CHAPTER 1

INTRODUCTION

Rice is a major food commodity throughout the world. Different cultures have preference for different types of rice. Scented rice or aromatic rice is popular in Asia and has gained wider acceptance in Europe and the United States. Because of their aroma, flavor and texture, aromatic varieties command a higher price in the rice market than do the non-aromatic rice varieties.

1.1 Rice [1-3]

Rice, like wheat, corn, rye, oats and barley belongs to Gramineae or grass family. The common rice, *O. sativa*, and African rice, *O. glaberrima*, are thought to be an example of parallet evolution in crop plants. The wild progenitor of *O. sativa* is the Asian common wild rice, *O. rufipogon*, which shows a range of variation from perennial to annual types. Annual types, also given a specific name of *O. nivara*, were domesticated to become *O. sativa*. There are three main varieties of Oryza sativa, Indica, Japonica and Javanica as shown in Figure 1.1. Indica rice concentrates in the worm climate belt, form Indochina, Thailand, India, Pakistan, Brazil and Southern U.S.A. Japonica is mostly grown in cold climate countries such as Japan, Korea, northern Chaina and California. Javanica is only grown in Indonesia.

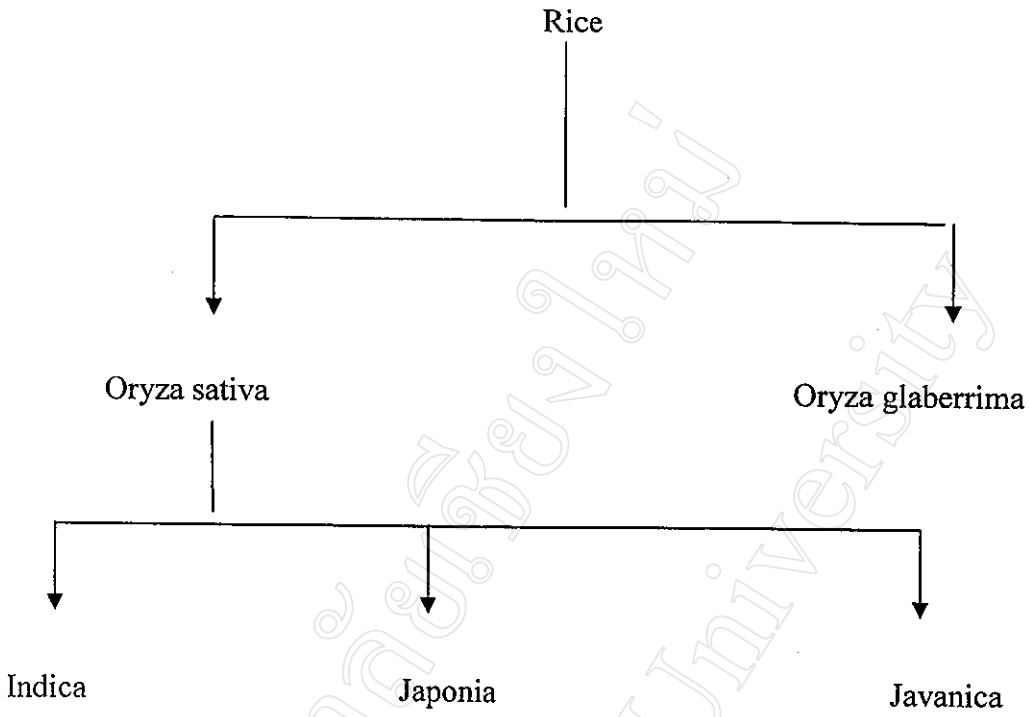

Figure 1.1 Varieties of rice in the world [4].

1.1.1 Aromatic rice

The aromatic rices are mainly grown and consumed in India, Pakistan, Thailand, Bangladesh, Afganistan, Indonesia, Iran and United States. Of these, India, Pakistan and Thailand export fine grain aromatic rices to mostly of Middle East, Europe and USA with the annual export worth millions of dollars. Bulk of aromatic rice from India and Pakistan consists of Basmati types, while Thailand is the supplier of Jasmine rice. Other important aromatic varieties in the world market are Khao Dowk Mali 105, Siamati (Thailand), Bahra (Afganistan), Sadri (Iran), Della, Texamati and Kasmati (USA).

1.1.2 Aromatic rice of Thailand

Rice has been in Thailand for more than 5000 years but the aromatic rice, locally known as Khao Hawm (fragrant rice) is considered to be the national pride of the Thai people. Formarly, it was grown in almost every household in Thailand mainly for their own consumption. In each region, farmer grew, several aromatic varieties in their fields, which suit their requirements. At present, aromatic rice was grown for commerce because it is great demand in the rice market. These varieties differ in cooking quality, maturity, responses to day-length and adaptability, etc. Only recently that the diversity of aromatic rice cultivars has been narrowed down to a few varieties in order to conform with domestic and international demands. In the north and northeast regions, rice is produced and consumed locally with little surplus for export.

1.1.3 History of KDML 105

Khao Dawk Mali (KDML) 105 is the most popular rice variety in Thailand. This is due mostly to its pleasant aroma, together with its white color and soft texture, which has resulted in its name "Khao Dawk Mali", meaning "as white as jasmine flowers". The name "jasmine rice" is, therefore, often used by foreign countries to refer to the KDML 105 Thai aromatic rice variety. KDML 105 is a traditional variety and its origin can be traced to 1945 when it was found by farmer in Chon Buri province in eastern Thailand. The seed of KDML was later distributed to the neighboring province of Cha Seong Sao where a district agriculture officer collected 199 panicles in 1950. The panicle-row method was employed and pure line selection initiated at Kok Samrong Rice Research Station in Lop Buri province. The out-

standing line, Khao Dawk Mali 4-2-105, was identified and further evaluated for yield potential and adaptability in the north, northest, and central regions. It was later released as Khao Dawk Mali 105 in 1995.

1.1.4 Aroma

Scented or aromatic rice is preferred in some areas of Asia and draws a premium price in certain specialty markets. The Middle East consumers prefer rices with strong aroma. They feel that rice without a distinctive aroma is like food without salt. For consumers in Europe, a trace of aroma is an objectionable trait, because for them any scent signals are spoilage and contamination. In most countries aroma is an important quality characteristic of high-quality rices command higher price. In the domestic Basmati and Jasmine rices command premium prices [5].

Among different quality traits, aroma is considered most important. A 'popcorn' like aroma component, 2-acetyl-1-pyrroline, has been reported as an important flavor compound of several aromatic varieties. Since the key aroma compound of rice, 2-AP, was first identified by Buttery and Ling [6] a few researchers have improved methods for quantification of 2-AP in rice, but none have reported the used of headspace gas chromatographic (HS-GC) technique. The structure of 2-acetyl-1-pyrroline (2-AP) is shown in Figure 1.2 [7].

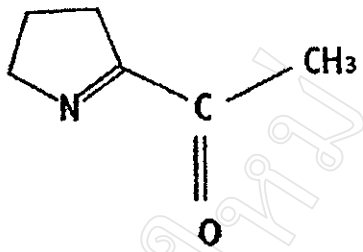

Figure 1.2 The structure of 2-acetyl-1-pyrroline.

Yajima *et al.* [8] studied volatile flavor component of cooked rice. Steam distillation of cooked rice was extracted with methylene chloride and the extract was separated into four fractions: acidic, weak acidic, basic and neutral fractions. All fractions were analyzed by combination of glass capillary gas chromatography and mass spectrometry. One hundred compounds were identified in this cooked rice extract without any components identified as a major aroma compound.

Yajima *et al.* [9] studied volatiles of cooked Kaorimai, *O. sativa* japonica variety, which were drawn off into a cold trap under reduced pressure. The resulting condensate was extracted with ethyl ether and the extract was separated into acidic, basic, and neutral fractions. All fractions were examined by gas chromatography and gas chromatography-mass spectrometry. One hundred and fourteen compounds were identified in the extract of this aromatic rice.

Buttery *et al.* [10] developed a relatively simple practical method for quantification of 2-AP in rice samples. The method used a steam distillation

continuous extraction device as isolation procedure with an acid-phase solvent extraction. 2-AP was determined for the first time using capillary column gas chromatography.

Tanchotikul and Hsieh [11] applied a rapid microscale steam distillation/solvent extraction (micro-SDE) procedure for extract ion of part per billion levels of 2-AP from milled aromatic rice samples. Then gas chromatographic separation and mass spectrometric sensitivity and specificity in the selected ion monitoring mode was utilized for quantification of 2-AP. Only 1 g of sample weight required for analysis. Selected aromatic rice samples were found to contain 2-AP in the range 76-156 ppb on the basis of equivalent weight of an internal standard, 2,4,6-trimethylpyridine, and dry weight of rice.

Laksanalamai and Ilangantileke [12] identified the aromatic compound, 2-AP, in fresh and aged Khao Dawk Mali (KDML)105, in nonaromatic rice and in pandan leaves, using a steam distillation extraction method. 2-AP was identified by gas chromatography-mass spectrometry. It was not found in nonaromatic rice and occurred in low concentrations in the aged KDML105 rice.

Bergman *et al.* [13] developed sample preparation by using solvent extraction. The extraction time and temperature as well as gas chromatographic parameters were optimized. The optimum extraction required 0.3 g of ground brown or milled rice in methylene chloride held at 85 °C for 2.5 hr. The complete gas chromatographic run requires 25 min, and 50 samples can be analyzed per day. Milled

and unmilled commercial and breeded aromatic rice samples contained 10-1104 ng/g of 2-AP and 148-2541 ng/g of hexanal, an off-flavor compound.

Mahatheeranont *et al.* [14] improved method for quantification of 2-AP in uncooked brown rice. The method was simplified by utilizing a solvent extraction procedure. 2-AP was extracted by acidic solvent at room temperature. Quantitative analysis was performed using a capillary gas chromatographic system employing a flame ionization detector with the aid of a more selective column, CP-Wax51 for amines. Amounts of 2-AP found in samples of Khao Dawk Mali 105 were in the range 0.05-0.34 ppm.

Jezussek *et al.* [15] indentified the aroma compounds in cooked brown rice of Nalagkit Sungsong (IMS), Basmati 370 (B370), Khaskhani (KK) and the variety Indica on the basis of aroma extract dilution analyses (AEDA). Forty-one order-active components were identified. 2-AP was confirmed as a further key aroma constituent in IMS, B370, and KK, but was not important in India.

Yoshihashi [16] analyzed 2-AP in aromatic rice samples by a stable isotope dilution method. The compound was extracted from seedlings, roots, and husks. The recovery of 2-AP was linear from 5 to 5000 ng/g with sensitivity of less than 0.1 ng/g. 2-AP was also found to show tautomerism with its imide form.

1.2 Introduction to headspace sampling [17]

Gas chromatography is an analytical technique suitable for the investigation of volatile compounds. Sample introduction must be instantaneous after all, if the sample vapor band introduced into the column already has a significant width, then analyte separation will be hindered by the initial broadness of the analyte mixture's band. Sample components must be volatile, because otherwise a solid residue will remain in the inlet system. Since this zone is heated, the solid residue may eventually decompose, thus creating volatile breakdown products that get into the column and thus, into the chromatogram, creating the impression that these compounds were present as such in the original sample. Also, sample residue may interfere with subsequent injections, as a result of adsorption and/or catalytic decomposition. Because of this problem, one may have to follow an indirect procedure in the case of complex solid samples, by extracting first the analyte(s) of interest and then introducing an aliquot of the resulting solution into the gas chromatograph. The resulting solution is injected and analyzed by gas chromatography. There are several problems with this approach. First, it is obviously a time-consuming procedure. Second, the analyte will generally be more diluted in the solution than it was in the original sample.

There is an indirect way in which we can study the volatile compounds present in an essentially nonvolatile matrix, without the necessity of carrying out liquid extraction, or without artificially separating the volatiles from the matrix upon sample handling. It is done by taking an aliquot of the gas phase, the volatile compounds were analyzed without interference by the nonvolatile matrix. The gas

phase in contact and in equilibrium with an essentially nonvolatile (or lesser volatile) sample was referred to as the "headspace" (HS), and its investigation as headspace analysis (HSA). Thus headspace analysis refers to the analysis of the gas (vapor) phase of a binary heterogeneous system in equilibrium. The other phase may be a liquid or a solid, and it was called the condensed phase.

If extraction is carried out with a liquid solvent, the analytes will be distributed between the sample and the solvent, by adjusting the conditions their distribution should favor the solvent phase. HSA is also an extraction procedure, only a gas is used instead of a liquid as the solvent because a gas is an ideal "solvent" for highly volatile compounds. In this case the analytes will be distributed between the condensed phase and the gas phase, and we adjust the conditions to favor the gas phase.

1.3 Introduction to headspace gas chromatography [17-18]

The headspace gas can be investigated by various methods. Gas chromatography (GC) is particularly well suited for such measurement, since GC is an ideal method for gas (vapor) analysis. In headspace-gas chromatography (HS-GC), the vapor (gas) phase in contact with a condensed (liquid or solid) phase is analyzed by gas chromatography.

HS-GC is applied for various gas extraction techniques, where volatile sample constituents are first transferred into a gas with subsequent analysis by gas chromatography. This technique widely used for samples that otherwise cannot be

handled with a syringe, such as solids and for samples that consist mainly of non-volatile material. The sample, placed in a closed container may be in contact and in equilibrium with the extraction gas (static or equilibrium headspace) or the volatile compounds may be stripped off in a continuous flow of an inert gas (dynamic headspace). HS-GC makes use of the equilibrium between the volatile components of a liquid or solid sample and the surrounding gas phase in a sealed vessel, aliquots of the gas phase being injection into GC for analysis.

HS-GC is a straightforward technique, when volatile compounds have to be separate from a solid or liquid matrix prior to GC analysis. As a gas extraction procedure it replaces a solvent extraction, thus avoiding the many problems with solvent. The sample is extracted by inert carrier gas with subsequent GC analysis. It is obviously better to employ a gas as a solvent with its ideal solubility for every volatile components as well as the advantage that a gas is normally available in a higher purity than any liquid solvents, which avoids problems with trace impurity on interferences.

In addition, this technique does not give a solvent peak with tailing in the chromatogram. Analytical procedures based on headspace GC are becoming increasingly popular with more and more trace determinations of volatile compounds. This is due to the increasing number of ecological problems nowadays. It is preferred if standard GC procedures cause problems with the samples matrix in respect of solubility or thermal stability. Use of HS-GC even permits the successful analysis of liquid samples, if the partition coefficient of the volatile is low enough to shift the equilibrium to the gas phase. In addition detection limits are very improved [19].

1.4 Type of Headspace Sampling [18,20]

There are three basic methods of headspace sampling namely, static headspace, dynamic headspace and purge methods. These are illustrated in Figure 1.3 which also includes illustrations of the methods of sample recovery; thermal desorption and cold trapping. Static headspace sampling was used in this work.

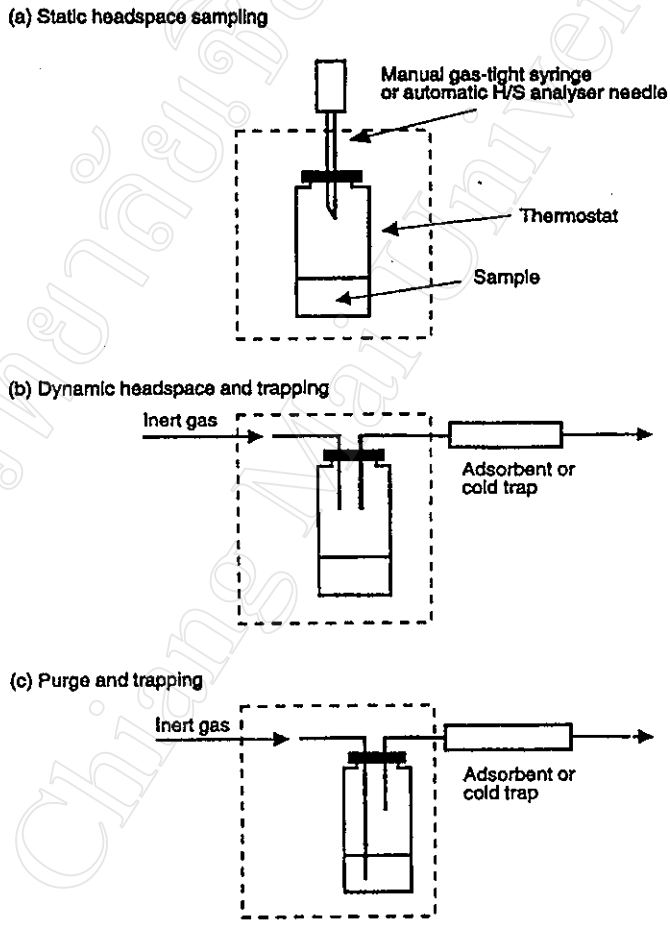

Figure 1.3 Illustration of headspace sampling techniques [20].

1.5 Principles and instrumentation of static HS-GC [18]

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.

The most popular device for headspace sampling is a gas syringe. Besides the risk of sample carry-over and significant memory effects there is the inherent problem that the internal pressure in the vial extends into the barrel of the syringe and after withdrawal from the vial, the pressurized headspace gas then expands through the open needle to the atmosphere. Part of the headspace gas will thus be lost. This drawback may be avoided by a using a gas-tight syringe equipped with a valve. Such syringe may be adequate for manual sampling, but are hard to automate.

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 then is 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 1.4.

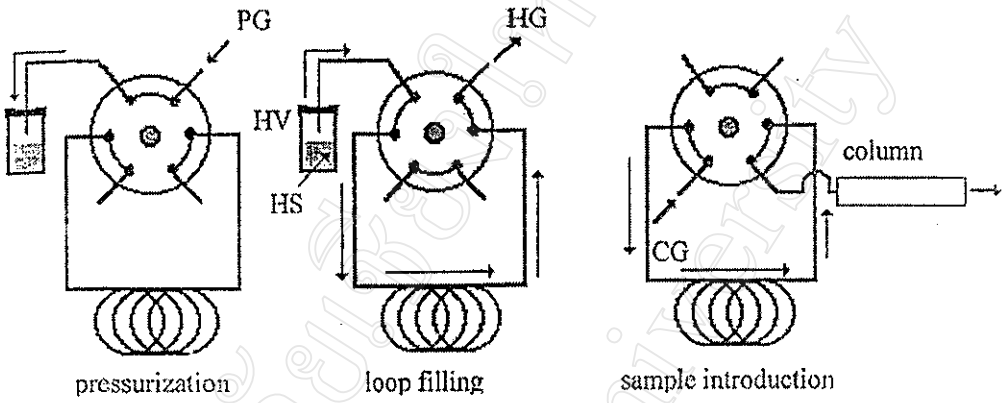

Figure 1.4 Schematic of the 'pressure/loop' headspace sampling system. PG= pressurization gas, HV=headspace vial, HS=headspace sample CG=carrier gas, HG=headspace gas

However, instead of filling a loop first, a pressurized headspace gas can expand directly into the column. Such a sampling system, called *balanced pressure sampling system* was already introduced in 1968 in the first automated headspace sampler by Perkin-Elmer and its principle is shown in Figure1.5, when techniques for cryogenic trapping are discussed.

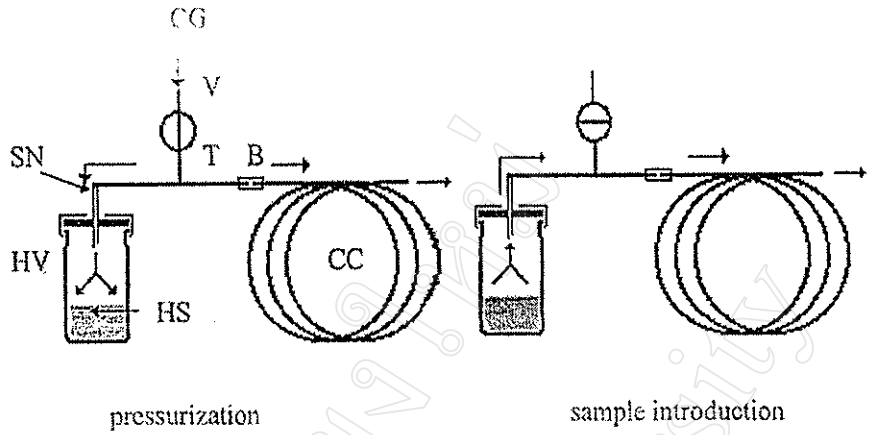

Figure 1.5 Schematic of the 'balanced pressure' headspace sampling system in splitless configuration. CG=carrier gas, V=solenoid valve, SN = sampling needle, HV=headspace vial, HS=headspace sample, T= fused-silica transfer line, B=butt connector, CC=capillary column.

Inert carrier gas enters the gas chromatograph through a solenoid valve V and branches before the column. Part of the gas is directed to the sampling needle SN. The needle penetrates the septum of the headspace vial and carrier gas pressurizes it usually up to the column head pressure, but any other pressure value may be applied as well during this cycle period. Sample transfer is subsequently performed by closing solenoid valve V for a short time (usually few seconds), thus disconnecting the carrier gas flow. The pressurized headspace gas in the vial expands now directly onto the column and no headspace gas is wasted by unnecessary expansion to atmosphere. The headspace gas replaces the carrier gas flow during the sampling time and the volume of headspace gas transferred into the column is therefore time-controlled and the injected volume can precisely be adjusted by varying the sampling time in a wide range. Any time event is part of a GC method and can thus be automatically varied

under computer control in an unattended run contrary to the pressure/loop system where the loop must be changed mechanically.

In this work, HP 7694 Headspace Sampler was used as sample injection system. Its schematic diagram is illustrated in Figure 1.6.

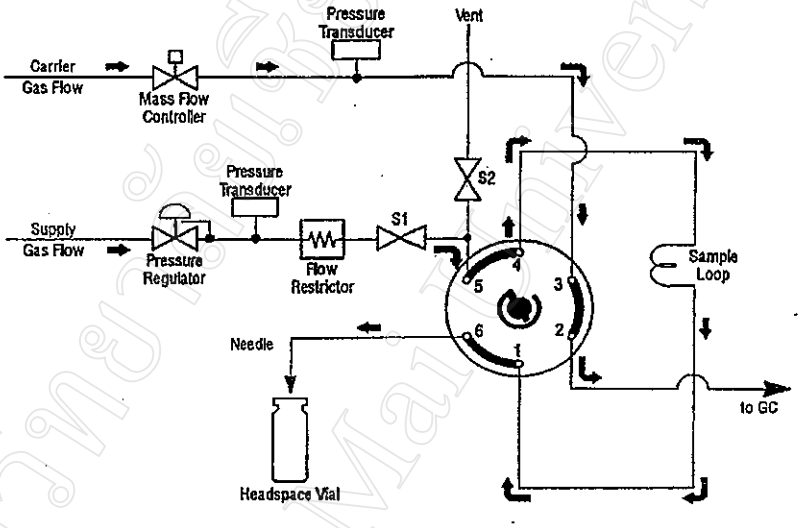

Figure 1.6 Schematic diagram of HP 7694 Headspace Sampler [20].

When the sampler is started, the robotic arm moves the first vial from the sample tray to be agitated in the oven for a required equilibration time. At the end of the vial equilibration time, agitation stops, the vial moves in-line with the sampling needle and is raised by the mechanical rod onto the needle. The pressurization valve closes. The sampling needle pierces the septum of the vial. The pressurization valve opens, allowing gas to enter the vial for required the pressurization time. The valve then closes again. The vent valve opens and the headspace gas fills the sample loop, it then vents to atmospheric pressure through the vent outlet. The sample valve brings

the sample loop in line with the carrier gas flow. The carrier gas flow through the sample loop and the transfer line into the GC inlet part. This sweeps the sample into the GC.

1.6 Theory of Headspace Analysis [17]

Figure 1.7 visualizes a headspace vial, with the two phases: the sample (condensed) phase and the gas (headspace). If the system contains volatile analytes that are soluble in the condensed phase, they will distribute between both phases according to the thermodynamically controlled equilibrium. The system represented by this vial is characterized by the following values:

$$V_V = \text{total volume of the vial} \quad (1.1)$$

$$V_S = \text{volume of the sample phase} \quad (1.2)$$

$$V_G = \text{volume of the gas phase} \quad (1.3)$$

$$V_V = V_S + V_G \quad (1.4)$$

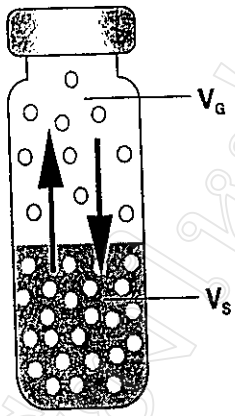

Figure 1.7 A headspace vial containing a liquid sample [17].

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

$$\beta = V_G / V_S \quad (1.5)$$

$$\beta = (V_V - V_S) / V_S = V_G / (V_V - V_G) \quad (1.6)$$

$$V_S = V_V / (1+\beta) \quad (1.7)$$

$$V_G = V_V [\beta / (1+\beta)] \quad (1.8)$$

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 change in the volume of the original sample.

$$V_o = V_S \tag{1.9}$$

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

$$C_o = W_o / V_S \tag{1.10}$$

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

$$C_S = W_S / V_S \tag{1.11}$$

$$C_G = W_G / V_G \tag{1.12}$$

$$W_S + W_G = W_o \tag{1.13}$$

The distribution of the analyte between the two phases upon equilibrium is expressed by the thermodynamically controlled equilibrium constant K, partition (distribution) coefficient.

$$K = C_S / C_G \tag{1.14}$$

$$K = (W_S/V_S) / (W_G/V_G) = (W_S/W_G)(V_G/V_S)$$
$$= (W_S/W_G)\,\beta \tag{1.15}$$

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

$$W_O = C_o V_S \tag{1.16}$$

$$W_S = C_S V_S \tag{1.17}$$

$$W_G = C_G V_G \tag{1.18}$$

$$C_S = K C_G \tag{1.19}$$

Thus, the material balance given by equation 1.13 can be written as follows:

$$C_o V_S = C_G (K V_S + V_G) \quad (1.20)$$

Expressing C_o and then C_G:

$$C_o = C_G (K + \beta) \quad (1.21)$$

$$C_G = C_o / (K + \beta) \quad (1.22)$$

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

$$C_G = (\text{constant})\, C_o \quad (1.23)$$

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

By following 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 our case, an aliquot of the headspace in analyzed in which the analyte's concentration is C_G. Thus for the obtained peak area A

$$A = (\text{constant})\, C_G \quad (1.24)$$

Where the constant incorporates the influence of a number of analytical parameters and the detector response factor.

$$A = (\text{constant})\, C_o \qquad (1.25)$$

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

There are two conclusions from equation 1.25. 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 a fundamental relationship on which quantitative HS-GC analysis is based. The second conclusion is related to the constant in equation 1.25, which indicated that it incorporates the influence of a number of parameters. Since their numerical evaluation would be very difficult, a prerequisite of reproducible analysis is the exact reproduction of the analytical conditions, and this is particular true if quantitative measurement is based on the comparative analysis of the sample and standard.

1.7 Gas Chromatography

1.7.1 Introduction to gas chromatography [21-23]

Gas chromatography (GC) is a seperation method. It involves a sample being vaporised and injected onto the head of the chromatographic column. The sample is

transported through the column by the flow of inert, gaseous mobile phase. Two types of GC are encountered which are gas-solid chromatography (GSC) and gas-liquid-chromatography (GLC). GLC finds widespread use in all fields of science, where its name is usually shortened to GC. GLC is based upon the partition of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid. This technique has also gained great interest due to its ease of automation. Because separation occurs in the gas phase, liquid and solid samples must first be vaporised. Applications of this technique are used in the fields of petrochemistry, pharmaceuticals, the environmental, flavor analyses, and so on.

Advantages of gas chromatography [24]

- Fast analysis, typical minutes
- Efficient, providing high resolution
- Sensitive, easily detecting ppm and often ppb
- Highly accurate quantitative analysis, typical relative standard deviation (RSD) of 1-5 %
- Requires small samples, typically μL
- Reliable and relatively simple
- Inexpensive

1.7.2 Principles of gas chromatography

Chromatography is a separation technique where component molecules (solute) in a sample mixture are transported by a mobile phase over a stationary phase. Mobile phase may be a gas or a liquid and stationary phase may be a liquid

film on surface of an inert support material or solid surface. Interaction occurs between the solute and stationary phase so that the solute is distributed between the stationary phase and mobile phase. Attraction of the solute for the stationary phase results in retardation of its movement through the chromatography system. Different components (solutes) will move at differing rates since each will have a slightly different affinity for the stationary phase with respect to the mobile phase. Each component or solute (A, B, C) is distributed between the two phases with an equilibrium established defined by the distribution ratio, thus for component A

$$[A_S] \longleftrightarrow [A_M]$$

where $[A_S]$ = the concentration of A in a unit volume of the stationary phase

$[A_M]$ = the concentration of A in a unit volume of the mobile phase

1.7.2.1 Some important terms in chromatography [23-24]

Distribution constant (K)

$$K = C'_S/C_M \qquad (1.26)$$

Where C'_S = the concentration of a component in the stationary phase/unit volume

C_M = the concentration of a component in the mobile phase/unit volume

The larger the distribution constant, the more the solute sorbs in the stationary phase, and the longer it is retained on the column.

Retention time and retention volume

Retention time and retention volume are measured from the time the sample is introduced into the chromatograph to when the components are eluted from the column; no allowance is made for the volume of mobile phase in the system nor the time the mobile phase takes to pass from the injector to the detector.

$$V'_R = V_R - V_M \quad (1.27)$$

$$t'_R = t_R - t_M \quad (1.28)$$

where V'_R = adjusted retention volume

V_R = retention volume, the volume of mobile phase required to carry the component molecules through the chromatographic system

V_M = void volume, the volume of the mobile phase within the length of the column

t'_R = adjusted retention time

t_R = retention time, the time taken by component molecules through the chromatographic system

t_M = hold-up time, the time taken by the mobile phase or unretained species to reach the detector

Capacity factor

The capacity factor is an important experimental parameter that is widely used to describe the migration rates of solutes on columns. For a solute A, the capacity factor k'_A is defined as

$$k' = (V'_S C'_S)/(V_M C_M) \quad (1.29)$$

where V'_S = volume of the stationary phase

V_M = volume of the mobile phase

From $K = C_S/C_M$

$k = KV'_S/V_M$

$k = (t_R - t_M)/t_M$

Selectivity factor

The selectivity factor (α) of a column for the two species A and B is defined as

$$\alpha = k_B/k_A \quad (1.30)$$

where k_B = the capacity factor for B

k_A = the capacity factor for A

The selectivity factor for the adjacent peak of components A and B is a function of the type of mobile phase, stationary phase and column temperature. For the separation to occur, α is more than 1.0.

Column efficiency

Two terms are used to indicate the column efficiency which are

- number of theoretical plate
- plate height

Number of theoretical plate

$$N = 16(t_R/W_b)^2 = 5.54(t_R/W_h)^2 \quad (1.31)$$

Where W_b = peak width at baseline

W_h = peak width at half height

A good column will have a large N.

Plate height

A relate parameter which expresses the efficiency of a column is the plate height, H.

$$H = L/N \quad (1.32)$$

Where L = the column length

H has the units of length and is better than N for comparing efficiencies of columns of differing length. It is also called the Height Equivalent to a Theoretical

Plate (HETP), a term which carried over from distillation terminology. A good column will have a large N and a small H.

Resolution

The resolution is measured to indicate the resolution power of the adjacent peaks for example, components A and B.

$$R_S = 2(t_{RB}-t_{RA})/(W_{BA}+W_{BB}) \quad (1.33)$$

Where t_{RB}, t_{RA} = retention time of component A and B, respectively

W_{BA}, W_{BB} = peak width at baseline of component A and B, respectively

1.7.2.2 Column and stationary phase [25]

The key to good GC separations is to use the most appropriate stationary phase and column at the optimum mobile phase velocity and column temperature. The main factors to consider when specifying the system for analysis of a given sample mixture are

- To note the boiling point range and vapour phase characteristics of the components and number of components.

- To identify the polar and non-polar characteristics of the components in the mixture and their functional groups.

- Selection of a stationary phase/column system and stationary phase film thickness to give the required selectivity and separation factors.

- Optimizing the carrier gas velocity for the fastest analysis time and minimum dead time set at the elution temperature of the critical pair of peaks, helium should be used as a carrier gas if at all possible.

- Optimizing the temperature programme or selecting the best temperature for isothermal analysis.

- Testing the system using a standard mixture either representative of he sample(s) to be analyzed or a mixture which includes compounds of differing polar character and boiling points.

1.7.3 Instrument of gas chromatography

Instrumentation of the gas chromatographic system is illustrated in Figure 1.8. The basic components of the gas chromatograph include: carrier gas; sample introduction; column and detector.

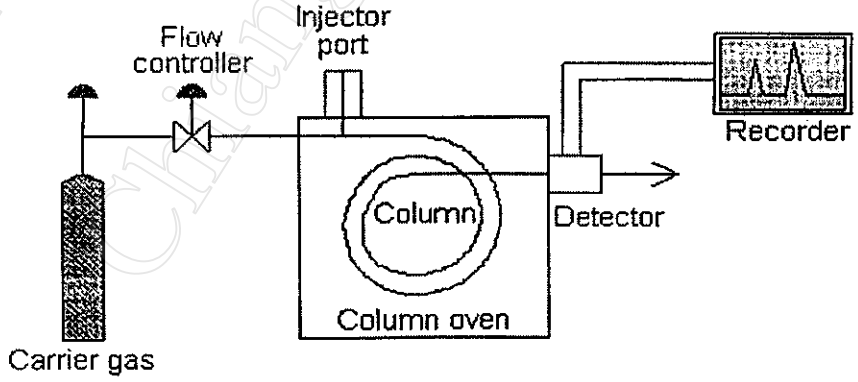

Figure 1.8 Schematic of a gas chromatograph [21].

1.7.3.1 Carrier gas and flow regulation [22]

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 which is used. In gas chromatography, the nature of the carrier gas does not significantly alter the partition coefficient K between the stationary and mobile phases. However, the velocity of the carrier gas and its flow rate have an effect on the analyte dispersion in the column thus affecting efficiency and sensitivity of detection which is illustrated in Figure 1.9. The pressure at the head of the column is stabilised either mechanically or through the use of an electronic device ensuring that flow rate in the column is at its optimal value. When a temperature program is used during analysis, the viscosity of the mobile phase is increased thus increasing the resistance to carrier gas flow.

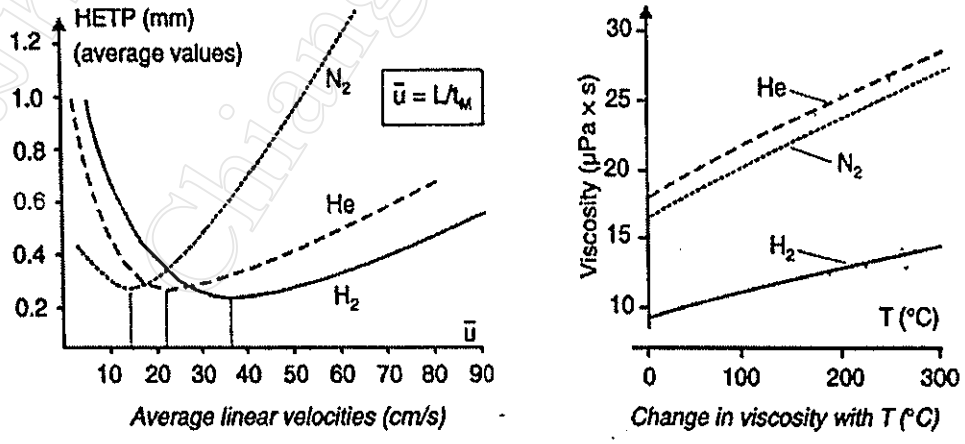

Figure 1.9 Optimum linear velocity of carrier gas [22].

The optimal mean linear velocities of the various carrier gas are dependent on the diameter of the column. The use of hydrogen as a carrier gas allows a faster separation than the use of helium while giving some flexibility in terms of the flow rate. This is why the temperature program mode is used. The significant increase in viscosity with temperature can be seen for gases. In addition, the sensitivity of detection depends on the type of carrier gas used.

1.7.3.2 Sample introduction [26]

In this work, the automated headspace sampler was used as sample injection system and connected to a gas chromatograph. HS-GC is illustrated in Figure 1.10.

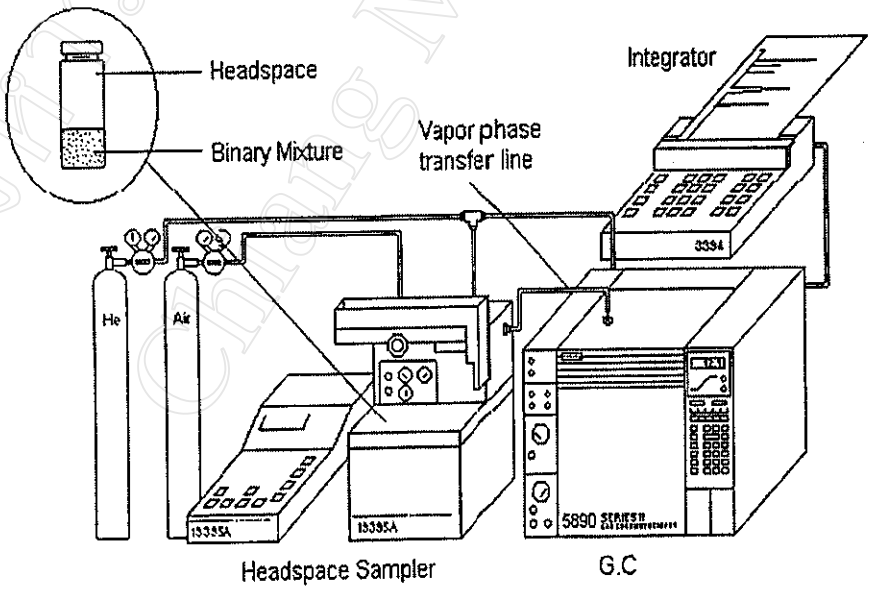

Figure 1.10 Schematic diagram of HS-GC [26].

When capillary column are used with small flow rates, even the smallest of injection volumes can saturate the column. Injectors that can operate in two modes, with or without flow splitting, are used (called split/splitless). In this work, splitless injection was used. Because the splitless is used for very dilute samples.

1.7.3.3 Column and column oven [22]

The column oven is used to control the column temperature to a few tenths of a degree for precise work with the aid of a thermostat. The temperature used in separation depends on the analyte's boiling point and the degree of separation required. In the broad boiling point range sample, the temperature program is required to provide reasonable separation and analytical time.

Packed and capillary columns are normally used in GC. The capillary column is preferred for today with its high resolution property. The capillary column is normally produced from fused silica with the length 10-100 m and 0.1-0.53 m internal diameter. The capillary column consists of three types namely, wall coated open tubular (WCOT), support-coated open tubular (SCOT) and porous-layer open tubular (PLOT) column. The WCOT is widely used. In WCOT, the liquid stationary phase is coated in the inner wall of column with 0.1-5 μm film thickness. But recently, the bonded phase of which the liquid stationary phase is chemically bonded with the signal groups onto the inner wall of the column is widely used. This provides more chemically inert and higher thermal stability. Many types of liquid stationary phase are commercial available. In this work, two types of liquid stationary phases were used; DB-1701 and DB-17MS containing 14% of cyanopropylphenylmethylpolysiloxane

and 50% of phenylmethylpolysiloxane, respectively. The chemical structures of both liquid stationary phases are shown in Figure 1.11-1.12.

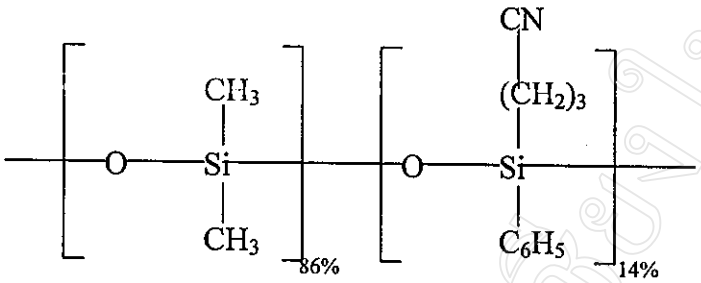

Figure 1.11 Structure of DB-1701 liquid stationary phase [27].

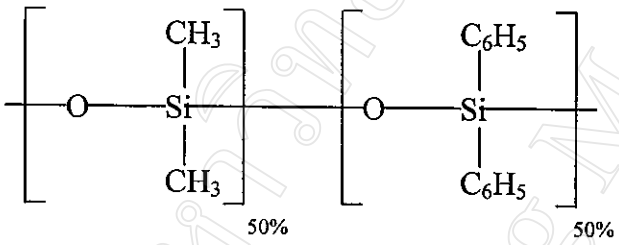

Figure 1.12 Structure of DB-17MS liquid stationary phase [27].

1.7.3.4 Detector [22, 23, 27]

The type of detectors that is chosen depends on the compounds of interest and the detection limits required for the analysis. Some detectors are universal. This is because they are sensitive to almost every compounds that elute from the column. However, most detectors are sensitive to a particular type of compound. These are called selective detectors. A selective detector is one that can detect only certain compounds, yielding a very simple chromatogram. The ideal determination of an

analyte is to have a detector that can detect only this type of analyte. All detectors give a response that is dependent on the concentration of an analyte in the carrier gas. In this work flame ionisation detector (FID) was used.

Flame ionisation detector [28]

The flame ionisation detector (FID) is the most universally used detector in capillary GC analysis because it is sensitive to all organic carbon containing compounds, it has a large linear dynamic range, easy to used, having a first response time, and a stable baseline. A diagram of the FID detector is illustrated in Figure 1.13.

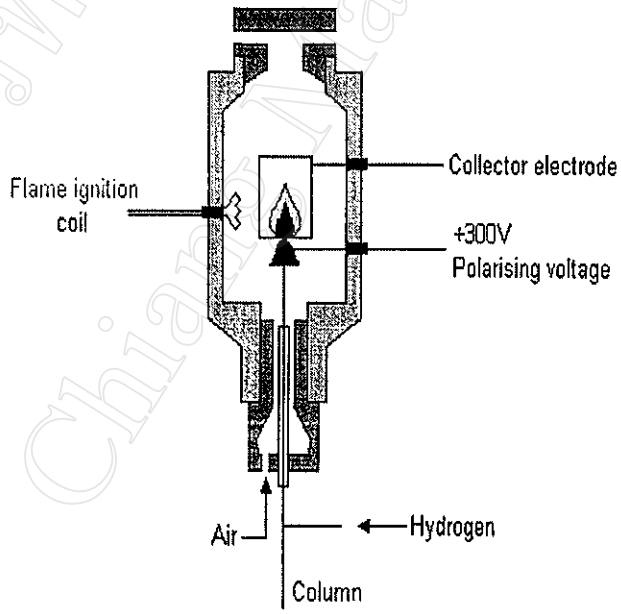

Figure 1.13 Flame ionisation detector [21].

The gas flow exiting the column passes through a small burner fed by hydrogen and air. This detector essentially destroys the sample. Combustion of the organic compounds flowing through the flame creates charged particles that are responsible for generating a small current between two electrodes. The burner, held at ground potential, acts as one of the electrodes. A second annular electrode, called the collector electrode, is kept at a positive voltage and collects the current that is generated. The signal is amplified by an electrometer that generates a measurable voltage. The detector is insensitive toward noncombusible gases such as H_2O, CO_2, SO_2, and NO_x. These properties make the FID a most useful general detector for analysis of most organic samples including those that are contaminated with water and the oxides of nitrogen and sulfur.

Alasalvar *et al.* [29] developed a static headspace analysis /gas chromatography/ mass spectrometry (SHA/GC/MS) for analyzing the volatiles of raw, stored and cooked carrot. A total of 35 different volatile compounds were identified in seven carrots. Of these, *trans*-ocimene, 2, 5-dimethyl styrene, camphor, borneol, α-santalene, α-selinene, γ-elemene and α-zingiberene in raw carrots and propanol in stored carrots were identified. Carrot volatiles did not change appreciably during the 28 day storage period at 5, 25, and 35 °C, except propanol that showed exponential increases at higher temperatures. Cooking resulted in 88.6, 93.0 and 95.5 % loss in total volatiles after cooking times of 10, 20 and 30 min, respectively.

Royer *et al.* [30] developed headspace gas chromatography for determination of dithiocarbamates in plant matrixes. This method offers a good level of accuracy and precision and is specific to the compounds determined. The limit of detection is below 0.020 mg/kg and the limit of quantification is below 0.050 mg/kg.

Alonso and Fraga [31] developed headspace gas chromatography-mass spectrometry for quantitation of the volatile compounds in yogurt. The relative standard deviation for individual flavor compounds range from 3.5% for acetaldehyde to 8.4% for aectone, with a total mean value of 52.4 $\pm$ 2.2 mg/kg. Recovery for individual flavor compounds ranges from 63.7% for acetone to 82.4% for acetic acid.

Koppen [32] determined ethephon in pesticide formulation by headspace gas chromatography. Ethephon (2-chloroethylphosphonic acid) is used as a growth regulator in argiculture and as a ripening accelerator in fruit and vegetable growing. This technique was used to determined ethylene formed from the decomposition of ethephon. Results from analyses of various pesticide formulation were compared with results obtained by using the official titrimetric method.

Zhong *et al.* [33] developed static headspace gas chromatography (SHS-GC) for the quantitation of residual solvents in pharmaceutical gel extrusion module (GEM) tablet formulations. Quantitation was performed by external standard analysis. The automated SHS-GC is simple and rapid. This method provides much more accurate and precise quantitative results.

Carrapiso *et al.* [34] used gas chromatography-olfactometry (GC-O) based on detection frequency (DF) for characterization the most order-active compounds from the headspace of Iberian ham. Twenty-eight odorants were identified. One of the most order-active compounds was identified as 2-AP.

Grimm *et al.* [35] used SPME/GC-MS for the screening of 2-AP in the headspace of rice. SPME was used to collect and concentrate the compounds in the headspace of rice. Then, the compounds were analyzed by GC-MS. This method uses less than 1 g quantitaties of rice which is important when rice varieties are screened as only a few grams of a particular variety may be available.

1.8 Qualitative Analysis [23]

The retention data of the reference standards and samples, retention time, relative retention time or index, is normally used for the qualitative purpose. But the retention data alone can only indicate the probability which the two substances, reference standard and solute in the sample, are the same. Because many compounds can give the same retention data in each GC column. Using the stationary phase with different polarities will give more accurate results. Nevertheless, the positive identification should be confirmed by using the spectroscopic methods such as MS or IR in conjunction with the gas chromatographic method.

1.8 Quantitative Analysis [28,36]

The analytical signal in gas chromatography is representative of the quantity of analyte present if several conditions have been met, for example:

- Injection error is minimized
- The analyte has been adequately resolved from matrix interferences
- The detector is reproducibly responding within its linear range

If the above conditions are true, several methods exist for measurement of peak size and for quantitative analysis, peak-size measurements include both manual methods and the faster and generally more accurate electronic integrations. Quantitative analysis can be performed using internal standard or external standard method.

1.9.1 Internal standard method

In this technique, a pure component (the internal standard) is added to the sample in a known amount. The ratio of analyte to the internal standard peak areas serves as the analytical parameter. With the suitable internal standard, the precision of better than 1% can be obtained. This technique has the advantage of carrying the standard through sample preparation, and consequently minimizes errors due to sample preparation and injection. It allows quantification of multiple components, and requires chromatographic resolution only of the components of interest and the internal standard. Requirements of an internal standard include the following:

- The internal standard must be nonreactive with all components of the sample
- The internal standard and the sample must be homogeneous
- The internal standard must be pure, and easily and accurately added to the sample
- The internal standard should be of similar retention time and of similar concentration to the component of interest

1.9.2 External standard method

For external standard method, the series of standards are injected to the gas chromatograph. The chromatographic response, i.e. peak height, is plotted versus the concentration. Then concentration of analytes in the sample is calculated from a constructed calibration graph. This technique allows the standard chromatographed separately from the sample. This necessitates the maintenance of constant chromatographic conditions and accurate measurement of syringe volumes. The standard referent compound is generally identical to the solute of interest. The concentration of the standard should approximate the concentration of the solute of interest, reducing errors from detector nonlinearity.

1.10 The scope and aims of this research

Aroma is an important quality characteristic of high-quality rices and the key aroma compound of rice is 2-acetyl-1-pyrroline (2-AP). As aromatic rice is in great demand in the rice market, Thai rice breeders have been trying to improve aromatic rice breeding program. In order to confirm aroma quality of the new

varieties obtained, analytical methods involving the determination of 2-AP in various rice varieties are utilized. A few researchers have improved method for quantification of 2-AP in aromatic rice, but none have reported the used of HS-GC technique. Recently, methods for determination of the aroma compound in rice have been developed. Conventional extraction methods employing steam distillation and solvent extraction required longer period of time for sample preparation and made degradation of aroma compounds. These methods had been replaced by solvent extraction. However, extraction of 2-AP by solvent requires many steps of sample preparation and, therefore, takes longer period of time[36]. In this work, HS-GC was applied for analysis 2-AP in rice seeds. The technique reduced sample preparation steps and time [37]. Thus, number of samples to be analyzed could be increased compared with those conventional methods.

The aims of this research work can be summarized as follows:

1) To investigate the extraction method and optimize the extraction conditions for extraction of 2-AP from rice seeds.
2) To optimize the automated headspace sampler conditions for the analysis of 2-AP in rice extracts by HS-GC using flame ionization as detector.
3) To quantify 2-AP in rice samples using the resulting analytical procedure.