CHAPTER 1
INTRODUCTION

1.1 Rice

Rice (genus *Oryza*) is the most important staple food for a large part of the world's human population. Most countries cultivate rice from the *Oryza* genus, which has more than twenty different species. *O. sativa* L. and *O. glaberrima* Steudel are the most widely grown and are cultivated primarily in Asia and Africa, respectively.[1] Rice is cultivated mostly in Indochina, Thailand, India, Pakistan, Brazil, Japan, Korea and Southern U.S.A. In Thailand, rice is grown in the most provinces, though the highest concentration is found in six plains; the upper northern, lower northern, central, upper northeastern, lower northeastern and southern plains. Due to different soil conditions and climate, each plain produces different rice varieties with different qualities. There are many different varieties of rice available, which contribute distinct characteristics to regional cooking. Aroma character of different rice varieties is directly caused by the total volatile compounds present in the grains as well as those generated when the rice is heated during cooking. Aromatic or fragrant rice has earned reputation and wide popularity in Thailand. This rice is highly valued throughout Asia and also has wider acceptance in Europe, Australia, USA and the Middle East.[2]

1.1.1 Morphology[3]

Cultivated rice is generally considered a semiaquatic annual grass, although in the tropics it can survive as a perennial, producing new tiller from nodes after harvest. At maturity the rice plant has a main stem and a number of tillers. Each productive tiller bears a terminal flowering head or panicle. Plant height varies by variety and environmental conditions, ranging from approximately 0.4 to over 5 meter in some floating rice. The morphology of rice is divided into the vegetative phases (including germination, seedling, and tillering stages) and the reproductive phases (including panicle initiation and heading stages).

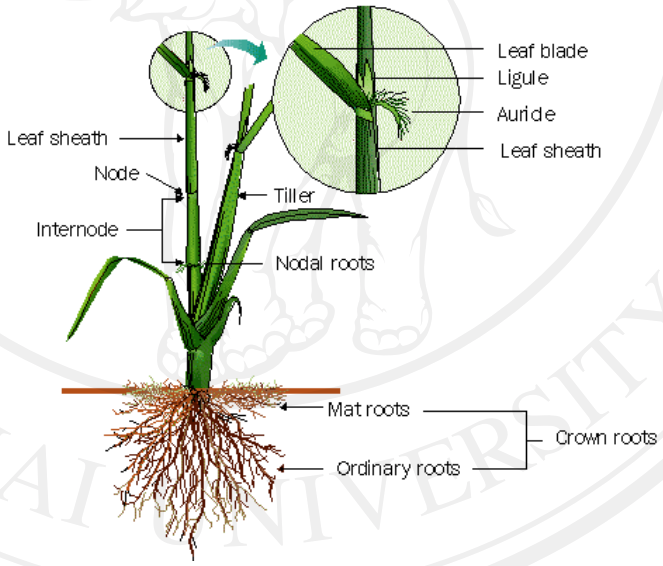

Figure 1.1 Morphology of rice[4]

The growth duration of the rice plant is 3-6 months, depending on the variety and the environment under which it is grown. During this time, rice completes two distinct growth phases: vegetative and reproductive. The vegetative phase is

subdivided into germination, early seedling growth, and tillering; the reproductive phase is subdivided into the time before and after heading, i.e., panicle exertion. The time after heading is better known as the ripening period.

Potential grain yield is primarily determined before heading. Ultimate yield, which is based on the amount of starch that fills the spikelet, is largely determined after heading. Hence, agronomical it is convenient to regard the life history of rice in terms of three growth phases: vegetative, reproductive, and ripening. A 120 days variety, when planted in a tropical environment, spends about 60 days in the vegetative phase, 30 days in the reproductive phase, and 30 days in the ripening phase.

The growth of rice plant is divided into three phases:

1. Vegetative phase
2. Reproductive phase
3. Ripening phase

These stages are numbered and described as follows:

Stage 0: Germination to emergence

Stage 0 is from germination to emergence (see Figure 1.2). Seeds are usually peregrinated by soaking for 24 hours and incubating at 25°C for another 24 hours. After peregrinations the radicle and plumule protrude through the hull. By the second or third day after seeding in the seedbed, the first leaf breaks through the

coleoptiles. The end of stage 0 shows the emerged primary leaf (still curled) and an elongated radical.

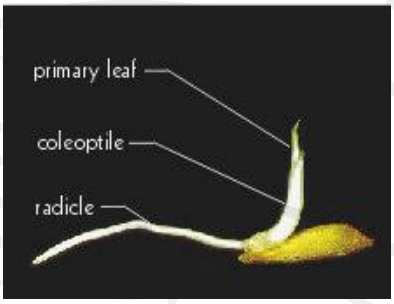

Figure 1.2 Germination to emergence stages of rice growth

Stage 1: Seedling

Stage 1 is called “seedling” (see Figure 1.3). This stage starts right after emergence and lasts until just before the first tiller appears. During this stage, seminal roots and up to five leaves develop. As the seedling continues to grow, two more leaves develop. Leaves continue to develop at the rate of 1 every 3-4 days during the early stage. Secondary adventitious roots that form the permanent fibrous root system rapidly replace the temporary radical and seminal roots.

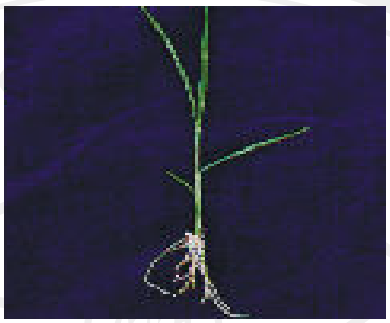

Figure 1.3 Seeding stage of rice growth

Stage 2: Tillering

Stage 2 is called “tillering” (see Figure 1.4). This stage extends from the appearance of the first tiller until the maximum tiller number is reached. Tillers emerge from the auxiliary buds of the nodes and displace the leaf as they grow and develop.

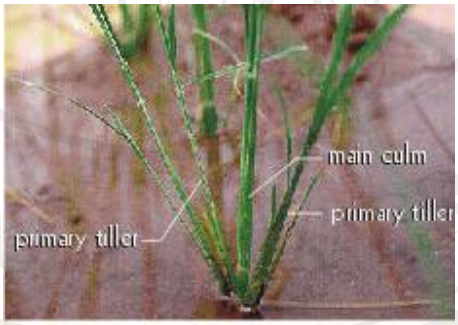

Figure 1.4 Tillering stage of rice growth

Stage 3: Stem elongations

Stage 3 is called “stem elongation” (see Figure 1.5). This stage may begin before panicle initiation or it may occur during the latter part of the tillering stage. Thus, there may be an overlap of stages 2 and 3. The tillers continue to increase in number and height, with no appreciable senescence of leaves noticeable. These first 4 stages make up the vegetative phase, the first phase of rice plant growth.

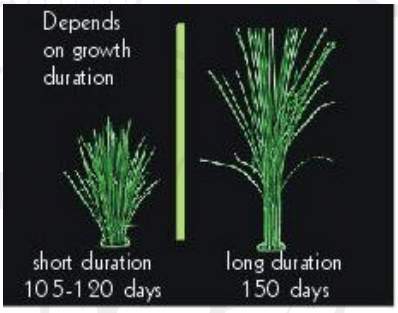

Figure 1.5 Stem elongations stage of rice growth

Stage 4: Panicle initiations to booting

The initiation of the panicle primordium at the tip of the growing shoot marks the start of the reproductive phase (see Figure 1.6). The panicle primordium becomes visible to the naked eye about 10 days after initiation. At this stage, 3 leaves will still emerge before the panicle finally emerges. In short-duration varieties, the panicle becomes visible as a white feathery cone 1.0 -1.5 mm long. This bulging of the flag leaf sheath is called booting. Booting is most likely to occur first in the main culm.

Figure 1.6 Panicle initiations to booting stages of rice growth

Stage 5: Heading or panicle exsertion

Stage 5, heading, is marked by the emergence of the panicle tip from the flag leaf sheath (see Figure 1.7). The panicle continues to emerge until it partially or completely protrudes from the sheath.

Figure 1.7 Heading or panicle exsertion stages of rice growth

Stage 6 flowering

Stage 6 is called “flowering” (see Figure 1.8). It begins when anthers protrude from the spikelet and then fertilization takes place. At flowering, the florets open, the anthers protrude from the flower glumes because of stamen elongation, and

the pollen is shed. The florets then close. The flowering process continues until most of the spikelets in the panicle are in bloom.

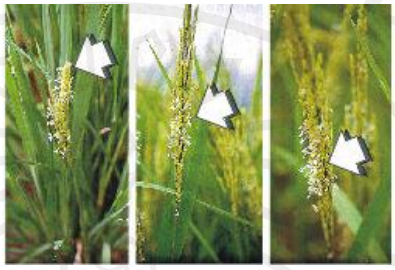

Figure 1.8 Flowering stage of rice growth

Stages 4, 5 and 6 constitute the reproductive phase, the second phase of rice growth.

Stage 7: Milk grain stage

The last 3 stages of growth, stages 7, 8, and 9 comprise the ripening phase. In this milk grain stage, the grain has begun to fill with a milky material (see Figure 1.9). The grain starts to fill with a white, milky liquid, which can be squeezed out by pressing the grain between the fingers. The panicle looks green and starts to bend. Senescence at the base of the tillers is progressing. The flag leaves and the two lower leaves are green.

Figure 1.9 Milk grain stage of rice growth

Stage 8: Dough grain stage

During this stage, the milky portion of the grain first turns into soft dough and later into hard dough (see Figure 1.10). The grains in the panicle begin to change from green to yellow. Senescence of tillers and leaves is noticeable. The field starts to look yellowish. As the panicle turns yellow, the last two remaining leaves of each tiller begin to dry at the tips.

Figure 1.10 Dough grain of rice growth

Stage 9: Mature grain stage

In this stage, the individual grain is mature, fully developed, hard, and turned yellow (see Figure 1.11). The upper leaves are now drying rapidly although the leaves of some varieties remain green. A considerable amount of dead leaves accumulate at the base of the plant.

Figure 1.11 Mature grain stages of rice growth

Stages 7 through 9 correspond to the ripening phase, the last phase in the development of the rice plant.[5]

1.1.2 Aromatic rice

Aromatic or fragrant rice is special rice sold at a premium price in local and export markets because of their superior grain qualities and pleasant and distinct aroma. Among them, the Basmati rice of India and Pakistan and the Jasmine type rice of Thailand are the aromatic cultivars commonly sold in world trade. These rice are highly valued throughout Asia[6] and also have wider acceptance in Europe[7], Australia[8] USA and the Middle East.[9]

Quality of aromatic rice are characterized not only by aroma but also several traits like grains length and width, elongation after cooking, amylase content and gelatinization temperature. Among different quality traits, aroma is considered the most important. A ‘popcon’-like aroma component 2-acetyl-1-pyrroline, has been reported as an important flavor component of several aromatic rice varieties.

Rice has been grown in Thailand for more than 5000 years and the Thai aromatic rice, locally known as Khao Hawm (fragrant rice), is considered to be the national pride of the Thai people. Khao Dawk Mali (KDML) 105, commonly known in food markets as ‘‘Jasmine Rice’’ or ‘‘Thai Hom Mali Rice’’, is the most popular aromatic rice variety consumed in Thailand and some nearby countries. It has, for the last decade, gained increasing popularity in many other countries in Asia, Europe and the United State of America, due to its pleasant aromatic character and nice texture.[10] Because of its famous reputation in appearance, cooking quality and high aroma level, the rice has also gained popularity throughout the world food market.[11]

1.2 2-Acetyl-1- pyrroline (2AP), a key aroma compound of rice

2AP was firstly identified by Buttery and co-workers[12] and suggested as the characteristic compound of aromatic rice. It is a five-membered *N*-heterocyclic ring compound with the molecular formula C_6H_9NO and has MW for 111.14 (see Figure 1.12). 2AP had also been identified among the volatiles of pandan (*Pandanus amaryllifolius*),[13] bread flowers (*Vallaris glabra*),[14] boiled potatoes,[15] roasted wild

mango seeds,[16] pan-fired green teas,[17] cured tobacco leaves[18] and boiled or fried mung bean.[19]

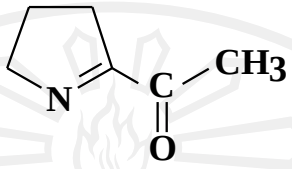

Figure 1.12 Structure of 2AP

Buttery and co-workers[20] isolated and identified 2AP as an important compound contributing to the aromatic odour. They suggested that 2AP was a major contributor to the popcorn-like aroma in several of the Asian aromatic rice varieties. Odour quality evaluation order of the amount of popcorn-like odour in 10 different rice varieties ranked them in general order of the concentration of this compound (Tsugita[21]). Orientals normally describe the aroma of the aromatic rice as being pandan-like. Paule and Powers[22] reported 2AP in Basmati 370, and aromatic rice from Pakistan, and positively correlated the 2AP concentration with the characteristic aroma of aromatic rice. Lin and co-workers[23], Ahmed and co-workers[24], and Tanchotikul and Hsieh[25] confirmed the reports of Buttery and co-workers[26] and Paule and Power[22] that 2AP was the characteristic odour of aromatic rice varieties. It is a common practice in Asia that Pandan (*Pandanus amaryllifolius*) leaves areincluded in cooking non-aromatic rice to give it an aroma. Buttery and co-workers[27] analysed Pandan leaves and found that the major volatile component was 2AP. They found a high correlation between the 2AP in Pandan leaves and aromatic rice. The

concentration of 2AP in Pandan leaves was 10 times greater than aromatic rice and 100 times greater than non-aromatic rice. The concentration of 2AP was lower in aged aromatic rice. Kim[28] reported that hydrocarbon compounds were not significantly different between aromatic and non-aromatic rice but aromatic rice had higher levels of alcohol (mainly *n*-pentanol,1-octen-3-ol, menthol and estragol), aldehydes and ketones (e.g. *n*-pentanal, *n*-heptanal, *n*-heptanal and *n*-nonanal), organic acids and other compounds. Aromatic rice had approximately 15 times more 2AP than non-aromatic rice.

Since the methods of food processing usually involve heating, the occurrence of 2AP in foods has been suggested to take place during cooking at elevated temperatures via a reaction between amino acids and carbohydrates, called the Maillard reaction.[29, 30, 31]

Some different studies on the genetic control of the typical aroma of rice have been reported.[32] A recessive gene (fgr) on chromosome 8 of rice, largely controlling the level of 2AP, has been identified in genetic studies. This gene corresponds with the gene that encodes for betaine aldehyde dehydrogenase (BAD), specifically with BAD2 in rice. The accumulation of 2AP in fragrant rice genotypes may be explained by the presence of mutations resulting in a loss of function of the fgr gene product.[33]

1.3 Extraction methods for aromatic rice volatiles

In order to assess aroma quality of rice products, many researchers have emphasized on the analysis of volatile components of rice grain, in which many techniques were employed to extract the rice volatiles such as steam distillation (SD), solvent extraction (SE), purge and trap and solid phase microextraction (SPME). The extracted volatiles were then analysed mainly by GC or gas chromatography-mass spectrometry (GC-MS).

In 1983, Tsugita and co-workers[34] studied volatile components of the stored rice at 40°C and 80% relative humidity for 60 days, which was sensorially evaluated as old rice. Result revealed a significant difference in cooking quality and texture of these stored rice when compared with 4°C-stored rice. Free phenolic acids were detected in a larger amount in the 40°C-stored rice, and it was proposed that the increase of phenolic acids during storage may partly contribute to the cooking properties of old rice. GC analyses of the volatiles of cooked rice showed that a larger amount of pentanal, hexanal, heptanal, alkenals, ketones, 2-pentylfuran and 4-vinylphenol and a smaller amount of 1-pentanol and 1-hexanol were found in 40°C stored rice more than those found in 4°C stored rice.

In 1986, Widjaja and co-workers[35] studied volatile components in cooked fragrant and non-fragrant rices. The volatile flavor compounds from the rice samples were isolated using the Likens-Nickerson simultaneous distillation-extraction (SDE) unit. The extracted volatiles were analyzed by GC and GC-MS. TMP was used as internal standard together with 500 g of rice mixed with $MgS0_4$ solution. The most important compounds found were alkanals, 2-pentylfuran, 2-acetyl-1-pyrroline and 2-

phenylethanol, along with many other compounds that contributed to the total aroma profile of the rice. It was noted by this research group that non-fragrant rice (Pelde) contained much more *n*-hexanal, (E)-2-heptenal, 1-octen-3-ol, *n*-nonanal, (E)-2-octenal, (E)-2,(E)-4-decadienal, 2-pentylfuran, 4-vinylguaiacol and 4-vinylphenol, than the fragrant rices (Basmati, Jasmine, Gool-arah and YRF9).

In 1995, Mahatheeranont and co-workers[36] studied the volatile compounds of uncooked Khao Dawk Mali 105 brown rice. The rice volatiles were extracted using simple steam distillation method under reduced pressure. After solvent extraction in dichloromethane, the extract was concentrated and subjected to analysis twice using capillary GC-MS. The first analysis was performed immediately after distillation and extraction, while the second analysis was done after the extract was left at room temperature until its aromatic volatile compounds dissipated. Comparison of these volatile compounds suggested that the compounds assumed to play an important role in aroma of Khao Dawk Mali 105 rice were 2AP as the major component, butyl acetate, diethyl carbonate, butyl cyclopropane, 1,4-dimethylbenzene, isocyanatomethylbenzene, hexanal, nonanal and 7-octen-4-ol and 2-(2-propoxyethoxy) ethanol.

In 2001, Grimm and co-workers[37] focused on the rapid screening of rice cultivars for the relative amounts of 2AP. SPME was used to collect and concentrate the compounds in the headspace of rice. Optimization was based upon the recovered levels of 2AP. By using this technique, samples of rice could be analysed as the flour, milled kernels, or brown rice. Twenty-one experimental rice varieties were analysed by this SPME method and then compared with a wet technique. Recoveries

of several nanograms of 2AP from 0.75 g samples of aromatic rice were observed, whereas only trace amounts of 2AP were recovered from non-aromatic rice.

In 2001, Mahatheeranont and co-workers[38] extracted volatile components of uncooked Khao Dawk Mali 105 brown rice using indirect steam distillation under reduced pressure and controlled temperature in order to prevent cooking. Analysis of the fresh extract by capillary GC-MS revealed that there were more than 140 volatile constituents. Among these, 70 volatiles were identified, including 2AP, a key aroma compound of cooked rice. The method was simplified by utilizing a solvent extraction procedure. Quantitative analysis was performed using a capillary gas chromatographic system employing a flame ionization detector with the aid of a more selective column, CP-Wax 51 for amines. This improved chromatographic system had remarkable detection sensitivity for 2AP in the rice extracts so that 2AP in an extract of only 0.5 g of uncooked Khao Dawk Mali 105 brown rice could be detected.

In 2004, Wongpornchai and co-workers[39] studied the effects of drying method and storage time on the aroma and milling quality of the aromatic rice, Khao Dawk Mali 105. A non-heated method of extraction, utilizing acidic solvent followed by re-extraction with organic solvent was employed for quantitation of 2AP in the rice samples which had been subjected to a designed set of drying methods and storage durations. At the same time, overall volatile constituents were examined by the use of an effective SPME device followed by GC-MS analysis. The six drying methods were modified: air at 30 and 40 ºC, hot air at 40, 50, and 70 ºC, and sun-drying. The results demonstrated that the drying methods that employed lower temperature appeared to

provide higher concentrations of 2AP and lower amounts of the off-flavour compounds, *n*-hexanal and 2-pentylfuran, regardless of the storage time. The sun-drying method yielded contrasting results. Overall, during 10 months storage, as the time increased, 2AP concentrations decreased whereas n-hexanal and 2-pentylfuran contents increased.

In 2006, Sriseadka and co-workers[40] developed and validated a method for quantitative analysis of 2AP in grains of fragrant rice using static headspace GC. This developed method excluded wet extraction, and the rice headspace volatiles were brought directly and automatically to GC analysis. The conditions of the static HS autosampler were optimized to achieve high recovery and sensitivity. The most effective amount of rice sample used was 1 g and a linear multiple headspace extraction (MHE) plot of the peak area of 2AP was obtained. The sensitivity of the method was enhanced by utilizing a megabore fused silica capillary column in conjunction with a nitrogen-phosphorus detector (NPD). The method was found to be effective when applied to the evaluation of aroma quality, based on 2AP concentrations, of some fragrant rice samples.

In 2008, Yang and co-workers[41] studied volatile profiles of cooked black rice and characterized their odor-active compounds. Thirty-five volatile compounds were identified by GC-MS using a dynamic headspace system with tenax trapping. Aldehydes and aromatics were quantitatively present in the greatest abundance of total relative concentration of volatiles. A total of 25 odor-active compounds, determined by gas chromatography-olfactometry, demonstrated significant difference between a black and a traditional white rice cultivar in terms of

aroma quality. 2AP, guaiacol, indole and p-xylene were largely influenced the difference between the aroma in cooked black and white rices. 2AP and guaiacol, thus, appear to be major contributors to the characteristic aroma of black rice due to their high intensity and the unique description.

In 2010, Mathure and co-workers[42] developed an efficient method for quantification of 2AP and other rice aroma volatiles by headspace (HS)-SPME/GC-flame ionization detector (FID). This technique was also used in analyzing aroma compounds among 33 scented and 2 non-scented rice samples. Extraction at 80 °C for 30 min and pre-incubation followed by 20-min adsorption from 1 g rice containing 300 µl of odour-free water were the optimum conditions for quantification. Rice types (Basmati, Ambemohar, Kolam, Indrayani and local variety) significantly contributed to the variation in 2AP, hexanal, nonanal, decanal, benzyl alcohol, vanillin, guaiacol and indole. However, in terms of analytical aspect, this method was rapid, sensitive and less expensive which could be effectively used for quality assurance of scented rice on the basis of 2AP content.

1.4 Headspace–Gas Chromatographic Technique (HS-GC)

1.4.1 Headspace (HS)

The headspace is the gas phase above the sample in a vial and the volatile components present in that gas. HS analysis is done by analyzing a portion of the upper gas phase being in equilibrium with the liquid phase in a closed vial.[43] This technique is used for the analysis of volatiles and semi-volatile organics in solid,

liquid and gas samples. Headspace technique consists of dynamic headspace and static headspace.

1.4.1.1 Theory of Headspace Analysis[44]

Figure 1.13 shows a headspace vial, with the two phases: the sample (condensed) phase and the gas phase (headspace), indicated by the respective subscripts of S and G. If the system contains volatile analytes that are soluble in the condense phase, these will distribute between both phases according to the thermodynamically controlled equilibrium. The system represented by this vial is characterized by the following values:

V_V = total volume of the vial

V_S = volume of the sample phase

V_G = volume of the gas phase

$$V_V = V_S + V_G \quad (1.1)$$

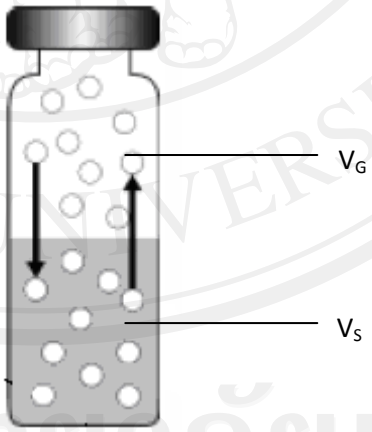

Figure 1.13 A headspace vial containing a liquid sample

The relative volumes of the two phases in the vial are characterized by the phase ratio β, representing the ratio of the volume of the two phases present:

$$\beta = V_G / V_S \quad (1.2)$$

$$\beta = \frac{V_V - V_S}{V_S} = \frac{V_G}{V_V - V_G} \quad (1.3)$$

$$V_S = \frac{V_V}{1+\beta} \quad (1.4)$$

$$V_G = V_V \cdot \frac{\beta}{1+\beta} \quad (1.5)$$

It is assumed that the volume of the sample phase after equilibrium is equal to the volume of the original sample V_o; in other words, the amount of analyte transferred to the gas phase during equilibration is not considered to result in any appreciable changes in the volume of the original sample:

$$V_o = V_S \quad (1.6)$$

The original amount of the analyte in the sample was W_o, and its original concentration was C_o:

$$C_o = W_o / V_S \quad (1.7)$$

After equilibration the respective amounts of the analyte in the two phases are W_S and W_G and their concentration are C_S and C_G:

$$C_S = W_S / V_S \quad (1.8)$$

$$C_G = W_G / V_G \quad (1.9)$$

$$W_S + W_G = W_o \quad (1.10)$$

The distribution of the analyte between the two phases upon equilibrium is expressed by the thermodynamically controlled equilibrium constant. In analogy to the common practice in gas chromatography, the synonymous term partition (distribution) coefficient (K) is preferred.

$$K = \frac{C_S}{C_G} \tag{1.11}$$

$$K = \frac{W_S}{V_S} \Big/ \frac{W_G}{V_G} = \frac{W_S}{W_G} \cdot \frac{V_G}{V_S} = \frac{W_S}{W_G} \cdot \beta \tag{1.12}$$

The partition coefficient is a fundamental parameter that expresses the mass distribution in the two-phase system. It depends on the solubility of the analyte in the condense phase. Compounds with high solubility will have a high concentration in the condense phase relative to the gas phase ($C_S >> C_G$). Hence, the value of K may be very high. On the other hand, C_S will be close to C_G, and might even be less than its value: hence, K will be small.

The above derived relationships can also be written as in the followings:

$$W_o = C_o \cdot V_S \tag{1.13}$$

$$W_S = C_S \cdot V_S \tag{1.14}$$

$$W_G = C_G \cdot V_G \tag{1.15}$$

$$C_S = K \cdot C_G \tag{1.16}$$

Thus, the material balance given by eq. 10 can be written as follows:

$$C_o \cdot V_S = C_G \cdot V_G + C_S \cdot V_S = C_G \cdot V_G + K \cdot C_G \cdot V_S$$

$$= C_G \cdot (K \cdot C_G + V_G) \quad (1.17)$$

Expressing C_o and then C_G:

$$C_o = C_G\left[\frac{K \cdot V_S}{V_S} + \frac{V_G}{V_S}\right] = C_G(K+\beta) \quad (1.18)$$

$$C_G = \frac{C_o}{K+\beta} \quad (1.19)$$

In a given system and under given conditions both K and β are constants, thus (K+β) and its reciprocals will also be constants.

$$C_G = (\text{const}) \cdot C_o \quad (1.20)$$

In other words, in a given system the concentration in the headspace is proportional to the original sample concentration.

If follows from the basic rules of gas chromatography that the peak area obtained for a given analyte is proportional to the concentration of the analyte in the analyzed sample. In this case, aliquot of the headspace is analyzed in which the analyte concentration is C_G. The obtained peak area A can thus be expressed as

$$A = (\text{const}) \cdot C_G \quad (1.21)$$

where the constant incorporates the influence of a number of analytical parameters and the detector response factor. Combining eqs. 20 and 21 results in

$$A = (\text{const}) \cdot C_o \quad (1.22)$$

where the combined constant incorporates the influence of headspace, GC and detector parameters.

Two conclusions can be drawn from eq 1.22. The first is that if an aliquot of the headspace at equilibrium is analyzed by GC, the obtained peak area of

the analyte will be directly proportional to its concentration in the original sample. This is the fundamental relationship on which quantitative HS-GC analysis is based. The second conclusion is related to the constant in eq. 1.22: as indicated, it incorporates the influence of a number of parameters. Since their numerical evaluation would be very difficult, a prerequisite of analysis is the exact reproduction of the analytical conditions, and this is particularly true if quantitative measurement is based on the comparative analysis of the sample and a standard.

Combining eqs. 1.19 and 1.21 yields the following equation.

$$A \alpha C_G = \frac{C_o}{K + \beta} \quad (1.23)$$

This equation represents the relationship at equilibrium between the peak area A obtained upon analyzing an aliquot of the headspace, the concentration of the analyte in the headspace C_G, the original sample concentration of the analyte C_o, the partition coefficient K and β, the phase ratio of the vial.

1.4.1.2 Static Headspace (SHS)[45]

Static headspace (SHS) analysis is a gas extraction method and it as a one-step gas extraction.[33] SHS is a technique that is suitable for determining volatile compounds in solids and in dirty liquid matrices such as blood, paints and sludge. In utilizing SHS, the sample is normally placed in a sealed vial, heated in a carefully regulated bath or oven and the volatiles in the liquid or solid phase are allowed to come to equilibrium with the gas phase.

Figure 1.14 A headspace autosampler – gas chromatography

The relative concentrations of the analyte in the two phases are determined by the partition coefficient defined as

$$K = \frac{C_l}{C_g} \qquad 1.24$$

where C_l is the concentration in the liquid phase and Cg is the concentration in the gas phase at equilibrium. An aliquot of the gas phase is removed and injected into the GC.

1.4.1.2.1 Principle and instrumentation of static HS[46]

A peculiar problem in static HS-GC is the internal pressure in the headspace vial generated during thermostatting by the sum of partial vapor pressures from all volatile sample constituents, from which in general the humidity of the sample is predominant. Thus, the vapor pressure of water contributes mostly to the internal pressure. Moreover, some sampling techniques pressurize the vial prior to sample transfer with the inert carrier gas. For these reasons it is necessary to close the

vial pressure tight by a septum (preferably PTFE-lined) and to crimp-cap it by an aluminum cap.

Instrument of headspace autosampler consists of oven, sample tray, sample loop and transfer line.

- Oven: To heat and control temperature of sample vial as shown in Figure 1.15.

- Sample tray: For input of sample vial and has automatic handle for sample vial as shown in Figure 1.15.

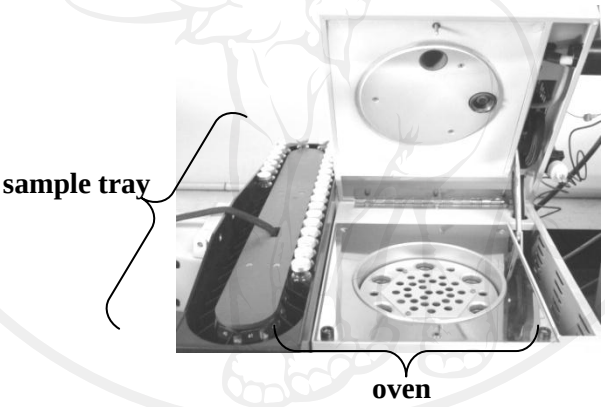

Figure 1.15 Oven and sample tray of headspace autosampler

- Sample loop: The volume of the headspace gas is controlled by size of loop and the flushing time as shown in Figure 1.16.

Figure 1.16 Sample loop of headspace autosampler

- Transfer line: To directly transfer volatile compounds from sample vial for analysis by gas chromatography as shown in Figure 1.17.

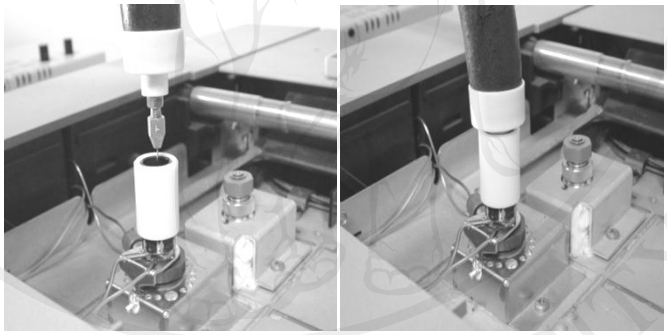

Figure 1.17 Transfer line of headspace autosampler

The headspace sample is a gas mixture and every sampling device to inject a gas sample into a gas chromatograph can in principle be used. Gas sampling valves with sample loops are very common for this application. Filling the loop with headspace gas is achieved by pressurizing the vials first up to a certain pressure level above the original pressure in the vial. The pressurized headspace is then temporarily connected to the sample loop and the pressurized headspace gas expands through the

loop to the atmosphere, thus filling the loop with headspace gas. By rotating the valve, the content of the loop is swept onto the column as shown in Figure 1.18.

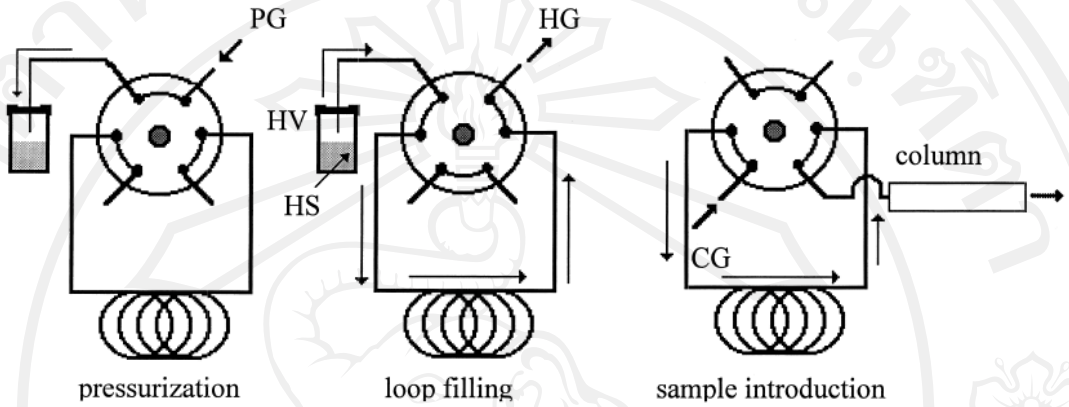

Figure 1.18 Schematic of the 'pressure/loop' headspace sampling system. PG = Pressurization gas, HV = headspace vial, HS = headspace sample, CG = carrier gas

1.4.1.2.2 The advantages of SHS

In comparison with other gas extraction technique, SHS has the following advantages:

- Headspace sampling is a solvent-free technique.

- Headspace is fast, simple and efficiently.

- SHS-GC also provides superior sensitivity and reproducibility.[47]

- SHS demonstrates good sensitivity, good recoveries of internal standards, good performance, and minimal waste.[43, 48]

1.4.2 Gas Chromatography (GC)[49, 50]

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. Figure 1.19 illustrates the major components of a general gas chromatographic system. A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, and the temperature of oven.

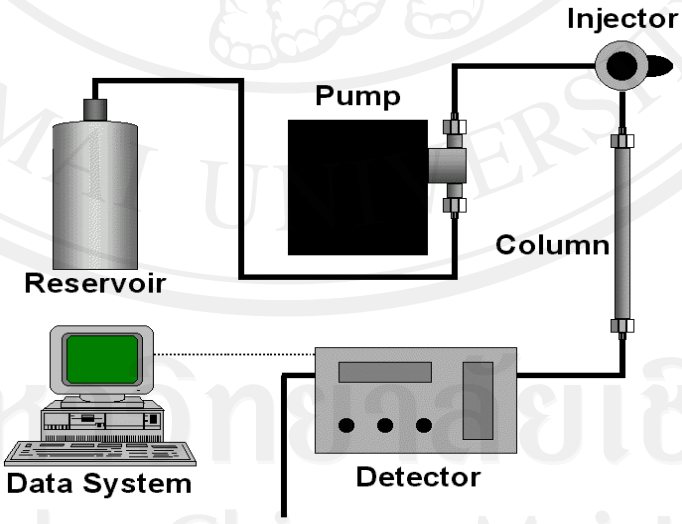

Figure 1.19 Schematic diagram of a gas chromatograph

1.4.2.1 Instrumental components of GC[51]

Instrumental components of GC consist of many parts which are carrier gas, injector, column, detector and recorder.

1.4.2.1.1 Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

1.4.2.1.2 Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapor. Slow injection of large samples causes band broadening and loss of resolution. The most common injection method is that where a microsyringe is used to inject sample through a rubber septum into a flash vaporization port at the head of the column. The temperature of the sample port is usually about 50 °C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a µL up to 20 µL. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} µL. For capillary GC, split/splitless injection is used, a schematic diagram of which is shown in Figure 1.20.

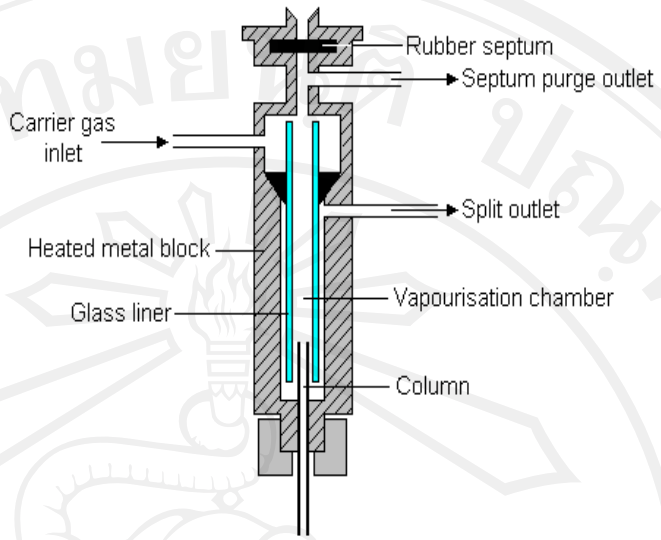

Figure 1.20 Schematic diagram of a split/splitless injection port

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vaporizes to form a mixture of carrier gas, vaporized solvent and vaporized solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

1.4.2.1.3 Columns

There are two general types of column, packed and capillary column (also known as open tubular). Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid

stationary phase. Most packed columns are 1.5 – 10 m in length and have an internal diameter of 2 – 4 mm. Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; wall-coated open tubular (WCOT) or support-coated open tubular (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns. In 1979, a new type of WCOT column was devised the fused silica open tubular (FSOT) column.

1.4.2.1.4 Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound. Detectors can also be grouped into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependent detector is related to the concentration of solute in the detector, and does not usually destroy the sample. Mass flow dependent detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependent detector is unaffected by make-up gas.

The most common GC detector is the flame ionization detector (FID). This detector is sensitive to a wide range of components, and works over a wide range of concentrations. FIDs are sensitive primarily to detecting hydrocarbons and other easily flammable components. They are mass sensitive rather than concentration sensitive. This gives the advantage that changes in mobile phase flow rate do not affect the detector's response. In general, FID is a useful detector for the analysis of organic compounds. It has high sensitivity, a large linear response range, and low noise. It is also robust and easy to uses, only one main limitation is that it destroys the sample.

Nitrogen-phosphorus detector (NPD), was showed in figure 1.21. This detector is very sensitive and specific for analysis of nitrogen and phosphorus compounds. It is similar in design to the FID, except it uses a thermionic NPD bead to generate ions in hydrogen and air plasma.[52] The actual NPD sensor is a rubidium or cesium bead contained inside a small heater coil. The helium carrier gas is mixed with hydrogen and passes into the detector through a small jet. The bead is heated by a current passing through the coil, which is situated above the jet, and the helium-hydrogen mixture passes over it. If the detector is to respond to both nitrogen and phosphorus, then a minimum hydrogen flow is employed to ensure that the gas does not ignite at the jet. In contrast, if the detector is to respond to phosphorus only, a large flow of hydrogen can be used and the mixture burned at the jet. A potential is applied between the bead and the anode. The heated alkali bead emits electrons by thermionic emissions, which are collected at the anode and thus produce an ion current. When a solute containing nitrogen or phosphorus is eluted, the partially

combusted nitrogen and phosphorus materials are adsorbed on the surface of the bead. This adsorbed material reduces the work function of the surface and, as a consequence, the emission of electrons is increased which raises the anode current.[53]

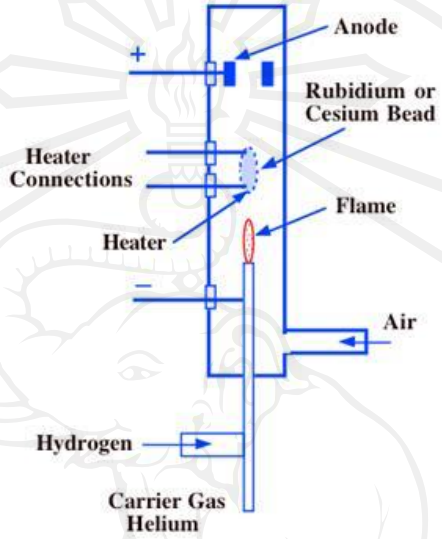

Figure 1.21 A diagram of NPD detector[54]

However, some detectors are also employed in GC system which is depicted in Table 1.1.

Table 1.1 Detectors in GC system

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, Nitrates, Nitriles, Peroxides, Anhydrides, Organometallics	50 pg	10^5
Nitrogen-phosphorus (NPD)	Mass flow	Hydrogen and air	Nitrogen, Phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, Phosphorus, Tin, Boron, Arsenic, Germanium, Selenium, Chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, Aromatics, Ketones, Esters, Aldehydes, Amines, Heterocyclics, Organosulphurs, Some Organometallics	2 pg	10^7

1.5 Aims and scope of this research

The objective of this research was to develop an automated headspace-gas chromatographic (SHS-GC) technique with nitrogen-phosphorous detector (NPD) for determination of an aroma compound, 2AP, in rice leaves sample. This developed method will be more convenient, rapid and requires less amount of sample which would be another choice used for figuring the level of aroma in hybrid rice samples obtained from the rice breeding programs.