could detect differences between the each of the complete aroma models and model mixtures in which single odorants or groups of odorants had been omitted. Forty untrained panels participated in the sensory evaluation

testing. Aroma model mixtures were served in 3-digit coded PTFE bottles in random order with all six possible combinations being presented to the panel (Meilgaard et al., 1991). Results were analyzed according to Roessler et al., (1978).

3.4.2 Study of trapping solvent

3.4.2.1 Sample preparation

Preparation of *Tian Op* Candle. *Tian Op* candle was prepared as described in 3.4.1.1

Smoke trapping process. The method of smoke collection was modified from that of Knoll and Talbot (1998). The smoke was generated from manual lighting of *Tian Op* for 30 seconds and then it was drawn by vacuum (pump) at the rate of 50 mL/min into a 100 mL flask containing 30 g trapping solvent. An internal standard solution (100 □L in 1,000 L) was added to the liquid smoke. Three different trapping solvents: propylene glycol, glycerol and odorless water, were used. All samples were kept at -60°C until analysis. The chemical compounds of these samples would be analyzed.

3.4.2.2 Analysis methods

For the analysis of volatile compounds, AEDA, GC-MS, compound identification, quantitative analysis, determination of odor detection thresholds, and sensory evaluation by quantitative descriptive analysis (QDA) were done as described in 3.4.1.2.

3.4.3 Optimization of combustion temperature and time for production of liquid smoke from *Tian Op*

3.4.3.1 Sample preparation

Beeswax (97g), benzoin (1g), dry kaffir lime peel (1g) and sandalwood (1g) were mixed and put in a round bottle. The mixture was then burned at different temperatures: 120±1, 140±1, 160±1, 180±1 and 200±1°C for 4, 6 and 8 hrs. Smoke was purged by nitrogen gas and trapped in 30 g suitable trapping solution selected from 3.4.2.

3.4.3.2 Analysis methods

For the isolation and analysis of volatile compounds, AEDA, GC-MS, compound identification, quantitative analysis, determination of odor detection thresholds, and sensory descriptive analysis were used as in 3.4.1.2. In the hedonic test, liquid smoke samples were

presented in Teflon lined screw cap coded with 3-digit random numbers. The sample orders were randomized for each panelist. All samples were presented at the same time. The panelists should evaluate the intensity of each aroma attributes in samples and marked the responses on 9-point numerical intensity scales anchored on the left with "dislike extremely" on the right with "like extremely". The intensity of all aroma attributes was expressed.

Statistical analysis. Statistical analysis of sensory analysis was done after Randomized Complete Block Design (RCBD) and mean difference was analyzed by Duncan's New Multiple Range Test using SPSS software to select the suitable combustion temperature and time.

3.4.4. Study on *Tian Op* smoke odor mimic

3.4.4.1 Flavor mimic analysis

Odor mimics were prepared by diluting and mixing synthetic standard solutions of different quantity and quality (Table 3.2). These mimic samples were analyzed comparing with liquid smoke from *Tian Op* using different from control test by marking responses on 10 point numerical intensity scales anchored on the left with "distinguish" on the right with "similar". Mimic solution model were adjusted related with flavor active compound in 3.1.

Table 3.1 Formulations of stock solutions used to prepare aroma models

Model	Heptanal (ppm)	Octanal (ppm)	Nonanal (ppm)	Decanal (ppm)	Undecanal (ppm)	Dodecanal (ppm)
1	9.0	9.5	8.5	5.5	4.0	3.5
2	4.5	4.75	4.25	2.75	2.0	1.75
3	18.0	19.0	17.0	11.0	8.0	7.0
4	0	9.5	8.5	5.5	4.0	3.5
5	9.0	0	8.5	5.5	4.0	3.5
6	9.0	9.5	0	5.5	4.0	3.5
7	9.0	9.5	8.5	0	4.0	3.5
8	9.0	9.5	8.5	5.5	0	3.5
9	9.0	9.5	8.5	5.5	4.0	0

CHAPTER 4

RESULT AND DISCUSSION

4.1 Study on flavor compounds in Thai dessert

4.1.1 Study on sensory attributes of *Tian Op* Smoked Desserts

The two types of unsmoked matrices (*Num Dok Mai* and *Gleep Lum Duan*) had markedly different sensory aroma profiles (Figure 4.1). Although waxy and floral notes were detected at moderate intensities in both matrices, the low-fat matrix (NDM) contained pronounced starchy, cooked rice and sweet aromatic notes, which were either found at low intensity or not detected in the high-fat matrix (GLD). Meanwhile, panelists detected a pronounced baked wheat note in GLD, but not in NDM.

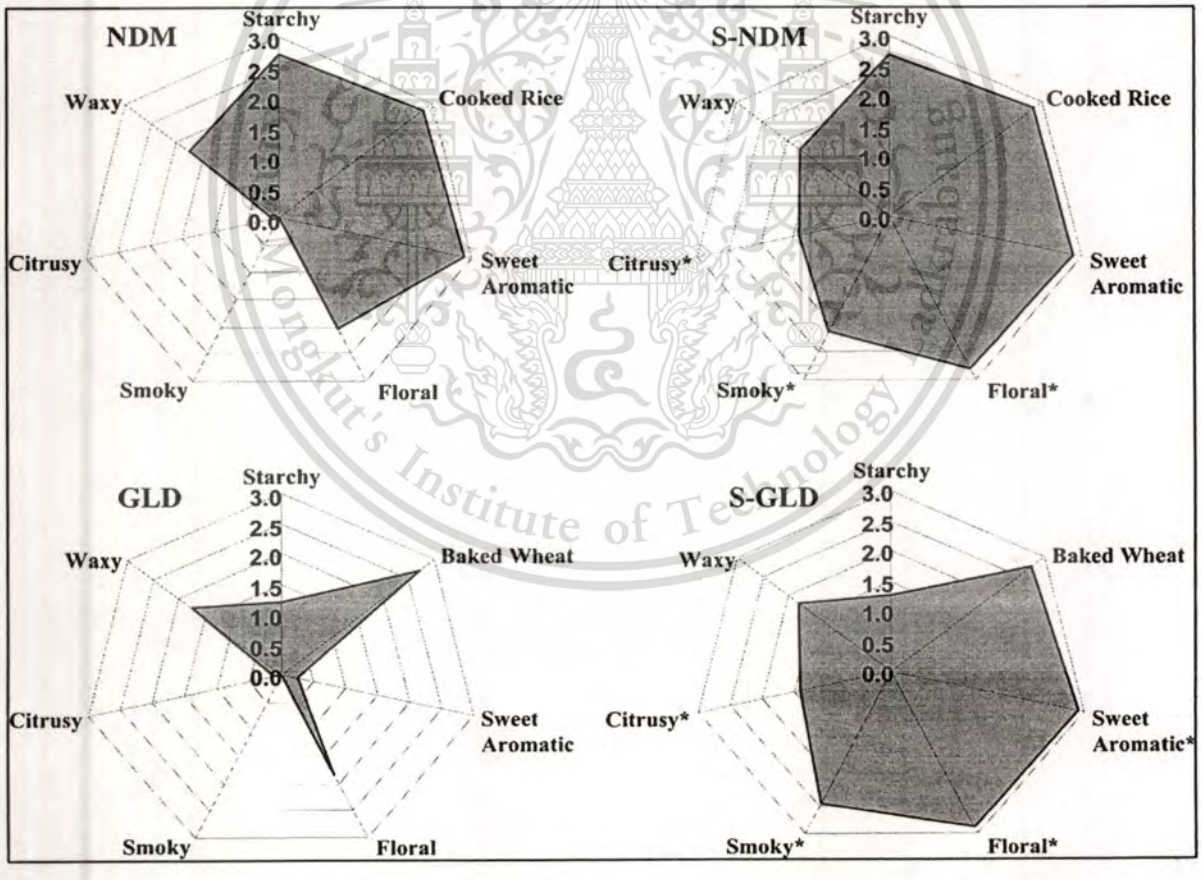

Figure 4.1 Sensory descriptive aroma profile comparison of unsmoked and smoked (S-) *Num Dok Mai* (NDM) and *Gleep Lum Duan* (GLD). Attributes marked with an asterisk are significantly different between unsmoked and smoked desserts ($p \leq 0.05$)

It is noteworthy that both matrices received only low intensity ratings for the citrusy attribute, neither had a perceivable smoky note. As a result of the *Tian Op* smoking process, the intensities of the citrusy, smoky and floral notes increased in both S-NDM and S-GLD. In addition, the smoking process caused an increase in the sweet aromatic attribute in S-GLD, but this note remained at about the same high intensity in S-NDM. Intensities of the remaining sensory attributes (i.e., starchy, waxy cooked rice, and baked wheat) were not affected by the smoking process. The end result was that S-NDM and S-GLD were nearly the same with respect to intensities of the citrusy, smoky, floral, and sweet aromatic notes imparted by the *Tian Op* smoking process.

4.1.2 MajorVolatile Components.

Treatment of NDM and GLD with *Tian Op* smoke resulted in a large increment in hydrocarbons and oxygenated compounds in the smoked desserts (S-NDM and S-GLD, respectively) (Table 4.1). For both S-NDM and S-GLD, the major volatile compounds were *n*-alkanes, R-1-alkenes (C_7-C_{14}), and *n*-aldehydes (C_5-C_{12}). Branched alkanes, R-1, ω-1-alkadienes, unsaturated aldehydes, methyl ketones, and vinyl ketones were some of the minor volatile constituents identified. The occurrence of the homologous series of *n*-alkanes, R-1-monoalkenes, R-1 and ω-1-dialkenes is in agreement with volatile patterns reported for the thermal degradation GC-MS analysis of beeswax (Asperger et al., 1999). It was previously demonstrated that the formation of R-1-monoalkenes in beeswax during thermal degradation is the result of either radical alkyl chain degradation or ester scission of the ester-bound fatty acids (Asperger et al., 1999). Meanwhile, methyl ketones and *n*-aldehydes (homologous series from C_4 to C_{17}) were reported as major degradation products of the thermal degradation of tristearin in air (Selke et al., 1975). It was concluded that there was no specific oxidation pattern and that all of the fatty acid methylene carbon atoms were susceptible to oxidation at high temperatures with preferential attack occurring near the center of the molecule. Similar results were observed for the thermal/oxidative degradation of methyl palmitate (Brodnitz et al., 1968).

4.1.3 Potent Odorants in Desserts Determined by AEDA

The predominant odorants of the unsmoked and smoked desserts were identified by means of GCO and AEDA (Table 4.1).

Table 4.1 Potent odorants in unsmoked and smoked *Num Dok Mai* and *Gleep Lum Duan*

no.[a]	compound	odor[b]	RI[c]			FD-factor[d]		
			WAX	RTX5	NDM	GLD	S-NDM	S-GLD
1	1-buten-3-one	plastic, painty	961	--[e]	nd[f]	nd	3	9
2	pentanal	painty	980	701	nd	nd	<3	9
3	1-penten-3-one	plastic, pungent	1021	680	nd	nd	3	81
4	*unknown*	potato	1042	--	nd	nd	3	27
5	hexanal	green, cut-grass	1081	799	3	3	27	81
6	1-hexen-3-one	plastic, pungent	1096	777	nd	nd	6561	2187
7	*unknown*	potato	1158	--	nd	nd	27	27
8	2-methyl-(*E*)-2-pentenal	dark chocolate	1165	831	nd	nd	81	243
9	heptanal	melon, citrus, orange	1181	902	nd	nd	9	27
10	*unknown*	dark chocolate	1186	--	nd	nd	9	27
11	1-hepten-3-one	plastic, pungent	1196	876	nd	nd	2187	2187
12	(*Z*)-4-heptenal	stale, crabby	1240	901	nd	nd	nd	27
13	styrene	plastic	1241	895	nd	nd	27	81
14	octanal	orange oil	1282	1002	<3	nd	81	729
15	1-octen-3-one	mushroom	1296	976	nd	9	6561	729
16	*unknown*	potato	1303	--	nd	nd	<3	9

Table 4.1 (continue)

no.[a]	compound	odor[b]	RI[c]				FD-factor[d]	
			WAX	RTX5	NDM	GLD	S-NDM	S-GLD
17	(*E*)-2-heptenal	pungent, fruity	1333	956	nd	nd	3	27
18	2-acetyl-1-pyrroline[g]	popcorn	1340	921	3	27	<3	9
19	7-octenal	fresh, melon	1354	996	nd	nd	243	729
20	nonanal	pungent, green	1388	1104	nd	nd	27	27
21	1-nonen-3-one	mushroom, pungent	1398	1078	nd	nd	243	729
22	(*E*)-2-octenal	fresh, melon, green	1436	1058	nd	nd	243	2187
23	8-nonenal	stale, fatty, plastic	1445	1095	nd	nd	6561	2187
24	3-(methylthio)propanal [methional]	potato	1462	906	3	3	nd	nd
25	decanal	pungent, green, soapy	1495	1208	nd	nd	9	9
26	(*Z*)-2-nonenal	hay, stale	1507	1151	nd	<3	243	729
27	(*E*)-2-nonenal	hay, stale, fatty	1535	1162	<3	3	243	729
28	(*E,Z*)-2,6-nonadienal	cucumber	1587	1155	3	3	nd	27
29	undecanal	green, pungent	1600	1303	nd	nd	27	27
30	(*E*)-2-decenal	pungent, soapy	1641	1264	nd	nd	27	27
31	phenylacetaldehyde	rosy, styrene	1653	1049	nd	9	nd	3
32	dodecanal	green, pungent, soapy	1701	1404	nd	nd	3	9

Table 4.1 (continue)

no.[a]	compound	odor[b]	RI[c]			FD-factor[d]		
			WAX	RTX5	NDM	GLD	S-NDM	S-GLD
33	unknown	hay, fatty	1735	- -	9	3	81	27
34	(*E*)-2-undecenal	cilantro, green, soapy	1754	1366	nd	nd	729	81
35	(*E,E*)-2,4-decadienal	fatty, fried	1817	1317	nd	9	27	81
36	2-methoxyphenol [guaiacol]	smoky	1862	1090	<3	<3	729	729
37	4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (HDMF)[g]	burnt sugar, caramel	2034	1071	3	9	243	81
38	(*Z*)-α-santalol[h]	incense, woody, cedar	2350	1674	nd	nd	3	nd
39	(*Z*)-β-santalol[h]	incense, woody, fragrant	2434	1716	nd	nd	9	3
40	vanillin	vanilla	2576	1404	3	nd	729	81

[a] Numbers correspond to those in Tables 4.2.

[b] Odor quality as perceived during GC-O.

[c] Retention indices were calculated from GCO data, WAX = Stabilwax column.

[d] Flavor dilution (FD) factor determined on Sabilwax column; NDM = unsmoked "*Num Dok Mai*", GLD = unsmoked "*Gleep Lum Duan*", S-NDM = smoked NDM, S-GLD = smoked GLD.

[e] - - = not available.

[f] nd = not detected.

[g] Compound was tentatively identified based on comparison of its odor property and retention indices with reference compound.

[h] Compound was identified based on mass spectra and retention indices determined for (*Z*)-α-santalol or (*Z*)-β-santalol in authentic sandalwood oil and by comparison to literature (Howes et al., 2004)

A combined total of 14 odorants were found at low flavor dilution (FD) factors (<27) in the two unsmoked desserts matrices. Six common odorants were detected in both matrices, with more odorants being detected in GLD than in NDM. The majority of the odorants in NDM and GLD were either lipid-derived or thermally generated compounds originating from the ingredients or produced during steaming/baking. The treatment of the NDM and GLD with *Tian Op* smoke caused a large increase in both the number and potencies of the odorants found in the smoked desserts.

A combined total of 39 odorants were detected by AEDA in the smoked desserts, with 36 found in S-NDM and 38 in S-GLD. With the exception of 2-acetyl-1-pyrroline, methional, and phenylacetaldehyde, all of the odorants detected in the unsmoked dessert matrices increased in potency as a result of the smoking process. S-NDM and S-GLD were similar with respect to the potent odorants identified (35 common odorants detected). Odorants with FD factors ≤ 729 in at least one of the two smoked desserts included octanal, 7-octenal, 1-nonen-3-one, (*E*)-2-octenal, (*Z*)-nonenal, (*E*)-2-nonenal, (*E*)-2-undecenal, 1-hexen-3-one, 1-hepten-3-one, 1-octen-3- one, 8-nonenal, guaiacol and vanillin. On the basis of their high overall FD factors ($\leq$ 729), the predominant odorants of both S-NDM and S-GLD were vinyl ketones (C5-C9), *n*-aldehydes (C5-C11), (*E*)-2-unsaturated aldehydes (C8-C11), and □-1-unsaturated aldehydes (C8 and C9).

The majority of the above-mentioned potent odorants were most likely produced as a result of the thermal oxidation of the beeswax components as discussed earlier. Saturated and (*E*)-2-unsatured aldehydes were reported as the most abundant odor active compounds formed during heating of triolein and trilinolein. Vinyl ketones (1-hepten-3-one, 1-octen-3-one, and 1-nonen-3-one), along with aldehydes (hexanal, octanal, nonanal, and (*E*)-2-nonenal) were reported as being primarily responsible for the off-odor associated with thermally oxidized polyethylene. 7-Octenal was previously reported as a volatile decomposition product of thermally oxidized triolein. Sanders et al., (2005) reported that 8-nonenal contributed a "plastic" off-odor to high-density polyethylene packaging. In addition to the above, some odorants may have originated from other ingredients of the *Tian Op* candle, such as vanillin from benzoin (*3*) and (*Z*)-R-santalol and (*Z*)-β-santalol from sandalwood. Likewise, guaiacol may have been derived from the thermal degradation of any of the lignin-containing ingredients (e.g., cotton wick or sandalwood).

4.1.4 Quantitative analysis.

Selected positively identified odorants from Table 4.1 were quantified in the unsmoked and smoked desserts (Table 4.2). The relatively high standard deviations are reflective of the fact that the average concentrations are based on the analysis of three totally independent samples for each type of dessert. NDM and GLD contained a low abundance of odorants. In both S-NDM and S-GLD, the *n*-aldehydes were in highest abundance, followed by vanillin, (*E*)-2-unsaturated aldehydes, □-1-unsaturated aldehydes, vinyl ketones, guaiacol, (*E,E*)-2,4-decadienal, phenylacetaldehyde, and 2-methyl-(*E*)-2-pentenal. All odorants were found at higher abundance in S-GLD than in S-NDM. The GLD matrix had a higher fat content than NDM, and because of its more lipophilic nature, the GLD matrix would be expected to more readily absorb the nonpolar odorants from the *Tian Op* smoke.

4.1.5 Odor-activity values (OAVs).

OAVs were calculated to estimate the potential sensory impact of each odorant based on the quantitative data (Table 4.2). Due to the complexities of the two dessert matrices, OAVs were calculated from both water and oil-based odor detection thresholds. Except for some odorants with low extraction recovery factors (e.g. no. 25, 29, 30, 32, and 34 in Table 4.1), the calculated OAVs in Table 4.2 are in good agreement with FD factors determined by AEDA. The above-mentioned discrepancies can be explained by the fact that recovery factors were not taken into consideration in AEDA, whereas they were used in the quantification method.

Therefore, poorly recovered odorants, the OAVs, which are based on accurate quantitative data, should be more indicative of an odorant's actual potency as long as the odor detection thresholds are reasonably accurate. The vinyl ketones, despite being present in relatively low abundance, had the highest OAVs in both S-NDM and S-GLD. This is due to the overall low odor detection thresholds (in both water and oil) of these odorants. Some other odorants in low abundance and having low odor detection thresholds in water, for example, 8-nonenal and (*E,E*)-2,4-decadienal, had relatively high OAVs.

Likewise, eight compounds found in high abundance, *n*-aldehydes and (*E*)-2-unsatured aldehydes, also had particularly high OAVs on the basis of their odor detection thresholds in water. However, when the odor detection thresholds in oil were considered, the above mentioned OAVs aldehydes were markedly lower. On the basis of the OAV concept, the above mentioned odorants should make the greatest impact on the overall aroma of the smoked

desserts. However, these data are recommended for sensory studies of model mixtures which are necessary to do fully assess of the actual contribution of these odorants for the overall aroma of S-NDM and S-GLD.

Table 4.2 Concentrations and odor-activity values (OAV) of selected volatile components of unsmoked and smoked *Num Dok Mai* and *Gleep Lum Duan*

no.[a]	compound	concentration (μg/kg)[b]				odor threshold (ug/kg)[c]	OAV[d]			
		NDM	GLD	S-NDM	S-GLD		NDM	GLD	S-NDM	S-GLD
3	1-penten-3-one	nd[e]	nd	38.0	311	1.3	--[f]	--	29.2	239
6	1-hexen-3-one	nd	nd	36.4	331	0.024	--	--	1520	13800
11	1-hepten-3-one	nd	nd	55.0	695	0.04	--	--	1380	17400
15	1-octen-3-one	nd	nd	40.4	329	0.005	--	--	5050	41125
21	1-nonen-3-one	nd	nd	55.3	369	0.008	--	--	6910	46100
2	pentanal	2.43	12.8	2100	6110	12	0.2	1.1	175	509
5	hexanal	69.0	94.2	1070	3350	4.5	15.3	20.9	238	744
9	heptanal	nd	nd	1080	2590	3	--	--	360	863
14	octanal	21.3	25.3	1580	5590	0.7	30.4	36.1	2260	7990
20	nonanal	69.5	220	2750	9830	1	69.5	220	2750	9830
25	decanal	15.2	76.1	2260	7150	2	7.6	38.1	1130	3580
29	undecanal	nd	nd	4330	9930	5	--	--	866	1990

Table 4.2 (Continue)

no.[a]	compound	concentration (μg/kg)[b]				odor threshold (ug/kg)[c]	OAV[d]			
		NDM	GLD	S-NDM	S-GLD		NDM	GLD	S-NDM	S-GLD
32	dodecanal	87.4	170	2470	20100	2	43.7	85.0	1240	10050
22	(*E*)-2-octenal	nd	nd	91.3	469	3	--	--	30.4	156
27	(*E*)-2-nonenal	3.90	29.9	76.1	601	0.08	48.8	374	951	7510
30	(*E*)-2-decenal	0.93	14.8	264	1330	0.3	3.1	49.3	880	4430
34	(*E*)-2-undecenal	nd	nd	616	4430	3.4[g]	--	--	180	1290
8	2-methyl-(*E*)-2-pentenal	15.1	31.8	35.5	91.6	580[g]	0.03	0.05	0.1	0.1
19	7-octenal	nd	nd	179	358	*21.5*[g]	--	--	8.3	16.7
23	8-nonenal	nd	nd	104	190	*0.0*[g]	--	--	3470	6330
35	(*E,E*)-2,4-decadienal	2.16	31.9	14.3	162	0.07	30.9	456	204	2314
31	phenylacetaldehyde	2.87	57.7	34.4	78.6	4	0.7	14.4	8.6	19.7
36	2-methoxyphenol	0.48	0.75	105	175	3	0.2	0.3	35.0	58.3
40	vanillin	212	35.7	2150	1550	25	8.5	1.4	86.0	62.0
41	2-pentanone	nd	nd	236	413	2300[h]	--	--	0.1	0.2

Table 4.2 (Continue)

no.[a]	compound	concentration (□g/kg)[b]				odor threshold (ug/kg)[c]	OAV[d]			
		NDM	GLD	S-NDM	S-GLD		NDM	GLD	S-NDM	S-GLD
42	2-hexanone	nd	nd	165	479	930[h]	- -	- -	0.2	0.5
43	2-heptanone	nd	nd	145	426	140	- -	- -	1.0	3.0
44	2-octanone	nd	nd	101	415	50	- -	- -	2.0	8.3
45	2-nonanone	nd	nd	106	330	200	- -	- -	0.5	1.7

[a] numbers correspond to those in Tables 4.1

[b] Average concentration (±percent relative standard deviation)of data from triplicate samples; NDM, un smoked *Num Dok Mai*; GLD, un smoked *Gleep Lum Duan*; S-NDM, smoked NDM ;S-GLD, smoked GLD.

[c]Orthonasal odor threshold in water or in oil (italics) (Guadagni and Buttery, 1978)

[d]Odor-activity value = concentration divided by odor detection threshold.

[e]nd, notdetected. [f] not available. [g]Orthonasal odor detection threshold in water determined in present study.

[h]Orthonasal odor detection threshold in canola oil determined in present study.

4.2 Study on trapping solvent

The three trapping solvents: propylene glycol, glycerol, and odorless water were used to trap smoke from *Tian Op* candle because they were safe, stable, and colorless (Buncel et al., 2005). The identified chemical compounds and quantities from these solvents after trapping are presented in Table 4.3

Table 4.3 The chemical compounds from *Tian Op* smoke in different solvents

compound	concentration (□g/kg)[a]		
	propylene glycol	glycerol	water
aldehyde			
heptanal	88.28±0.69	4.06±0.51	-[b]
octanal	94.58±0.55	3.93±0.62	-
nonanal	82.11±0.76	8.28±0.84	-
decanal	51.95±0.92	-	-
undecanal	36.09±0.55	-	-
dodecanal	33.09±0.86	-	-
tridecanal	35.82±0.56	-	-
tetradecanal	41.19±0.93	-	-
pentadecanal	29.38±0.88	-	-
hexadecanal	32.63±0.42	-	-
heptadecanal	29.56±0.83	-	-
alkane			
decane	335.40±2.37	39.31±0.98	16.62±0.34
undecane	329.20±1.92	36.13±1.94	8.47±0.60
dodecane	419.62±0.75	25.36±1.74	9.81±0.71
tridecane	273.81±0.81	44.27±1.45	7.76±0.62
tetradecane	264.46±0.99	62.79±0.89	3.44±0.12
pentadecane	752.36±1.08	72.98±1.1	3.69±0.27

Table 4.3 (continue)

compound	concentration (□g/kg)[a]		
	propylene glycol	glycerol	water
hexadecane	316.14±0.91	128.11±0.71	4.56±0.38
heptadecane	486.13±1.58	72.15±1.20	3.66±0.68
octadecane	332.09±1.80	248.31±0.78	3.94±0.29
nonadecane	446.94±1.30	76.26±1.36	4.51±0.42
eicosane	282.26±2.79	168.41±0.70	8.31±0.18
heneicosane	511.46±2.14	37.70±0.92	6.67±0.85
docosane	299.63±1.98	121.79±1.22	8.04±0.53
tricosane	1,499.35±1.72	289.57±1.08	12.81±0.78
tetracosane	410.13±1.85	52.87±1.26	24.21±0.75
heptacosane	10,856.73±2.22	79.15±0.42	9.25±0.61
octacosane	448.92±2.80	99.22±1.11	21.73±0.43
nonacosane	5,302.39±2.63	281.93±1.19	65.22±0.96
pentacosane	3,128.65±7.41	292.76±2.03	10.55±0.51
hexacosane	558.24±5.13	69.77±2.63	4.28±0.58
alkene			
1-nonene	8,803.78±1.74	328.54±2.18	131.19±1.27
1-decene	1,627.20±3.02	361.28±1.17	93.73±1.23
1-undecene	10,146.29±1.70	999.13±1.35	46.16±0.10
1-dodecene	9,230.75±2.76	1,926.57±1.07	49.69±0.71
1-tridecene	8,022.33±2.71	442.74±1.02	166.23±0.45
1-tetradecene	5,201.23±1.98	627.92±1.28	48.06±0.52
1-pentadecene	6,017.71±2.32	476.51±1.32	77.63±0.95
1-hexadecene	4,724.78±1.40	1,875.84±2.45	36.70±1.55
1-heptadecene	3,319.98±1.29	2,336.77±1.54	31.89±1.04
5-eicosene	3,732.91±2.01	1,969.67±1.09	57.25±0.42
1-nonedecene	2,956.81±4.84	1,358.45±2.38	45.62±0.69

[a] average concentration (±percent relative standard deviation)of data from triplicate samples; propylene glycol, glycerol and water

[b] not available

The alkenes were found at the highest quantity in all solvents, followed by alkanes and aldehydes. However, the data showed that the solvents had different trapping ability for aldehydes. The odorless water could not trap any aldehyde, while the glycerol could trap three aldehydes (heptanal, octanal, and nonanal) at low concentration, but the propylene glycol could trap many aldehydes at high concentration.

It is not surprising to find more chemical compounds from liquid smoke when using propylene glycol as trapping solvent because most of the volatile compounds are nonpolar. Propylene glycol [$(CH_2)_3(OH)_2$] has lower polarity than glycerol [$C_3H_5(OH)_3$] and water (H_2O). It has two separate hydroxyl (-OH) groups while glycerol has three hydrophilic alcoholic hydroxyl groups (OH^-) and water has two hydrogen atoms covalently bond with a single oxygen atom. The low polarity of propylene glycol is near the polarity of C_7-C_{17} aldehyde, alkene, and alkane in *Tian Op* smoke so that propylene glycol can trap the chemical compounds in *Tian Op* smoke better than glycerol or water (Mcmurry, 1989).

The aldehyde group could be considered a polar functional group because it is a terminal carbonyl group (C=O). The oxygen which is more electronegative than carbon pulls the electrons from the carbon-oxygen bond towards itself, creating an electron deficiency at the carbon atom. However, the aldehydes found in *Tian Op* smoke have low polarity because the R groups of these aldehydes consist of nonpolar hydrocarbons at the point of attachment to the carbonyl group. The longer the R-group, the greater the nonpolar nature of the compound. For this reason, the C_7-C_{17} aldehydes, which have long chain R groups, are less soluble in water but are soluble in less polar solvents (e.g. propylene glycol) (Mcmurry, 1989).

Alkanes and alkenes are chemical compounds that consist only of the elements carbon I and hydrogen (H) (i.e. hydrocarbons), wherein each of these atoms are linked together exclusively by single bonds (i.e. they are saturated compounds). Each carbon atom must have 4 bonds (either C-H or C-C bonds), and each hydrogen atom must be joined to a carbon atom (H-C bonds). A series of linked carbon atoms is known as the carbon skeleton or carbon backbone. Typically the number of carbon atoms is often used to define the size of the alkane (e.g. C_2-alkane).

Alkanes can be linear (general formula C_nH_{2n+2}) where the carbon atoms are joined in a snake like structure, cyclic (general formula C_nH_{2n}, n>2) where the carbon backbone is linked so as to form a loop, or branched (general formula C_nH_{2n+2}, n>3) where the carbon backbone splits off in one ore more directions. Alkanes can also combine any of the linear, cyclic (e.g. polycyclic) and branching structures (no general formula). Saturated oils and waxes are example of larger

alkanes where the number of carbons in the carbon backbone are great than 10. Alkane and alkene are high solubility in oil, propylene glycol, glycerol and water respectively (Mcmurry, 1989). From above mentionable reason propylene glycol is the suitable trapping solvent.

4.3 Study on potent aroma on *Tian Op* smoke

4.3.1 Study on potent odorant desert by AEDA

Extracted smoke was analyzed for the aroma-active components using aroma extract dilution analysis (AEDA). Log_3FD chromatograms are illustrated in Figure 4.2. The log_3FD-factor, characteristic, and concentration of each aroma compound are presented in Table 4.4.

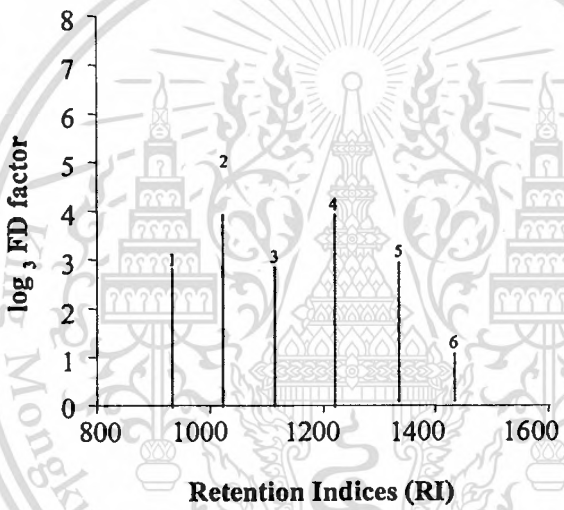

Figure 4.2 Log_3 FD chromatograms of smoke extract. 1= heptanal, 2= octanal, 3= nonanal, 4 = decanal, 5= undecanal, 6= dodecanal

Table 4.4 Identification of aroma-active compounds in smoke extract

no	compound	characteristic[a]	RI[b]		Log_3FD factor	concentration (□g/kg)[c]
			DB-5	DB-wax		
	aldehyde					
1	heptanal	fatty, sweet, fruity	904	1179	3	8.83±0.49
2	octanal	citrus oil	1004	1284	4	9.46±0.74
3	nonanal	fatty-rose like order	1104	1362	3	8.21±0.22
4	decanal	strong, waxy, fatty citrus	1205	1487	4	5.19±0.52
5	undecanal	orange peel	1306	1692	3	3.61±0.73
6	dodecanal	flowery-waxy order	1409	1746	1	3.31±0.40
7	tridecanal	sweet, waxy-fatty-citrus	1512	1804	nd[c]	3.58±0.53
8	tetradecanal	-[d]	1607	1919	nd	4.12±0.24
9	pentadecanal	-	1818	2004	nd	2.94±0.29
10	hexadecanal	-	1921	2126	nd	3.26±0.31
11	heptadecanal	-	2023	2224	nd	2.96±0.65
12	octadecanal	-	2124	2329	nd	2.91±0.36
	alkanes					
13	decane	-	1000	1000	nd	3.35±0.20
14	undecane	-	1100	1100	nd	3.29±0.48
15	dodecane	-	1200	1200	nd	4.20±0.33
16	tridecane	-	1300	1300	nd	2.74±0.19
17	tetradecane	-	1400	1400	nd	2.64±0.25
18	pentadecane	-	1500	1500	nd	7.52±0.84
19	hexadecane	-	1600	1600	nd	3.16±0.21
20	heptadecane	-	1700	1700	nd	4.86±0.84
21	octadecane	-	1800	1800	nd	3.32±0.66
22	nonadecane	-	1900	1900	nd	4.47±0.60
23	eicosane	-	2000	2000	nd	2.82±0.20
24	henicosane	-	2100	2100	nd	5.11±0.97
25	docosane	-	2200	2200	nd	3.00±0.39
26	tricosane	-	2300	2300	nd	15.00±0.29

Table 4.4 (continue)

no	compound	characteristic[a]	RI[b]		Log_3FD factor	concentration (□g/kg)[c]
			DB-5	DB-wax		
27	tetracosane	-	2400	2400	nd	4.10±0.07
28	pentacosane	-	2500	2500	nd	31.27±2.40
29	hexacosane	-	2600	2600	nd	5.58±0.76
30	heptacosane	-	2700	2700	nd	108.57±2.52
31	octacosane	-	2800	2800	nd	4.49±0.60
	alkenes					
32	1-nonene	-	902	1176	nd	8.8±1.24
33	1,9decadiene	-	982	1282	nd	1.63±0.73
34	1-decene	-	991	1360	nd	10.15±1.13
35	1-undecene	-	992	1484	nd	9.23±0.52
36	1-dodecene	-	1091	1689	nd	8.03±0.48
37	1-tridecene	-	1192	1744	nd	5.20±0.16
38	1-tetradecene	-	1295	1802	nd	6.02±0.10
39	1-pentadecene	-	1393	1916	nd	4.73±0.09
40	1-hexadecene	-	1493	2001	nd	3.32±0.05
41	1-heptadecene	-	1546	2124	nd	3.74±0.08
42	1-octadecene	-	1697	2221	nd	2.96±0.16
43	5-eicosene	-	2006	2327	nd	3.02±0.59
44	1-nonadecene	-	1994	2279	nd	2.84±0.34
45	1-heneicosene	-	2094	2458	nd	5.11±0.20
46	1,21-docosadiene	-	2430	2614	nd	2.59±0.71

[a]Retention indices were determined on HP-5 and DB-wax using C_7-C_{15} as external references.

[b]Identification was based on GC-O with an authentic compound.

[c]not detected.

[d] not available.

From Table 4.4, octanal (no. 2, citrus oil) and decanal (no. 4, strong, penetrating, sweet, orange peel odor) were found at the highest odor potency (Log_3FD-factors >3), followed by heptanal (no. 1, Fresh herbaceous), nonanal (no. 3, fresh, citrusy, floral), undecanal (no. 5, soft,

oily-citrusy), and dodecanal (no. 6, floral, sweet). These potent aldehydes were produced from oxidative breakdown pathway of myricyl palmitate which was the main fatty acid in beeswax. The formation of aldehydes could be explained by the autoxidation reaction routes (Kimoto and Gaddis, 1969; Pan et al., 2004). When the fatty acids in beeswax were broken by the heating or autoxidation, alkene and alkane were found (Power and Hauber, 1932; Kimoto and Gaddis, 1969; Ferber and Nursten, 1977; Fine et al., 1999).

Ferber and Nursten (1977) had studied on aroma compounds produced at 65°C and found that octanal, nonanal, and decanal were the aroma-active compounds in molten beeswax. This agrees with the C_7-C_{13} aldehydes found in the present study as the major aroma-active compounds in *Tian Op* smoke. Fine et al., (1999) characterized the particles emitted from burned beeswax candle and also discovered that the compounds in smoke candle were alkenes (C_8-C_{20}) followed by alkanes (C_4-C_{20}) and aldehydes (C_8-C_{11}). He explained that all of the compounds from burned beeswax came from incomplete combustion.

Thus the chemical compounds of smoke from beeswax candle were as same as *Tian Op* smoke candle. From frying palm oil process at 170°C, Fullana et al., (2004) found heptanals, octanals, and nonanals which were formed from oxidation of linoleic acid. They were same chemical compounds found in *Tian Op* smoke which were formed from cleavage of fatty acids in beeswax.

4.3.2. Quantitative descriptive analysis

Tian Op liquid smoke was evaluated by sensory descriptive analysis. The characterization and intensity of *Tian Op* liquid smoke were presented in Table 4.5.

Table 4.5 The intensity and odor attributes of *Tian Op* liquid smoke by descriptive sensory analysis

characteristic	descriptive	reference		intensity
		physical	chemical	(Score)
sweet	sugar sweet	2% sugar	100 ppm nonanal	2.9
wax	wax from beeswax	bees wax	100 ppm octanal	1.87
smoke	burning cotton (Candlewick)	burning cotton	-	2.4
flower	sweet of chrysanthemum flower	chrysanthemum	100 ppm undecanal	2.87
sour	lime peel	lime peel	100 ppm decanal	1.4

Ma et al., (2004) studied the sweet and floral aroma of beeswax and found that these aromas came from the fatty acids in beeswax which were cleaved by heat which caused aldehydes to be produced. Undecanal (sweet floral), one of the aroma-active compounds found in the present study, was also reported in chrysanthemum flower (Lorjaroenphon, 2004). The smoke character might be produced from burned cotton (wick) in candle. It smelled like burned cellulose or burned grass (Meilgaard et al., 1991). The sour smell was one of the important characteristic at moderate intensity. The potent aroma compounds of lime peel were nonanal, octanal, and decanal (Lawrence et al., 1971). These compounds were found as the major aroma-active compounds in *Tian Op* smoke. Meanwhile, waxy and slightly caramel aroma notes had been reported in beeswax that came from a beehive that was composed of wax and sugar from pollen (Tulloch, 1980).

4.4 Optimum combustion conditions (temperature and time) for *Tian Op* liquid smoke production

4.4.1 Quantification of aroma compound by Gas chromatography-mass spectrometer

To eliminate unstable format used in *Tian Op* candle, the mixed ingredients of 97% beeswax, 1% sandal wood, 1% dry kaffir lime peel and 1% benzoin were used instead of the *Tian Op* candle. Propylene glycol was used as trapping solvent from mentionable issue (4.2). The trapping solvent was analyzed by GC-MS and 80 aroma compounds were detected (Table 4.6).

Alkanes were the most abundant chemical compounds found under all conditions. The higher the burning temperature, the more alkanes, alkenes and aldehydes were found. The highest quantity was found at 160°C for 4 hours followed by at 140°C for 6 hours and then at 160°C for 6 and 8 hours.

At low burning temperature, less alkenes and aldehydes were detected because the temperature was not high enough for cleavage of myricyl palmitate in beeswax and other compounds. However, when burning temperature was higher than 160°C, fewer alkenes and aldehydes were found because they were further degraded alkanes (Yoo et al., 1989).

Burning at over 180 °C resulted in more acids and esters being detected because myricyl palmitate in beeswax and other compounds are degraded to short chain acids (Pan et al., 2004).

Table 4.6 The quantity and quality of chemical compounds in extracted smoke at different time and temperature

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
alkanal															
heptanal	-[a]	34.98	274.15	9.20	64.59	81.34	465.20	270.68	69.29	105.73	11.36	168.53	189.24	37.61	94.27
octanal	57.64	143.82	1240.89	99.08	476.59	1177.02	3058.00	1355.25	714.75	557.69	291.51	715.01	952.49	238.63	25.27
nonanal	-	238.93	2098.12	-	1245.50	667.49	5926.87	139.26	955.81	988.19	749.59	303.78	2150.56	1076.54	237.57
decanal	152.29	1021.55	5226.75	891.78	4379.39	11477.72	12525.79	5418.50	3603.65	3258.42	2379.83	4140.96	4644.61	16409.28	70.82
undecanal	-	-	-	-	159.56	2952.77	912.78	206.39	122.74	78.01	74.68	217.74	41.00	-	-
dodecanal	-	-	-	-	230.78	441.55	1110.51	148.49	-	187.18	533.50	-	-	72.50	609.88
tridecanal	-	-	-	-	-	341.83	-	-	101.34	29.86	351.72	101.05	39.39	-	818.45
alkenal															
heptenal	-	-	-	-	-	-	51.69	74.50	9.59	33.49	-	24.98	60.55	-	-
nonenal	-	-	-	-	-	690.17	1390.91	635.00	181.19	236.88	-	298.72	399.28	-	300.83
detenal	-	-	454.69	4.22	123.28	3043.06	9066.63	1445.23	1196.63	765.56	-	966.49	585.62	-	767.56
decadienal	-	-		-	-	207.12	945.32	45.34	65.48	-	-	-	-	-	-
undecenal	-	-	-	-	583.44	2613.50	3566.75	38.94	90.85	-	-	72.72	-	-	453.94

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
tridecenal	-	-	-	-	-	7911.69	-	-	-	-	-	-	-	-	-
alkene															
sabinene	-	-	64.39	-	58.39	-	157.67	139.77	20.43	-	-	-	76.89	-	-
cymene	-	-	-	-	-	54.72	-	-	-	-	-	-	-	-	-
limonene	-	19.56	126.22	13.43	69.83	34.12	-	753.28	-	15.06	-	35.96	638.56	90.56	94.50
benzene	-	-	48.72	7.44	-	-	-	-	-	-	4.76	-	-	-	-
terpene	-	23.33	294.17	13.50	193.22	-	45.88	23.50	47.18	104.88	27.66	-	269.28	45.67	-
sabinenehydrate	-	48.48	154.72	7.99	128.67	96.23	74.61	274.17	91.33	-	86.86	-	-	23.72	-
cubebene	8.40	86.82	301.55	325.61	1178.39	5.73	302.78	342.33	183.09	290.94	589.39	381.54	332.61	417.28	431.28
copaene	17.50	187.30	782.66	70.50	1480.56	397.74	40.55	394.11	351.30	232.39	646.72	351.50	268.50	646.72	22.66
caryophelene	-	131.50	508.55	34.39	870.44	892.35	126.77	314.61	160.27	97.09	530.50	1025.67	77.28	243.40	1655.45
aromadendrene	60.33	453.83	1328.50	156.26	4535.17	7405.73	82.45	2233.45	719.14	6.22	2391.33	65.33	-	846.27	247.78
nephthalene	-	-	-	4.50	-	144.24	30.56	100.50	34.56	8.65	-	33.59	29.28	-	-

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
cadinene	-	123.53	-	8.17	424.72	299.73	97.78	100.89	76.69	1438.68	421.67	-	5.39	228.99	2580.56
muurolene	-	-	235.66	-	-	-	-	-	44.08	-	-		-	-	
alkanes			131.66									67.26			-
nonane	-	-	-	-	-	-	-	-	-	92.06	75.39	61.78	-	-	-
decane	-	-	175.66	-	-	-	-	-	-	71.68	75.39	124.47	20.55	-	-
undecane	-	-	105.72	-	-	104.78	-	-	-	75.56	-	56.09	116.23	-	-
dodecane	-	285.33	88.71	-	335.45	-	124.83	-	-	37.43	-	119.38	1688.50	-	57.72
tridecane	-	416.40	121.99	-	1202.34	329.92	82.39	86.61	20.57	79.14	140.66	62.56	80.22	-	-
tetradecane	-	424.63	475.12	47.81	1526.23	496.75	94.55	140.56	64.31	94.47	285.83	906.87	105.28	37.55	2028.99
pentadecane	71.15	2433.95	295.33	247.61	8340.28	2155.24	43.39	578.61	531.55	747.64	3030.55	251.80	492.22	274.66	117.45
hexadecane	40.71	242.70	674.64	57.09	989.34	511.60	101.45	693.72	290.42	138.68	321.67	1746.72	75.50	115.12	545.64
heptadecane	83.28	209.81	20.55	495.89	4968.44	3727.59	331.44	1587.72	998.80	139.86	3296.72	74.64	795.67	-	739.55
octadecane	9.06	120.92	393.25	82.97	562.44	317.95	55.45	169.27	110.72	118.67	202.72	2989.50	27.34	-	370.70
nonadecane	-	280.76	124.22	120.66	1756.61	1532.79	95.67	1581.28	499.68	1734.30	1610.33	77.13	485.44	1314.77	-

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
eicosane	-	92.06	238.67	27.27	354.11	22.48	895.33	100.11	56.77	417.07	75.28	4428.54	69.28	-	30921.27
henecosane	-	91.00	111.26	46.50	552.61	175.63	472.44	741.94	512.41	2339.31	823.72	774.76	464.33	1345.17	46999.38
docosane	-	31.32	418.47	23.01	177.45	87.93	83.44	136.40	866.49	12.60	118.50	418.03	186.72	185.33	6328.45
tricosane	-	124.67	-	154.72	19.13	306.97	177.39	1967.61	621.74	156.41	2192.06	1027.66	-	-	10918.00
tetracosane	-	-		-	-	-	121.79	117.30	72.79	853.83	172.72		434.34	4293.25	
pentacosane	-	89.57	616.59	173.83	531.12	892.35	1109.56	2314.00	77.14	5743.34	3598.12	10637.65	391.44	4821.33	3611.22
hexacosane	-	-	-	-	-	-	-	-	145.55	712.70	241.29	47.12	-	-	6770.56
bis	154.50	14142.24	14389.43	7658.03	30.387.22	2831.14	5790.77	1633.39	188.11	39502.21	31766.66	3289.97	760.66	1090.95	1302.88
heptacosane	-	665.74	1165.24	716.69	601.92	1711.31	2473.45	5001.44	2756.66	7879.95	8654.72	1333.86	4358.17	9267.83	50196.22
nonacosane	-	61.50	249.52	691.05	912.52	735.67	1728.02	2231.83	705.33	3287.42	3020.21	245.68	98.28	314.66	385.55
octacosane	-	-	-	-	-	138.55	895.33	274.44	28.87	337.66	166.605	667.11	3467.98	890.52	11688.40
acid															
burenoic acid	-	-	-	-	-	-	-	-	33.44	-	17.39	-	82.39	-	25.50
benzoic acid	71.22	-	-	115.39	-	-	44.23	1347.39	1237.31	3771.90	-	-	11340.61	-	-

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
nonanoic acid	-	-	-	-	-	-	-	-	-	-	-	-	15.34	-	-
propanoic acid	-	-	183.86	-	-	-	-	-	-	-	-	-	14.89	-	-
decanoic acid	-	-	118.45	-	-	-	-	-	-	33.37	-	-	33.66	-	169.11
undecanoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	187.06
dodecanoic acid	-	-	-	-	-	-	-	-	-	41.37	-	99.13	15.01	-	286.50
tetradecanoic acid	-	-	-	-	-	-	-	-	-	39.01	-	115.64	-	-	549.50
pentadecanoic acid	-	-	-	18.77	-	-	98.61	111.98	-	-	-	-	-	74.56	-
hexadecanoic acid	-	-	-	-	-	81.00	43.28	-	-	1736.83	-	105.55	520.45	386.50	18.645.72
oleic acid	-	-	-	-	-	-	-	-	-	180.55	-	51.57	20.28	-	9344.55
heptanoic acid	-	-	-	-	-	-	-	-	-	-	715.94	-	-	-	-
prepenoic acid	-	-	242.21	-	-	-	-	-	15.59	-	-	-	-	-	-
ester															
methyl benzoate	-	132.15	-	-	3170.22	1076.96	207.67	1536.61	-	-	3327.67	6332.37	-	168.45	33924.40
pentyl benzoate	-	-	-	-	-	-	-	-	-	42.48	-	-	92.78	-	-

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
allyl benzoate	-	-	-	-	132.76	-	-	-	-	17.84	-	-	-	-	-
methyl caprate	-	-	-	-	137.17	-	-	-	-	-	-	-	-		-
benzyl benzoate	-	-	119.31	51.80	604.12	1602.20	197.33	1500.39	593.19	598.54	673.88	1085.142	408.11	364.61	6106.39
methyl palmitate	-	-	-	-	438.04	-	-	151.67	2827.42	2827.43	348.42	6807.22	-	-	-
methyl Stearate	-	-	-	-	-	-	-	-	25.14	5.14	-	-	-	-	-
oxide															
cis linaloxide	90.03	188.02	611.50	86.44	1340.39	1550.67	1170.67	329.39	771.36	611.85	1109.23	782.43	1219.61	292.50	-
trans-linaloloxide	36.79	70.87	262.06	221.55	705.61	-	654.28	608.39	864.14	-	533.72	-	-	-	1405.89
rose oxide	19.61	-	576.28	105.44	446.56	300.47	317.50	380.61	360.33	359.50	77.83	498.28	-	114.44	843.50
furan															
furfural	-	-	-	-	63.50	-	108.78	-	24.82	36.75	-	0.53	164.57	155.00	110.06
furancarboxaldehyde	-	-	335.44	-	-	-	-	-	-	-	-	262.50	-	-	-
alcohol															
linalool	-	-	-	-	315.06	-	-	112.50	92.29	-	181.73	-	-	-	-

Table 4.6 (continue)

volatile compounds	volatile compounds quantity (ppm)														
	120°C			140°C			160°C			180°C			200°C		
	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr	4 hr	6hr	8 hr
isopulegol	-	-	81.33	4.28	88.67	-	100.50	219.50	42.61	71.81	120.44	-	117.72	114.45	251.39
terpineol	-	-	252.34	-	171.39	-	124.83	464.72	128.77	-	81.06	-	-	-	-
beta-citronellol	-	-	-	-	-	-	-	77.83	-	-	84.22	-	-	-	-
other															
camphor	-	-	-	-	-	32.15	124.17	134.55	-	-	-	-	-	-	-
citronella	-	188.76	283.18	50.61	1135.61	507.78	487.05	849.06	506.52	-	180.34	36.78	259.72	248.83	-
unknown	-	71.22	82.21	71.08	124.53	300.89	321.14	200.14	115.36	43.22	-	-	-	-	-
unknown	-	21.36	35.67	22.35	89.47	208.35	241.03	108.27	88.74	57.54	-	-	-	-	-
unknown	-	107.24	110.39	108.43	208.64	1054.36	1070.24	412.32	201.32	79.83	23.22	-	-	-	-
unknown	-	64.14	72.83	71.49	249.54	708.28	788.21	200.39	233.35	59.21	18.54	-	-	-	-
unknown	-	47.54	64.22	63.13	199.28	700.21	701.47	187.32	189.74	44.14	-	-	-	-	-
unknown	-	33.68	50.09	48.99	108.47	987.32	999.22	178.21	105.21	32.23	-	-	-	-	-

- not available

4.4.2 Potent Odorants by AEDA

Burning at 160°C for 4 hours was the best combusting condition for the formation of prominent aroma-active compounds. The liquid smoke provided as odor which was nearly the same as that from *Tian Op* smoke. Furthermore, the two other conditions, burning at 140°C for 8 hours and 160°C for 6 hours, also gave similar odors (Table 4.7).

The $\log_3$FD factor of octanal, decanal, nonanal and undecanal from liquid smoke were the same as in *Tian Op* smoke while heptanal and dodecanal of liquid smoke gave $\log_3$FD factor higher and lower than in *Tian Op* smoke, respectively. However, both heptanal and dodecanal did not affect the overall odor.

The aroma-active compounds of liquid smoke samples burned at 120, 180, and 200°C were different. The samples burned at 120°C and 140°C for 4 hours contained lower levels and intensities for the volatile compounds while the samples burned at 160°C for 8 hours and at 180°C and 200°C had lower levels of aldehydes, acids and esters because the aldehydes were oxidized at high temperature to alkanes and alkenes. They also contained benzoic acid, heptanoic acid, and benzyl benzoate as aroma-active compounds. The other compounds such as dodecanoic acid, tridecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, and oleic acid had $\log_3$FD-factors less than 2, which was means they had only minor aroma impact.

From the burning temperature studies it was found that the quantity of benzoic acid was the highest followed by oleic acid and heptanoic acid, respectively. Sabinene, limonene, terpene, sabinene hydrate and cadinene which came from breakage of palmitate chain by heat in directly burning process were also found (Table 4.6).

The oxidative reactions of myricyl palmitate were responsible for the formation of alkanes and 1-alkene series, aldehydes, ketones, alcohols, esters, and fatty acids (Parliment et al., 1989). If the mixed ingredients (97% beeswax, 1% dry kaffir lime peel, 1% sandal wood, and 1% benzoin) were heated at high temperature, the saturated fatty acids were oxidized. It was believed that the oxidative mechanism involved the formation of monohydroperoxides and oxygen attack occurred for all chemical groups of the fatty acid chain (Parliment et al., 1989). Power and Hauber (1932) stated that formation of the odorous aldehydes and ketones (sweet volatile) came from the myricyl palmitate in beeswax.

Table 4.7 Identification of aroma-active compounds in extracted smoke from burning the ingredients at different time and temperature

active compounds	RI		characteristic	$\log_3$FD-factor														
				120 °C			140 °C			160 °C			180 °C			200 °C		
	DB-5	DB-wax		4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr
alkanal																		
heptanal	904	1179	green-leafy-waxy	-[a]	-	1	-	-	1	1	-	-	-	-	-	-	-	-
octanal	1005	1284	citrus, orange-lemon. green	-	-	2	-	1	4	4	4	2	2	1	1	2	1	-
nonanal	1104	1362	fresh, citrus, floral, green	-	1	1	-	3	3	3	1	2	2	3	1	3	3	1
decanal	1208	1487	strong, penetrating, sweet, orange peel odor	-	1	1	1	2	3	4	2	2	2	2	2	2	3	-
undecanal	1306	1692	soft, oily-citrusy odor	-	-	-	-	2	3	4	2	2	2	2	2	1	-	-
dodecanal	1409	1746	sweet, waxy-fatty-citrus	-	-	-	-	1	3	3	1	-	1	3	-	-	-	2
tridecanal	1512	1804	citrus-peel, fatty-waxy	-	-	-	-	-	-	-	1	1	-	1	1	-	-	2
alkenal																		
nonenal	1507	1151	cucumber, green, watermelon	-	-	-	-	-	1	1	1	1	1	-	1	1	-	1
undecenal	1641	1264	mild citrus-fruity	-	-	-	-	1	1	-	1	-	-	-	-	-	-	1

Table 4.7 (continue)

active compounds	RI		characteristic	$\log_3$FD-factor														
				120 °C			140 °C			160 °C			180 °C			200 °C		
	DB-5	DB-wax		4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr
alkene																		
sabinene	980	1117	woody	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-
limonene	1036	1195	sweet, citrus	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-
terpene	1065	1242	Pungent, glass	-	-	1	-	1	-	1	1	1	-	-	-	1	-	-
sabinenehydrate	1075	1464	Citrus	-	-	1	-	1	1	1	2	1	-	1	-	-	-	-
cadinene	1543	1755	dry, woody, slight burnt,	-	-	1	-	1	1	1	1	1	2	1	-	-	1	-
acid																		
benzoic acid	1221	-	very weak, balsamic odor	-	-	-	-	-	-	-	-	1	1	-	2	2	2	3
oleic acid	1673	-	Pungent	-	-	-	-	-	1	1	1	-	2	2	1	1	1	4
ester																		
benzyl benzoate	1311	-	faint, sweet, balsamic	-	-	1	-	2	3	1	3	2	2	2	3	2	2	4
oxide																		
cis linaloxide	1123	-	Kaffir lime, sweet, floral	-	-	1	-	3	3	3	1	2	2	3	2	3	1	-
rose oxide	1452	1404	Sweet floral	-	-	3	1	2	2	2	2	2	2	1	2	-	1	3

Table 4.7 (continue)

active compounds	RI		characteristic	$\log_3$FD-factor														
				120 °C			140 °C			160 °C			180 °C			200 °C		
	DB-5	DB-wax		4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr	4hr	6hr	8hr
alcohol																		
isopulegol	1168	1574	Kaffir lime, camphoraceous sweet, floral, green	-	-	-	-	-	-	1	1	-	-	-	-	1	1	1
terpineol	1178	1677	Kaffir lime, pungent, dry, green weedy	-	-	1	-	1	-	1	1	1	-	-	-	-	-	-
unknown	790	1034	fruity, berry, woody	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-
unknown	815	1143	green, fruity, Sweet	-	-	-	-	-	1	1	1		-	-	-	-	-	-
unknown	1189	1520	floral, Woody	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
unknown	1307	1652	sweet, Fruity, Woody	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
unknown	1324	1759	fruity, herbal, floral	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
unknown	1336	1826	fruity, sweet	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-

- not available

4.4.3 Sensory analysis

4.4.3.1 Quantitative descriptive analysis

From Table 4.8 it can been seen that wax, oil, sour, sweet sugar and balsamic notes were the positive aroma characteristics of liquid smoke, while medical, metallic, cool, and sharp were negative characteristics. Liquid smoke produced from burning at 160°C for 4 hours resulted in the intensity of the characteristic compounds being nearly the same as *Tian Op* smoke. Samples burned at low temperature (<140°C) had less odor while samples burned at higher than 160°C had low sweet sugar but high medical, metallic, cool, and sharp characters.

Liquid smoke produced from the sample burned at high temperature (>160°C) gave unpleasant odors. Comparing between sensory evaluation result and aroma-active compound analysis it was found that burning at 160°C for 4 hours was the most suitable condition because the liquid smoke obtained gave nearly the same aroma as *Tian Op* smoke.

Table 4.8 Quantitative analysis of 15 conditions in extracted smoke at different time and temperature

descriptor	reference		score												
	physical	chemical	120 °C			140 °C			160 °C			180 °C			200 °C	
			4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
wax	beeswax	100 ppm octanal	1	1	1.07	1.42	1.56	2.64	2.91	2.89	2.75	2.82	2.73	2.65	2.62	2.51	2.50
oil	oil	100 ppm nonanal	1	1	1.05	1.32	2.38	2.80	2.93	2.72	2.64	2.71	2.51	2.31	2.34	2.21	2.17
sour	lime peel	100 ppm decanal	1	1	1	1.73	2.47	2.53	2.55	2.32	2.15	2.01	1.99	1.88	1.92	1.89	1.73
sweet	2% sugar	100 ppm undecanal	1	1	1.04	1.05	2.38	2.75	2.94	2.44	1.73	1.52	1.43	1.32	1.35	1.23	1.07
balsamic	benzoin	1000 ppm benzyl benzoate	1	1	1	1.32	1.42	1.48	1.49	1.78	1.99	2.04	2.43	2.59	2.587	2.78	2.85
medical	medicine	terpineol	1	1	1	1	1	1	1	1.12	1.37	1.43	1.76	1.97	2.12	2.34	2.49
metallic	metal	1000 ppm rose oxide	1	1	1	1	1	1	1	1.23	1.42	1.51	1.73	1.92	2.21	2.29	2.53
cool	mint	isopuagol	1	1	1	1	1	1	1	1.08	1.12	1.24	1.31	1.39	1.42	1.45	1.53
sharp	100 ppm methyl benzoate	100 ppm methyl benzoate	1	1	1	1	1	1	1	1.21	1.54	1.43	1.76	2.08	2.45	2.59	2.71

4.4.3.2 Sensory Analysis on preference of liquid smokes

The sensory evaluation result of 15 liquid smoke samples created at different temperatures and times tested by 50 panelists using hedonic analysis is shown in Table 4.9.

Table 4.9 Preference score of liquid smoke produced under different burning conditions

temperature (^{o}C)	time (hr)	preference score
120	4	4.10 ± 0.87^{c}
	6	4.04 ± 0.79^{c}
	8	4.26 ± 0.57^{c}
140	4	3.99 ± 0.84^{c}
	6	7.04 ± 0.85^{b}
	8	7.47 ± 0.51^{b}
160	4	8.09 ± 0.59^{a}
	6	7.37 ± 1.45^{b}
	8	7.24 ± 2.17^{b}
180	4	4.24 ± 0.84^{c}
	6	4.16 ± 0.76^{c}
	8	2.27 ± 0.86^{d}
200	4	2.12 ± 0.74^{d}
	6	1.96 ± 0.56^{d}
	8	1.52 ± 0.42^{e}

mean value within the same column followed by different superscript differs significantly by Duncan's multiple range test ($p\leq0.05$)

The sample burned at 160^{o}C for 4 hours received the highest score of acceptance followed by the samples burned at 140^{o}C for 8 hours, 160^{o}C for 6 hours, and 140^{o}C for 6 hours respectively. This was because the liquid smoke produced by burning at 160^{o}C for 4 hours had not only higher sweet sugar characteristic which was the important characteristic *Tian Op* smoke but it also had lower intensity of sour, balsamic, medical, metallic, and sharp characteristics. The samples produced from burning at over 180^{o}C got lower score because they had high intensity of sour, balsamic, medical, metallic, and sharp characteristics.

4.5. Study on flavor mimic

Tian Op aroma was mimicked by mixing together standard compounds as found in *Tian Op* smoke. There were nine different models of mixing as following: same quantity as in smoke (model 1), half quantity (model 2), twice quantity (model 3) while in model 4-9 heptanal, octanal, nonanal, decanal, undecanal and dodecanal were not included respectively. These samples were evaluated by 50 panelists using Different from Control Test and Preference Test. The result was shown in Table 4.10.

Table 4.10 Difference from control and preference evaluation between flavor mimic model and *Tian Op* liquid smoke

Model*	score	
	mean difference	likeness
1	0.07	8.79 ± 0.25^{a}
2	-4.26**	2.01 ± 0.97^{b}
3	4.30**	2.14 ± 1.02^{b}
4	-2.22**	3.67 ± 0.57^{b}
5	-2.26**	3.54 ± 1.32^{b}
6	-2.24**	3.09 ± 0.94^{b}
7	-2.30**	3.57 ± 0.87^{b}
8	-2.27**	3.24 ± 0.67^{b}
9	0.04	8.54 ± 0.43^{a}

*models of odor mimics was shown in Table 3.1

**The mean difference is significant different at 0.05 levels

mean value within the same column followed by different superscript differs significantly by Duncan's multiple range test ($p\leq0.05$)

The sensory score as show in Table 4.10 came from comparing between *Tian Op* smoke and nine different flavor mimic samples. The mean difference score equal to zero meant that the flavor mimic model was not different from *Tian Op* flavor. However, a plus score meant the intensity was greater and a minus score meant less intensity from control (*Tian Op* smoke). Models 1 and 9 received a mean difference score near zero which meant that these two samples had about the same aroma attributes as *Tian Op* smoke. These also received the highest likeness

score. Although dodecanal was not included in model 9, the score was not significantly different from model 1. This showed that dodecanal had little or no aroma impact on *Tian Op* smoke aroma.

CHAPTER 5

CONCLUSIONS

1. The quantity of hydrocarbons and oxygenated compounds in smoked *Gleep Lum Duan* was higher than in smoked *Num Dok Mai* dessert. Most of the volatile compounds in smoked *Num Dok Mai* which contained no lipid were alkenes, followed by acids, aldehydes, alkanes, ketones, and alcohols while most of volatile compounds in smoked *Gleep Lum Duan* which contained oil were alkenes, followed by acids, aldehydes, alkanes, ketones, and alcohols.

2. Octanal ($\log_3$ FD factor = 4), decanal ($\log_3$ FD factor = 4), heptanal ($\log_3$ FD factor = 3), nonanal ($\log_3$ FD factor = 3), and undecanal ($\log_3$ FD factor = 3) were the prominent aroma-active compounds in *Tian Op* smoke. From descriptive analysis, it was found that the prominent characteristics of *Tian Op* smoke were sweet, flower, smoke, wax and sour.

3. Propylene glycol was the suitable trapping solvent because it could trap aldehydes which were the aroma-active compounds in *Tian Op* smoke better than glycerol and water.

4. The optimum condition to produce *Tian Op* smoke was burning at 160 $^{\circ}$C for 4 hours from three reasons that the AEDA result, quantitative descriptive analysis and sensory analysis, were all positive. The AEDA result showed the same characteristics as *Tian Op* smoke. The quantitative analysis gave the wax, oil, sour, sweet sugar and balsamic which were the positive characteristics. While the preference score from sensory analysis was the highest.

5. From the mimic flavor test, two models (model 1 and 9) gave the same aroma as *Tian Op* smoke. Model 1 composed of 9 ppm heptanal, 9.5 ppm octanal, 8.5 ppm nonanal, 5.5 ppm decanal 4.0 ppm undecanal and 3.5 ppm dodecanal and model 9 composed 9 ppm heptanal, 9.5 ppm octanal, 8.5 ppm nonanal, 5.5 ppm decanal and 3.5 ppm dodecanal. It was also found that dodecanal had no effect on the overall aroma.

CHAPTER 6

SUGGESTIONS

1. Thus, the predominant odor in *Tian Op* smoke were *n*-aldehydes, (*E*)-2-unsaturated aldehydes, □-1-unsaturated aldehydes, and vinyl ketones which can be produced from beeswax. Therefore, only beeswax can be used in *Tian Op* liquid smoke production, while the other ingredients are not necessary.

2. Although the liquid smoke may useable to satisfy the Thai desserts market demand, it stills is not the easy to use. From a marketing point of view, the *Tian Op* smoke in powder form will have greater ease of use. However, a marketing survey should be done to confirm that this idea will be valuable for production.

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APPENDIX

APPENDIX A

Method for smoking dessert with *Tian Op*

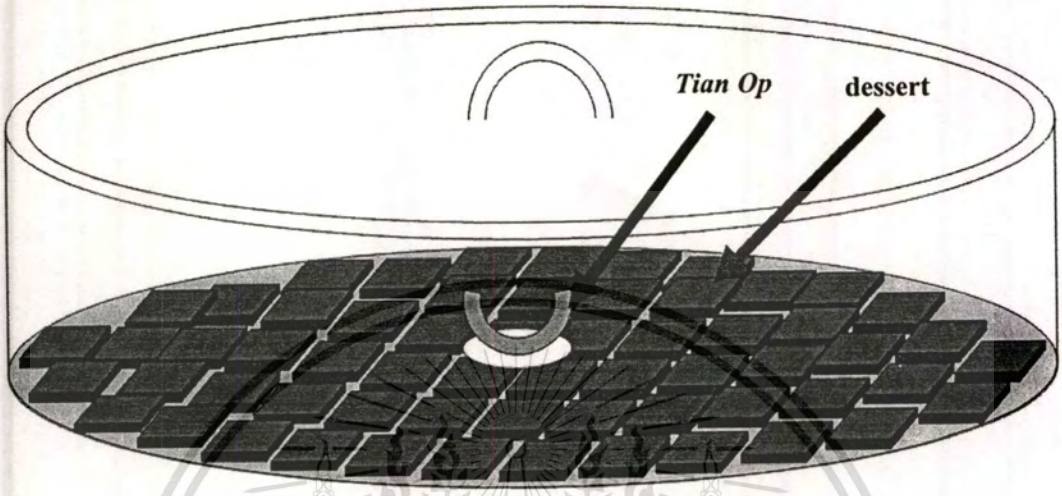


Figure A Smoking Apparatus used in the experiment

APPENDIX B

Sensory evaluation

Appendix B1 Sensory description of quantitative descriptive analysis

Table B1 Attributes, definitions, and references for the sensory descriptive analysis of unsmoked and smoked *Num Dok Mai* and *Gleep Lum Duan*.

attribute	definitions	reference
starchy	aroma associated with starchy grains	raw rice or wheat grains
cooked rice	aroma of cooked rice	cooked rice
baked wheat	aroma of baked wheat	baked wheat
sweet aromatic	aroma associated with the aroma of sugar	Cane sugar
smoky aroma	Associated with wood smoke	burnt cotton
foral	Aroma associated with flowers	ylang-ylang flower (*Cananga odorata*)
citrusy	aroma associated with lime peel	fresh lime peel
waxy	aroma associated with beeswax	beeswax

Appendix B2 Questionnaire for sensory evaluation

Appendix B2.1

QDA test for unsmoked and smoked *Num Dok Mai* and *Gleep Lum Duan*.

Name..... Date .....

Instruction:

1. Taste the sample labeled control first. The intensity for each attribute is assigned as zero
2. Taste the test samples, one at a time.
3. Rate the size of the different for each attribute between each test sample and the control
4. Mark on the scale to indicate the size of difference

Starchy

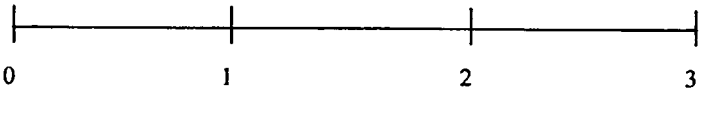
Cooked rice

Sweet aromatic

Smoky aroma

Foral

Citrusy



Waxy

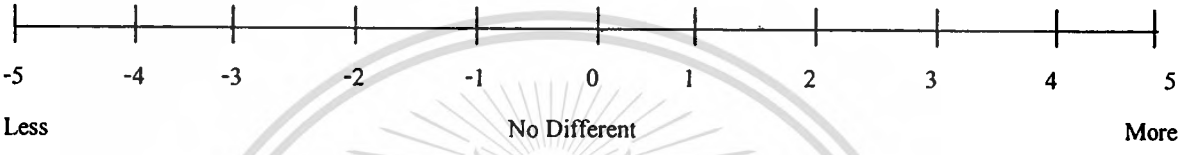
Appendix B2.2

Different from control test for flavor mimic solution model

Name.....Date .....

Instruction:

1. Taste the sample labeled control first. The intensity for each attribute is assigned as zero
2. Taste the test samples, one at a time.
3. Rate the size of the different for each attribute between each test sample and the control
4. Mark on the scale to indicate the size of difference



Appendix B2.3

Preference test for flavor mimic solution model

Name.....Date .....

Instruction: Taste the sample from left to right and put the score

9 = like extremely 8 = like very much 7 = like moderately

6 = like slightly 5 = neither like nor dislike 4 = dislike slightly

3 = dislike moderately 2 = dislike very much 1 = dislike extremely

Sample Code	Score

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