Imparting Aromas into Raw Milled Rice: An Experimental Study

by

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PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
Dedication

To my husband, Tamrin Latief, and daughters, Ayeshah Augusta Rosdah and Elisha Rosalyn Rosdah, who inspired and encouraged me during my course of study.

Because of their love and understanding, I received the strength to complete my study.
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Declaration and Publications

The research reported in this thesis is my own work and, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in whole or in part, for a degree at any other university or institution.

Filli Pratama

Publications that have arisen from this thesis:


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Abstract

This thesis describes a series of experiments concerned with the production of aromatised rice, in which one or more aroma compounds were introduced into raw milled rice. The end product, which is potentially marketable, showed no visible difference from the untreated rice, and the cooked product had a perceivable aroma to consumers. The aromatisation process used liquid carbon dioxide as a vehicle to deliver the aroma, and eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde were used as the principal model aroma compounds in this study. Liquid carbon dioxide at a pressure of 8 MPa and an equilibration time of 5 minutes were found to be the optimum conditions for imparting these compounds into rice, and it was found that the aromas penetrated the cores of the rice grains. This was significant, as it potentially provided a longer period for the aroma compounds to migrate toward the rice surface and be lost to the open air. The stability of the injected aroma compounds in aromatised rice was investigated through a kinetic study, which showed that aroma loss was effectively a first-order process, although some of the model compounds showed evidence of two binding modes, with two distinct phases of aroma loss. These kinetic data, which showed different rate constants for the loss of the various aroma compounds, were used to calculate effective diffusivities for the aromas in rice.

To further assess the strength of aroma binding to rice, rice-flour, amylose, amylopectin and β-cyclodextrin gas-chromatography columns were developed to model the binding of aromas to the aromatised rice. Solution-phase experiments were also carried out for comparison. The relative retention times for aromas in these novel GC columns were used to evaluate the aroma interactions with rice and
starch. The results confirmed that the model aroma compounds interact with solid starch, both amylopectin and amylose. The aromas were better retained by the rice-flour column, possibly due to the structural organisation of amylose and amylopectin in the starch granules, or due to binding to the protein, or perhaps a combination of these two possibilities. Factors such as vapour pressure and the physicochemical properties and functional groups of the aroma compounds are also significant, and hydrogen bonding appeared to play a role in aroma-rice binding. The model aroma compounds had no difficulty fitting into the pores of the whole rice grains. Shelf-life studies demonstrated that eugenol and cinnamaldehyde in aromatised rice showed no significant changes in concentration after being stored for 6 months in sealed vacuum aroma-barrier plastic bags, and the aromas could be detected by the human olfactory system after the rice had been cooked by boiling and steaming.
Chapter 1

Introduction

1.1. General Introduction and Overall Aims

This thesis describes a series of experiments concerned with the production of aromatised rice, wherein extra aromas were added to raw rice. This includes a study of possible methods for imparting aromas into white milled rice, with the aim of ensuring a substantial level of aroma absorption by the rice while causing minimal damage to the grains. A series of model compounds, some of which occur in well-known spices, were evaluated for their level of absorption, strength of binding to rice, and rate of loss from the aromatised rice. The aroma concentrations in stored and cooked aromatised rice were also measured to evaluate its stability during storage and perceivability by the human olfactory system. The results have the potential to be used in the production of fragrant rice with a new range of aromas. This rice would be competitive in price with naturally grown fragrant rice, have an acceptably long shelf life, and attract consumer interest in a variety of international markets.

A wide range of experimental methods and modern analytical instrumentation were used in this project; for example, nuclear magnetic resonance (NMR) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, gas chromatography (GC) and pressurised liquid carbon dioxide technology. Liquid carbon dioxide was found to be an effective aroma carrier for introducing aromas into white milled rice, as it can deliver the aroma compound into rice grains in a short time and have a
negligible impact upon the integrity and appearance of the grains. In addition, the method can be applied on an industrial scale to accommodate a large quantity of rice.

Rice is one of the most important cereal crops in the world and approximately one third of the world’s population, especially in East, Southeast and South Asia, depend on rice as a staple food (Juliano, 1985a; Ronald, 1997). Rice from different cultivars can produce cooked rice with different characteristics in the areas of aroma, tenderness and hardness (Juliano, 1985a). Based on the intensity of the aroma after cooking, rice can be grouped into either non-fragrant or fragrant rice varieties. Fragrant rice is popular in some countries such as in East and Southeast Asia and the Middle East (Paule and Powers, 1989). The aroma of fragrant rice can be exuded from the leaves and seeds (grains) of a rice plant, and perceived by the human olfactory system (Pinson, 1994). The aroma of the volatile compounds can also attract insects and rats to feed on the plant. This can occur during seedling, harvesting (when rice is piled in the fields for drying) or storing, resulting in a low yield of production. Hence farmers, especially in developing countries where rice is a staple food, are reluctant to grow fragrant rice. However, the demand for fragrant rice is increasing due to the pleasant aroma in the cooked rice. This results in a higher price for fragrant rice than non-fragrant rice. Two alternative approaches to increasing the availability of inexpensive fragrant rice are (1) selective breeding or other genetic modification of fragrant rice varieties; or (2) development of aromatised rice products.

Research on genetically modified fragrant rice has been carried out to improve the quality of the rice, such as the cross breeding of non-fragrant and
fragrant rice varieties to combine the best characteristics from each variety (Nagaraju et al., 1975; Reddy and Sathyanarayanaiah, 1980; Pinson, 1994). There is general agreement that there are a number of factors that might affect the properties of the cross-bred fragrant rice; for example, the strength of the aroma in the original fragrant rice cultivar, and the genes that are related to the inheritance of the fragrant rice character. One example of a new variety of fragrant rice is YRF9 which was hybridised from Goolarah (an Australian fragrant rice). The YRF9 variety has a similar fragrance to that of Jasmine rice and a reasonable resistance to disease (Widjaja et al., 1996a). We have not as yet found a published paper on aromatising grains, especially rice. This could be due to the difficulty of modifying or processing rice grains without significantly changing their physical appearance and properties; for example, by cracking them and making them brittle. The method of imparting aromas into rice that was developed in this project can be used to impart various aroma compounds into rice to obtain a wider variety than is provided by the naturally grown fragrant rice. The procedure offers the further possibility of masking the unpleasant aroma of poor-quality rice.

The aroma of cooked naturally grown fragrant rice smells like popcorn or fragrant pandan leaves (Pandanus amaryllifolius Roxb) (Butterly et al., 1983a; Laksanalamai and Ilangantileke, 1993). Therefore, pandan leaves are added while cooking non-fragrant rice in most rice consuming countries in Asia. In addition, spices or herbs such as cloves, cinnamon, coriander and turmeric may also be added to the rice during cooking. This suggested the possibility of adding aromas to the uncooked rice before packaging, so that cooked aromatised rice can be produced without the need to add extra ingredients.
In the present project, raw milled rice and aroma compounds were used to produce aromatised rice. As mentioned earlier, it is desirable for aromatised rice to be similar in appearance to naturally grown fragrant rice, therefore the initial step of the project was to develop a method of imparting aroma compounds into raw rice without significantly changing the appearance and properties of the grains. Once the method was established the next step was to identify the types of aroma compounds that could be imparted into the rice, that have a low rates of loss during storage, and that have good sensory properties.

Use of carbon dioxide (either liquid carbon dioxide or supercritical fluid (SCF) carbon dioxide) to penetrate and wash the off-odours out of raw milled rice has been reported by a number of researchers (Yoshitsugu, 1988; Katsushi et al., 1990; Hiroki et al., 1992; Youichirou et al., 1994). In addition, carbon dioxide gas has also been found to be capable of penetrating some cereal grains, including rice (Yamamoto, 1976). These studies inspired us to use forms of carbon dioxide as aroma carriers to carry aroma compounds into the raw milled rice. Specifically, carbon dioxide gas and liquid carbon dioxide were used as carriers, and air was also used as a comparison.

A series of experiments — for example the effect of the degree of milling of rice on aroma retention in aromatised rice, and the effect of injected aroma compound concentrations — were undertaken in order to optimise the method for producing aromatised rice (see Chapter 2). Experiments were carried out afterwards to evaluate the stability of the aroma compounds in the aromatised rice; for example,
kinetic measurements of aroma loss (see Chapter 3) and measurements of the
effective diffusivities of aroma compounds in aromatised rice (see Chapter 4). We
also evaluated the relative strengths of the interactions between various injected
aroma compounds and rice components, by developing a number of starch (amylose,
amylopectin, β-cyclodextrin and rice-flour) gas chromatography (GC) columns (see
Chapter 5). The starch GC columns were used as media for evaluating which aroma
compounds could bind most readily to the rice, as well as what components of the
rice were most responsible for binding. Production of aromatised rice is assumed to
have been successful if the aroma is still perceivable after the rice has been cooked.
Hence, we carried out measurements of the aroma concentrations in cooked
aromatised rice (see Chapter 6). Some of these experiments were very time
consuming, particularly the kinetic measurements of aroma loss during storage, since
five model aroma compounds were used, with five replicates. The aroma
concentrations in the aromatised rice were measured at intervals that progressed from
an hourly through to a daily basis. For this reason, a simple method of aroma
extraction and analysis using methanol coupled with gas chromatography was used,
to ensure that the experiments could be carried out in a reasonable period of time.

The aromatised rice consists of a solid matrix with various non-volatile
components and added aroma compounds, resulting in a complex system that was
hard to reproduce consistently when experiments were replicated. This, coupled with
the natural variation between rice grains and the inherent imprecision of aroma
extraction and analysis, introduced significant statistical errors to many of our results.
Therefore the standard deviations of some results, especially the measurements of
aroma concentrations during storage (kinetics of aroma loss), were reasonably large;
however, we were still able to evaluate the results and obtain useful conclusions due to the availability of a sufficient number of data points.

As stated above, various starches, for example amylose and amylpectin, were used as stationary phases in GC columns with the aim of investigating the rice components that affected the interaction between aroma compounds and rice starch. The development of the starch and rice-flour GC columns was innovative and aimed to maintain the stationary phase under conditions that were relatively similar to the aromatised rice, rather than carrying out solution-phase studies, which has been the standard approach in earlier work. Due to the presence of a variety of rice components in the rice-flour GC column, we did not expect to obtain sharp peaks in the aroma compound chromatograms. We were only interested in evaluating the retention times of the aroma compounds in the various columns, to explore the hypothesis that these retention times reflected the strength of interactions between the compounds and components of the rice starch. It was assumed that longer retention times indicated stronger binding than shorter retention times.

The results from the various experiments undertaken in this project can be used by food manufacturers to guide the production of quality aromatised rice that is economically marketable. The project establishes a method of imparting aromas into rice, collects data on measuring the kinetics of aroma loss during storage, examines factors that might affect the strength of binding of aromas to rice, and measures the aroma concentrations in stored and cooked aromatised rice, all of which can contribute to greater control over aromatised rice quality.
1.2. Introduction to the Literature Review

Rice consumers are becoming more selective in purchasing rice, on the basis of judging the sensory quality of the cooked rice (Juliano, 1972a; Paule and Powers, 1989). The sensory quality of cooked rice is determined by factors such as colour, texture and aroma. In the absence of additives such as turmeric, the colour of cooked rice relates to the degree of milling of the raw rice, where degree of milling refers to the extent to which the outer layers are removed from brown rice during the milling process (Wadsworth, 1994). Brown rice that has had all of the outer layers (see Figure 1.1) removed during milling is called white milled rice. White milled rice, which is mostly consumed as boiled or steamed whole grains, is principally a source of energy and protein rather than vitamins (de Lumen and Chow, 1991). The vitamins, which are mostly located in the outer layer of a rice grain, are removed during the milling process. Although the majority of rice consumers prefer white milled rice, brown rice contains more protein, vitamins, minerals and lipid than milled rice (Spadaro et al., 1980; Wadsworth, 1994).

Factors affecting the texture of cooked rice have long been recognised, owing to extensive research in this area (Kumer et al., 1976; Okabe, 1979; Chrastil, 1990; Juliano, 1990). The texture of cooked rice depends, among other factors, on the ratio of amylose and amylopectin in the rice (Juliano, 1972b); cooked rice with a higher amylose content is dry, fluffy and less cohesive, whereas the texture of cooked rice with a lower amylose content is more sticky and soft (Lorenz et al., 1978). The preference for soft or fluffy cooked rice varies from one culture to another. The other attribute that contributes to the preference for a particular cooked rice is its aroma. The aroma released by a hot serving of cooked rice can increase its palatability.
Research on some aspects of the aroma of both fragrant and non-fragrant rice has been well reviewed (Yajima et al., 1978 and 1979; Tsugita et al., 1980; Buttery et al., 1982, 1983a, b, 1986, and 1988; Laksanalamai and Ilangantileke, 1993; Widjaja et al., 1996a, b). These papers mostly discussed the isolation and identification of aromas from rice. In a significant experiment, Buttery et al. (1985) imparted synthetic 2-acetyl-1-pyrroline into non-fragrant rice in order to compare the aroma of cooked aromatised rice with naturally grown fragrant rice by adding 25 mL of a 0.05 ppm water solution of synthetic 2-acetyl-1-pyrroline while cooking the non-fragrant rice. A sensory evaluation showed that the addition of 2-acetyl-1-pyrroline to non-fragrant rice during cooking gave the cooked rice the aroma of the naturally grown fragrant variety. We were interested in using 2-acetyl-1-pyrroline during the present study that unfortunately it was not possible to impart this aroma compound into the rice due to its instability. A detailed description of 2-acetyl-1-pyrroline, and problems associated with it, is presented in Section 1.3 below.

This chapter will summarise research on volatile components of rice that has been carried out over the last 25 years. A brief review will also be presented of studies of the improvement of rice aroma using liquid or supercritical carbon dioxide, as well as the physicochemical characteristics of rice and carbon dioxide.

1.3. Structure and Volatile Components of Rice

Rice grains consist of approximately 20% hull and 80% brown rice. According to Wadsworth (1994), the brown rice kernel (caryopsis) consists of bran (several histologically identifiable soft layers and the soft embryo or germ)
surrounding the hard starchy endosperm, which constitutes the milled rice kernel. Three distinct layers make up the caryopsis coat: the pericarp (consisting of the epicarp, mesocarp and endocarp), the seed coat or tegumen (consisting of the spermoderm and perisperm) and the aleurone layer which encloses both the starchy endosperm and the embryo. The cross section of a rice grain is depicted in Figure 1.1. Approximately 10% of the surface portion of brown rice, which is nutritionally superior to milled rice (Table 1.1.), is removed by friction or by abrasive milling to produce white milled rice (Juliano, 1985a). The degree of milling is defined as the amount of bran and polish removed from the brown rice during rice milling. The by-product of milling the brown rice to produce white milled rice is bran. The process itself is termed whitening. A further milling process of white milled rice in order to obtain a “glossy satin-like appearance” is defined as polishing, and the by-product during this process is polish (Spadaro et al., 1980; United Nations Industrial Development Organization, 1985). Besides producing white milled rice, milling can also prolong rice shelf life due to the removal of lipids which are mostly found in the outer layers of the rice endosperm (Juliano, 1992). Lipids in brown rice are subject to hydrolytic and oxidative deterioration (Champagne, 1994) which give rise to off-flavours. Furthermore, milling can also reduce cooking time and result in rice with a chewier texture, as well as removing the strong odour associated with the bran layers (Wadsworth, 1994).
Source: Juliano (1972b).

**Figure 1.1.** A cross section of a rice grain.

**Table 1.1.** Composition of brown and milled rice, rice bran and rice germ (%).

<table>
<thead>
<tr>
<th>Components</th>
<th>Brown rice</th>
<th>Milled rice</th>
<th>Rice bran</th>
<th>Rice germ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>8.3 - 9.6</td>
<td>7.3 - 8.3</td>
<td>13.2 - 17.3</td>
<td>17.7 - 23.9</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>2.1 - 3.3</td>
<td>0.4 - 0.6</td>
<td>17.0 - 22.9</td>
<td>19.3 - 23.8</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.7 - 1.2</td>
<td>0.3 - 0.6</td>
<td>9.5 - 13.2</td>
<td>2.8 - 4.1</td>
</tr>
<tr>
<td>Crude ash</td>
<td>1.2 - 1.8</td>
<td>0.4 - 0.9</td>
<td>9.2 - 11.5</td>
<td>6.8 - 10.1</td>
</tr>
<tr>
<td>Starch</td>
<td>77.2</td>
<td>90.2</td>
<td>16.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>19.1</td>
<td>4.5</td>
<td>27.6 - 33.3</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Pomeranz and Ory (1982).
One of the problems encountered in processing or modifying rice grains, especially white milled rice, is the cracked appearance of the grains after some types of treatment. The treatment, in this case, is the addition of substances (in particular those that incorporate water, such as gum arabic suspensions for coating) to the rice grains. It is known that rapid moisture adsorption causes low-moisture rice grains to crack or fissure easily (Kunze and Wratten, 1985). Aside from the moisture content of rice, Spadaro et al. (1980) claimed that the temperature and humidity of the environment and the condition of the milling machine could affect the breakage of rice during milling. However, Indudhara Swamy and Bhattacharya (1980) found that the number of broken grains after milling rarely exceeded the number of defective grains before milling. Defective or imperfect (common term) rice grains are those that have some deviations from the common grain shape, size, colour and lustre (Hoshikawa, 1993). Rice cracking was studied extensively by Stahel (1935). He studied the correlation between the moisture content of paddy (unhulled rice) and the age of rice on the breakage of rice during milling on eleven Suriname rice varieties. The results showed that low breakage percentages were obtained from rice varieties with moisture contents of from 10 to 11 percent, and also that the optimum age for rice to be harvested for milling was from one to three weeks.

The critical moisture content that causes rice to crack is different for paddy, brown and milled rice of the same lot or variety. For unhulled rice, the diffusion of moisture into the rice grain is slow as it must pass through the hull and pericarp before entering the endosperm. For this reason, unhulled rice is not easy to crack. Moisture penetration into brown rice is more rapid because the hull has been removed. Cracks appear most rapidly in milled rice, since the moisture can penetrate
the endosperm easily. Therefore, when hulled, brown, and milled grains are submerged in water together and then drained off, the milled rice grains will show substantial fissures while the brown and hulled rice may not (Kunze and Wratten, 1985). This feature makes it difficult to use water or moisture when processing rice, and indeed our own experiments using gum arabic as a potential aroma carrier caused the milled rice grains to crack.

The volatile components of rice, especially those in rice bran, were first studied by Fujimaki et al. (1977). These workers fractionated the whole steam-volatile concentrate from rice bran into basic, acidic, phenolic and neutral fractions. They found that the neutral fraction had a rice-bran like odour, the phenolic fraction had a guaiacol-like odour with the characteristic unpleasant odour of decayed straw, and the acidic fraction had a rancid and butter-like odour. Fujimaki et al. (1977) further confirmed that the major component which was identified from the acidic fraction — 4-vinylphenol — is what produces the unpleasant odour in rice bran. The following year, Tsugita et al. (1978) successfully isolated and identified aroma components of rice bran, using neutral and basic fractions. They further found that of 146 isolated compounds, 2-acetylthiazole and benzothiazole were identified as the key aroma compounds of rice bran.

Research on volatile compounds of rice was also continued using cooked rice. The characterization of the aroma compounds of fragrant rice was first attempted by Yajima et al. (1978). He and his co-workers identified more than one hundred volatile components from cooked Koshihikari rice (non-fragrant rice), including 13 hydrocarbons, 23 alcohols, 16 aldehydes, 14 ketones, 14 acids, 8 esters, 5 phenols, 3
pyrazines and 8 other compounds using steam distillation. The extracted compounds were then analysed using capillary gas chromatography (GC) and mass spectrometry (MS).

One year later, Yajima et al. (1979) reported an extension of this research using another variety of rice in order to compare the volatile components in non-fragrant rice *Koshihikari* (*Oryza sativa* var *Koshihikari*) to those in fragrant rice *Kaorimai* (*O. sativa* var. *Kaorimai*). The volatile components were extracted using diethyl ether, and separated into acidic, basic, and neutral fractions. GC and GC-MS were used to analyze all of the components that were extracted. They successfully identified 114 compounds in these fractions. However, they were unable to identify the characteristic volatile compounds in *Kaorimai* fragrant rice that gave *Koshihikari* non-fragrant rice a different odour.

Unfortunately, none of these earlier researchers identified the key aroma compound in most fragrant rices, until Buttery *et al.* (1982) established that it is 2-acetyl-1-pyrroline (abbreviated as 2AP in the rest of this thesis). 2AP was isolated from rice using continuous-extraction steam distillation, and analysed by capillary GC-MS. According to Buttery *et al.* (1983b), although 2AP is a clear colourless liquid while stored under vacuum at −20°C, it turns red and then becomes gradually darker with longer storage. It was suspected that the changes of the 2AP during storage are caused by the formation of a conjugated pyrroline polymer. Buttery and co-workers also found that 2AP was unstable under general GC conditions involving silicone or carbowax coated columns, but was reasonably stable with a glass capillary GC system. It is for this reason that 2AP was not detected in the extraction of
volatile compounds from rice in the earlier studies. After this discovery, further extensive research was carried out on the aroma compounds of fragrant rice, particularly 2AP. Once the methods for the isolation and identification of 2AP were successfully established, research was carried out to compare the concentrations of aroma volatile compounds in both fragrant and non-fragrant rice varieties (Lin et al., 1990; Widjaja et al., 1996a, b), and to improve the methods for isolating and quantifying 2AP (Buttery et al., 1986; Buttery et al., 1988; Tanchotikul and Hsieh, 1991; Petrov et al., 1996). Some researchers studied the similarity of the aroma of 2AP to that of other natural products (Laksanalamai and Ilangileke, 1993). They confirmed that 2AP which was isolated from fragrant pandan leaves (Pandanus amaryllifolius) also existed in fragrant rice. They also found that the intensity of the 2AP aroma tended to decrease during storage, however, they did not study the effect of the storage conditions (for example, temperature) on the degradability of the 2AP.

The dominant aroma volatile (2AP) was obtained from freshly cooked rice by vacuum steam distillation and continuous extraction with hexane (Buttery et al., 1982). According to Buttery et al. (1986), the release of 2AP from rice occurs during the cooking process. In fact, the measured levels of 2AP refer not to the concentration of 2AP in the raw rice, but to the total amount of 2AP released from a given mass of rice during a 2-hour heating period at atmospheric pressure, which occurs in the isolation process. This finding was in agreement with Schieberle (1990, cited in Schieberle, 1991), who found that the 2AP in bread crusts was actually formed during the baking of bread as a result of the reaction of amino acids (proline and ornithine) with a sugar degradation product (2-oxopropanal). Buttery et al. (1983b) stated that, although rice technologists agreed that raw fragrant rice also
contains the aroma volatile compounds that are present in the cooked fragrant rice, Buttery and co-workers did not detect 2AP in raw fragrant rice. This suggests that 2AP is released when a stable precursor compound is decomposed (perhaps by hydrolysis) during cooking. This would explain how fragrant rice has an acceptable shelf life, even though free 2AP is comparatively unstable.

![Structural formula of 2-acetyl-1-pyrroline (2AP).](image)

**Figure 1.2.** Structural formula of 2-acetyl-1-pyrroline (2AP).

The molecular structure of 2-acetyl-1-pyrroline, as presented by Buttery et al. (1985), is shown in Figure 1.2. When fragrant rice is cooked, much of the 2AP is released, and only small concentrations are left in the cooked fragrant rice. Therefore, the smell of fragrant rice is much more intense during cooking than in the final cooked product. It has been found that the natural product that is most similar in odour to 2-acetyl-1-pyrroline is the *pandan* leaf (*Pandanus amaryllifolius*). This is the reason why in most Asian countries, including Indonesia, *pandan* leaves are added while cooking non-fragrant rice. Adding *pandan* leaves not only produces fragrant rice but also helps to mask the stale odour of old rice. The conventional practice of using *pandan* leaves was further studied by Laksanamalamai and Ilangantileke (1993), who isolated the aroma volatiles of frozen *pandan* leaves and then compared them with the aroma volatiles of Thai fragrant rice (*Khao Dawk Mali-105*). They found that the GC retention time of the principal aroma volatile
compound extracted from pandan leaves and the authentic 2AP were reasonably identical. Therefore it seemed very likely that 2AP is the aroma compound in the oil of pandan leaves. The concentration of 2AP in pandan leaves was 10 times greater than that found in cooked fragrant rice, and 100 times greater than cooked non-fragrant rice. Buttery et al. (1983b) found that fragrant-rice varieties contained 0.04-0.009 ppm of 2AP whereas common rice varieties had approximately 10 times less (0.006 to < 0.008 ppm). The odour threshold of 2AP was 1.0 µg L⁻¹ in water (Buttery et al., 1982). The concentrations of 2AP found in some fragrant varieties of rice from different countries are presented in Table 1.2.

There is a worldwide preference for milled rice rather than brown rice, although brown rice is much more nutritious (Sabularse et al., 1991; Wadsworth, 1994). Tsugita et al. (1980) found that the degree of milling of rice affected the amount of aroma volatiles in brown and milled rice. For four degrees of milling of rice (8, 15, 25 and 50%), the highest levels of volatile compounds were found for the 8% degree of milling, and decreased with further milling. These results are generally consistent with those in Table 1.2. Thus, Tsugita et al. (1980) concluded that the outer rice layers play an important role in the formation of the cooked rice odour. An 8% degree of milling means that 8% of the mass of the brown rice is discarded during milling.
Table 1.2. Concentration of 2-acetyl-1-pyrroline in fragrant rice varieties.

<table>
<thead>
<tr>
<th>Rice Varieties</th>
<th>Milled Rice (ppm*)</th>
<th>Brown Rice (ppm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malagkit Sungsong</td>
<td>0.09</td>
<td>0.2</td>
</tr>
<tr>
<td>IR 841-76-1</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>Khao Dawk Mali 105</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>Milagrosa</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Basmati 370</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Seratus Malam</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Azucena</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>Hieri</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Texas Long Grain</td>
<td>&lt;0.008</td>
<td></td>
</tr>
<tr>
<td>Calrose</td>
<td>&lt;0.006</td>
<td></td>
</tr>
</tbody>
</table>

*ppm = parts per million of 2AP in rice (dry weight); these values refer to 2AP released during cooking (see text).

Source: Buttery et al. (1985).

The extraction of aroma compounds from rice was continued by Lin et al. (1990). They identified and quantified the compounds from cooked “popcorn like” fragrant rice (Della variety), and non-fragrant rice (Lemont). The cooked Della rice contained almost 0.3 ppm of 2AP (i.e., 300 ng/g dry weight), whereas the Lemont rice contained only 0.004 ppm of 2AP. These results indicate that Della white rice contained about 4 times as much 2AP as Jasmine white rice from Thailand (0.073 ppm).

Recent research on the volatile aroma compounds of raw rice has been carried out by Widjaja et al. (1996a), who observed the volatile components of paddy, brown and white fragrant rice during storage, and carried out comparative studies on the
volatile components of non-fragrant (Pelde) and fragrant rices (Basmati, Jasmine, Goolarah, YRF9). They reported that non-fragrant rice contains much more hexanal, heptanal, 6-methyl-5-hepten-2-one, (E)-2-heptenal, 1-octen-3-ol, nonanal, (E)-2-octenal and (E)-2, (E)-4-decadienal than fragrant rices. Also, levels of pyridine, pentanol, 2-pentylfuran, 4-vinylguaiacol and 4-vinylphenol were higher in Pelde rice than in the three fragrant rices. Widjaja et al. (1996b) also reported that there was generally an increase in levels of most of the carbonyl compounds in rice during storage. Increases also occurred in n-pentanol, 2-pentylfuran, 1-octen-3-ol, and 4-vinylguaiacol, which were higher in the paddy rice than in brown and white rice. Widjaja and her colleagues successfully identified the major aroma component (2-acetyl-1-pyrroline) in fragrant rices Goolarah (691 µg kg\(^{-1}\)) and YRF9 (670 µg kg\(^{-1}\)) and non-fragrant rice Pelde (15 µg kg\(^{-1}\)). Although 2AP was also found in non-fragrant rice, the concentrations were far below that in fragrant rice, with the outcome that the odour of this compound is virtually undetectable in cooked non-fragrant rice. They also confirmed that the higher concentration of 2AP in brown rice indicated that the outer layers of brown rice contain more 2AP or its precursors, and that this amount decreases with an increase in the degree of milling.

In summary, the current project of imparting aromas into rice has built upon the knowledge that rice is a complex system consisting of various non-volatile and volatile components, and treatments incorporating water can cause the rice grains to fissure easily. Previous work has also established that the distinctive difference between non-fragrant and fragrant rice varieties is the pleasant odour of 2-acetyl-1-pyrroline, which is much more intense in cooked fragrant rice. Our initial aim in this project was to study the possibility of aromatising non-fragrant rice to produce an
inexpensive product that resembled current varieties of fragrant rice, however, it
became clear that any attempts to impart 2AP into a non-fragrant variety of rice
would be difficult, since pure 2AP is unstable, and the product would probably have
a short shelf life. In any case, a more promising way of achieving this particular aim
would be to try to produce better-yielding and hence less expensive varieties of
fragrant rice through selective breeding or genetic modification; other workers are
working along these lines. We therefore sought to produce alternative aromatic rice
products that would have acceptable shelf life and marketable sensory properties.
Based on the findings of the earlier studies of the aroma volatiles of rice, we were
able to select some aroma compounds that possess similar molecular structures to the
naturally occurring aroma compounds in rice, which should therefore facilitate the
aromatisation process. Another aspect of this study was the possibility of improving
the aroma and palatability of poor-quality or aged rice.

1.4. Improvement of the Aroma of Rice

Improvement of the aroma of rice has long been practised by rice consumers.
For example, in many Asian countries it is customary to add pandan leaves, lemon
gress, turmeric or coconut milk during the cooking of rice in order to produce
pleasant aromas during eating. Another purpose for adding aromatic ingredients is to
mask any stale flavour (off-flavour) of the rice, and this can improve the palatability
of cheap but poor-quality rice. Hori et al. (1995) studied the sensory evaluation by
Japanese consumers of different types of rice. One of the types of rice that they used
was a mixture of stale rice with fragrant rice. However, this was not very successful
at masking the off-flavour of rice. So far, we have not found any published studies
of the deliberate use of pandan leaves or other aromatic ingredients, such as turmeric,
clove or cinnamon, to mask off-flavours in stale raw rice. However, there has been some research carried out to reduce the stale flavour of cooked rice, for example by adding acetic acid bacteria during cooking (Nomura et al., 1995). They found that the bacterial cells effectively reduced the unpleasant aroma of cooked rice by oxidising the compounds of the off-flavour, such as aldehydes, into their corresponding carboxylic acids which have higher odour thresholds than aldehydes. This results in reduced detection by the human olfactory system. Although the technique significantly reduced the stale flavour of cooked rice, it was not easy to apply in the everyday cooking of rice. This was due to the procedural requirement of soaking the rice in water (50ºC, pH 6) containing the acetic acid bacteria cells for one hour before cooking.

Some other methods for improving rice flavour have been studied by Japanese researchers. Yoshitsugu (1988) carried out a study of washing rice grains with liquid carbon dioxide. His main purpose was to remove fat in order to produce a good quality of rice for making sake. Two years later, Katsushi et al. (1990) studied the processing of brown rice in supercritical carbon dioxide in order to produce "an easy water-penetrated brown rice". Brown rice has hard outer layers that lead to a longer cooking time, since water cannot penetrate easily into the brown rice. Katsushi et al. (1990) obtained results that were not entirely satisfactory, since water still could not easily penetrate into the brown rice after processing. In addition, they found that some fat remained in the brown rice, so the aroma of the brown rice was preserved. Only the fats on the outside of the brown rice were washed off during processing.
Youichirou et al. (1994) also studied methods for removing the aroma of brown rice. They developed a manufacturing process to produce brown rice that does not need to be washed thoroughly before cooking, and has a similar quality to polished rice in taste, but with the same nutritional components as brown rice except for the fat content. The rice was treated with liquid or supercritical carbon dioxide and, as a consequence, only the fat components of the brown rice were expected to be washed off. However, the results showed that the fat content of brown rice was not significantly reduced by this processing. Youichirou et al. (1994) also used milled rice in the experiments, and they found that the fat content in milled rice was more significantly reduced than that of the brown rice using this technique, due to the ease with which the liquid or supercritical carbon dioxide could penetrate the grains. This result had significance for the present project.

A diagram of the apparatus used by Youichirou et al. (1994) is presented in Figure 1.3. Initially, carbon dioxide gas is admitted into the apparatus by opening the valve of a carbon dioxide cylinder (1). The gas is passed through a pressure valve (2) and condenser (3) to produce liquid carbon dioxide. The liquid carbon dioxide travels to chamber (4) which has already been filled with rice. The liquid carbon dioxide and rice are held for a certain period in this chamber. Then the liquid carbon dioxide is sent to a container (6) at a reduced pressure which is controlled by a second valve (5). Under these conditions, the fats which dissolve in the liquid carbon dioxide chamber (4) will be precipitated as a solid in container (6). The carbon dioxide in container (6) is passed through a filter (7) and further filtered by absorbent (8) in order to clean the gas. After that, the carbon dioxide passes through a condenser (9) in order to reduce its temperature, and it is subsequently released or
recycled. The apparatus that was used by Youichirou and co-workers seemed to allow the liquid carbon dioxide to penetrate well into milled rice, as indicated by the reduction in fat content. A modified version of this apparatus was used as a basis for the present project for carrying aromas into milled rice to produce aromatised rice, as the liquid carbon dioxide has the potential to penetrate the grains and deposit aroma compounds in the cores of the grains.

The physicochemical properties of rice, such as swelling, cooking time, stickiness, chemical and sensory properties, are subject to change during storage (Chrstil, 1994; Villareal et al. 1976). Widjaja et al. (1996b) studied the changes in levels of volatile compounds in paddy, brown and white fragrant-rice during storage. They found that during storage in air, there was an increase in the levels of most carbonyl compounds (for example 2-hexanone, 2-heptanone, 2-nonanone and acetophenone) in the rice. The increase was greatest in white milled rice, followed by brown and paddy rice. During storage, the rate of oxidation was greater for white milled rice, due to the removal of the hull and bran during the milling process.

Hiroki et al. (1992) tried to improve the aroma of stale (oxidised) rice by getting rid of the off-flavours through washing the rice with supercritical and liquid carbon dioxide (CO₂). During this study, they also combined the liquid carbon dioxide with short-chain n-alkane co-solvents such as ethane, propane or butane. They found that the addition of the co-solvents to the liquid carbon dioxide could significantly reduce the levels of fat and the carbonyl compounds which caused the unpleasant aroma in the rice. This raises the possibility of using co-solvents in cases
where liquid carbon dioxide is not an effective aromatising agent. In practice, we did not find this necessary.

Source: Youichirou et al. (1994).

**Figure 1.3.** Scheme of apparatus for processing rice to remove fats.
As mentioned above, most rice consumers prefer white milled rice to brown rice, although many know that brown rice is superior in nutrient value. Champagne (1994) reported that a major deterrent to greater use of brown rice is its short shelf life of 3-6 months, due to rancid off-flavours and off-odours being imparted to the rice as its oil deteriorates through oxidation. The off-odours are due to rancidity of the lipids in the rice, which are concentrated in the outer layers (Chrstil, 1994). This susceptibility to rancidity has limited the commercial production, marketing, and consumption of not only brown rice kernels, but also of its products: flour, bran, and oil.

When processing rice, the most important factors that the manufacturer should know are the structure and physicochemical properties of the rice. These properties are helpful in choosing the equipment and chemical agents to be used in the processing. Since the present study used carbon dioxide for processing rice (as do several earlier studies, as summarised above), it is important to review the properties of carbon dioxide and its interaction with rice.

The adsorption of carbon dioxide gas by cereal grains has been studied by Mitsuda et al. (1973), and Yamamoto and Mitsuda (1980). The results of their experiments are reviewed below. They found that the carbon dioxide gas adsorption could be due to the dissolution of carbon dioxide gas in water and fat, diffusion of carbon dioxide gas to porous tissues of the grains, and biological fixation of carbon dioxide gas. Mitsuda et al. (1973) studied the mechanism of carbon dioxide gas adsorption, and found that fifty to sixty percent of the maximum adsorption takes place within the first six hours in the case of rice. Desorption curves show that the
carbon dioxide gas adsorption is almost completely reversed when the grains are allowed to stand in air (Figure 1.4). The rate of desorption is larger than that of adsorption. The amount of carbon dioxide gas adsorbed by grains depends on temperature, and increases at lower temperatures. However, the rate of adsorption is not as strongly temperature dependent. When rice is heated at 120°C for 3 hours and then cooled in a desiccator for 30 minutes prior to carbon dioxide determination, the amount of carbon dioxide adsorbed increases remarkably (nearly two-fold) which is due to the increased porosity of the rice grains.

The terms ‘absorption’ and ‘adsorption’ are used throughout this thesis and thus require definition. Adsorption is defined as a process in which the molecules of a substance attach to the surface of a matrix, while the removal of the molecules from the surface is called desorption. Absorption, on the other hand, describes the penetration of the substance into the matrix. In most published papers dealing with the penetration of carbon dioxide gas into cereal grains (as discussed below), the term ‘adsorption’ is used. However, since it was found in the present study that the injected aroma compounds penetrated into the rice grains (see Section 2.4.3), the term ‘absorption’ may be more applicable.

The adsorption of carbon dioxide depends on the moisture content of the rice, as shown in Figure 1.5. Adsorption increases almost linearly in brown rice but decreases almost linearly in paddy (hulled rice) as the moisture content of the rice grains increases; presumably this effect is due to the dissolution of carbon dioxide gas in water. The effect of temperature on carbon dioxide gas adsorption by rice was confirmed by Yamamoto and Mitsuda (1980). They found that temperature can
affect the amount of carbon dioxide gas which is adsorbed by rice grains, with increased adsorption at lower temperatures (Figure 1.6). However, the rate of adsorption was not significantly affected by temperature.

Source: Yamamoto and Mitsuda (1980).

Figure 1.4. Carbon dioxide gas adsorption and desorption by cereal grains as a function of time at 25°C.
Figure 1.5. Effect of moisture content on carbon dioxide adsorption by rice at 25°C.

Mitsuda et al. (1973) found that the lipid content in rice played a minor role in the adsorption of carbon dioxide gas by rice grains, and the most important factor in the adsorption phenomenon is the porosity of the grains. They further stated that the carbon dioxide remained in a solid solution after the gas was absorbed by the porous tissue of the grains. The amount of carbon dioxide gas adsorbed by various grains is presented in Table 1.3, with the results showing that peanut, soybean and
sesame seeds are more adsorbent than rice. Yamamoto and Mitsuda (1980) found that starch in purified forms failed to adsorb carbon dioxide gas and therefore the better adsorbency of peanuts, soybean and sesame seeds might be due to the participation of fat in the interaction with the carbon dioxide gas. However, the findings contrasted with their other experiments on the adsorption of carbon dioxide gas by undefatted and defatted brown rice. They found that undefatted brown rice adsorbed less carbon dioxide gas than defatted brown rice. This suggested that there was another factor affecting the adsorption process. According to Mitsuda et al. (1973) and Yamamoto and Mitsuda (1980), it was the porosity of the rice grains that had an influence on the carbon dioxide gas adsorption phenomenon. The defatted rice grains had more pores (spaces) between starch granules and hence carbon dioxide gas could penetrate more readily into the grains. The capacity of brown rice to adsorb carbon dioxide was also observed by Mitsuda et al. (1973). Although the experiments were not carried out on whole brown rice grains (separated parts of the grains were used), they confirmed that the carbon dioxide gas is not located in a single part of a brown rice grain, but rather distributed throughout the grain (Figure 1.7).
Source: Mitsuda et al. (1973).

**Figure 1.6.** Effect of temperature on carbon dioxide adsorption by rice.

Source: Mitsuda et al. (1973).

**Figure 1.7.** The relative capacities of different components of brown rice to adsorb carbon dioxide.
Table 1.3. Amount of carbon dioxide gas adsorbed by various grains.

<table>
<thead>
<tr>
<th>Grains</th>
<th>Amount of CO₂ adsorption at 20°C during 3 hours (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddy rice</td>
<td>86</td>
</tr>
<tr>
<td>Brown rice</td>
<td>90</td>
</tr>
<tr>
<td>Polished rice</td>
<td>70</td>
</tr>
<tr>
<td>Rice flour</td>
<td>60</td>
</tr>
<tr>
<td>Wheat</td>
<td>75</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>60</td>
</tr>
<tr>
<td>Corn</td>
<td>170</td>
</tr>
<tr>
<td>Peanuts</td>
<td>560</td>
</tr>
<tr>
<td>Soybeans</td>
<td>440</td>
</tr>
<tr>
<td>Soybean flour</td>
<td>216</td>
</tr>
<tr>
<td>Red beans</td>
<td>64</td>
</tr>
<tr>
<td>Coffee beans</td>
<td>123</td>
</tr>
<tr>
<td>Sesame seeds</td>
<td>230</td>
</tr>
<tr>
<td>Black tea</td>
<td>115</td>
</tr>
</tbody>
</table>

Source: Mitsuda et al. (1973).

One of the most important aspects of imparting an aroma into food is how to keep the aroma stable in the food material. Many approaches have been developed for trapping aromas within food materials; one of these is encapsulation. When encapsulating an aroma into food, carriers are needed to coat or entrap the aroma. According to Bhandari and D'Arcy (1996), maltodextrins, gum arabic, modified starches, sucrose, common salt, gelatine, vegetable and milk protein, waxes and fats can be used as carriers for encapsulation. It should, however, be noted that encapsulation methods often involve the use of water. Since moisture absorption or desorption by rice can cause the development of fissures in rice grains (Spadaro et al., 1980), treatments that involve liquid can cause the grains to crack. In a
preliminary study within this research project, a range of methods were tested in an attempt to impart aromas into rice; for example, coating the raw rice with gum arabic, which is an effective coating for entrapping aromas. Unfortunately, the final product exhibited fissures across the rice grains due to the water that was used in the treatment. We did not persist with other encapsulation methods, since water is often involved in the encapsulation process, especially in manufacturing the coating materials (since starch dissolves in water) which are used to encapsulate the matrix.

There are several factors that should be considered when choosing a method of imparting an aroma into raw rice. Firstly, as mentioned above, it is important to determine whether or not fissures will occur in the raw rice grains after treatment. Secondly, the raw rice is mostly consumed as whole boiled or steamed rice, therefore the aroma should be bound tightly to the rice during storage and released slowly during cooking. Thirdly, the rice should retain a perceptible aroma until it is consumed.

One of the methods that is proposed by us involves using a carrier to carry aromas into raw rice without causing fissures in the grains. As reviewed in this chapter, Yoshitsugu (1988), Katsushi et al. (1990), Hiroki et al. (1992) and Youichirou et al. (1994) used various forms of carbon dioxide to treat rice, especially to remove unpleasant odours. This research indicated that it might be possible to develop a method for imparting aromas into raw rice using carbon dioxide as a carrier. Further discussion of preliminary work on the use of carbon dioxide, especially gas and liquid carbon dioxide, is presented in Chapter 2 of this thesis.
In the present chapter, the properties of carbon dioxide are briefly reviewed. Possible states of carbon dioxide are solid, liquid (subcritical or near critical), and supercritical. The location of the critical point for carbon dioxide is illustrated in Figure 1.8. Supercritical carbon dioxide exists at pressures and temperatures above the critical values (73.8 bar, 31.06°C) for the substance, whereas the liquid phase occurs between the triple point and the critical point. Carbon dioxide can be maintained in the liquid phase under the relatively modest pressure of about 950 psi at 25°C (Hyatt, 1984). However, as is well known, liquid carbon dioxide does not exist at normal atmospheric pressure (1 atm; 101 kPa). Liquid carbon dioxide behaves like any other liquid, whereas the supercritical phase (the supercritical fluid, SCF) has higher diffusivity, lower viscosity and lower surface tension than the liquid (Rizvi et al., 1986). Both liquid and supercritical carbon dioxide have good solvent properties for a variety of solutes, as discussed below.

According to Moyler (1993), compressed carbon dioxide (liquid and supercritical) can penetrate materials from which aroma compounds are being extracted, and can then be easily removed without leaving any solvent residue. In addition, by varying the temperature and pressure of the carbon dioxide during extraction, its solvent properties can be tailored to suit the particular volatile compound that is to be extracted. In the present project we aim to use this process in reverse, where the ability of liquid carbon dioxide to penetrate foodstuffs allows it to deposit aroma compounds, rather than removing them.
Source: Snow et al. (1997) and Rizvi et al. (1986).

Figure 1.8. Phase diagram for carbon dioxide.

Carbon dioxide has been widely used in the food industry for a variety of purposes. For example, near-critical carbon dioxide is used in the deodorization and deacidification of edible oils (Ziegler and Liaw, 1993), and carbon dioxide gas can be used for extending the shelf-life of food during storage (Penney et al., 1993; Bremner and Statham, 1987; Gill, 1990). Liquid and supercritical carbon dioxide are mostly used as solvents to extract heat-labile aroma volatile compounds from food, such as the removal of free fatty acids from rice bran and cotton seed oils (Mukhopadhyay and Nath, 1995). In addition, carbon dioxide, especially in a supercritical state, is also used for the quantification of aroma vapours absorbed in packaging films (Johansson et al., 1993).
The wide use of carbon dioxide, especially in the liquid and supercritical states, results from its being easily available, non toxic, relatively inert, and environmentally acceptable (Hyatt, 1984). The properties of some compounds that are commonly used as supercritical fluids are listed in Table 1.4. The extensive use of carbon dioxide in industry is also due to its physical properties such as viscosity and diffusivity, which make it an effective solvent. The details of some physical properties of carbon dioxide in gas, liquid and supercritical phases are summarised in Table 1.5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Boiling point (°C)</th>
<th>Critical temperature (°C)</th>
<th>Critical Pressure (atm)</th>
<th>Critical density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>-78.5</td>
<td>31.3</td>
<td>72.9</td>
<td>0.448</td>
</tr>
<tr>
<td>N₂O</td>
<td>-89.0</td>
<td>36.5</td>
<td>71.4</td>
<td>0.457</td>
</tr>
<tr>
<td>SF₆</td>
<td>-63.8</td>
<td>45.6</td>
<td>37.1</td>
<td>0.752</td>
</tr>
<tr>
<td>NH₃</td>
<td>33.4</td>
<td>132.3</td>
<td>111.3</td>
<td>0.240</td>
</tr>
<tr>
<td>Water</td>
<td>100.0</td>
<td>374.4</td>
<td>226.8</td>
<td>0.344</td>
</tr>
<tr>
<td>Methanol</td>
<td>64.7</td>
<td>240.5</td>
<td>78.9</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Source: Snow et al. (1997).

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Carbon dioxide phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>0.6 – 2.0</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>0.01 – 0.03</td>
</tr>
<tr>
<td>Diffusivity (cm²/s)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Most recent research has centred on carbon dioxide in the supercritical phase because it is thought to have, in general, better solvent properties than subcritical carbon dioxide. These studies dealt mostly with the extraction of aroma volatiles. In contrast, liquid carbon dioxide has been used as a vehicle to carry aromas into the rice grains in this project. Liquid carbon dioxide was chosen as the carrier due to its relatively high diffusivity and its good solvency for some aroma volatile compounds, such as aldehydes, esters, ketones, and short-chain hydrocarbons (Hyatt, 1984). We used it in preference to supercritical carbon dioxide because the construction of the equipment was less expensive than that required for supercritical carbon dioxide, and the liquid was found to perform very well, with minimal damage to the rice grains.

A knowledge of the properties of the carrier is not enough to give a good understanding of how an aroma is imparted into rice. Besides the selection of the right type of aroma, it is also important to determine the capacity of the medium (rice) to bind the aroma.

White milled rice consists mainly of a starchy endosperm, of which more than 80% is starch (Juliano, 1985a). The other major components are fat (0.3-0.5%), protein (5-10%), water (12%) and ash (0.4%) (Rijkens and Boelens, 1975). It was therefore suspected that the starch in the endosperm of rice plays a major, and perhaps dominant part, in the binding of the aroma compounds which were injected into samples of white milled rice. However, in this thesis we have also considered the role that fat and protein may play in aroma binding. Starch, which is a polysaccharide that is found in plants, consists of amylose (Figure 1.9) and amylopectin (Figure 1.10). The amylose is composed of linear chains of D-glucose
with $\alpha$(1-4) linkages, whereas amylopectin is a highly branched chain of glucose units. The linear linkages in amylopectin are $\alpha$(1-4), whereas the branch linkages are $\alpha$(1-6) (Belitz and Grosch, 1999). Much research has been carried out which shows that starch, especially amylose, is capable of forming inclusion complexes with volatile compounds (Osman-Ismail et al., 1961; Osman-Ismail and Solms, 1973; Rutschman et al., 1989; Rutschman and Solms, 1990; Escher et al., 1999).

Source: Szejtli (1971).

**Figure 1.9.** The helical conformation of amylose.
(a) amylopectin with parallel double helices.  (b) an enlarged segment of (a).

Solid lines indicate $\alpha$-1,4 glucosidic chains and arrows indicate $\alpha$-1,6 glucosidic bonds.

Source: Banks and Muir (1980).

Figure 1.10. The cluster model of amylopectin.

The amylose content of rice varies, and depends on the rice variety. The amylose content of low and intermediate amylose rice is 7-20% and 20-25%, respectively, while the content of high amylose rice is above 25% (Juliano, 1979 cited in Juliano, 1985b). With the aid of X-ray diffraction analysis, native starch granules reveal three crystallographic patterns, denoted as A, B, and C (Belitz and Grosch, 1999). The A pattern is usually present in cereal starches, including rice. The B pattern is found in potatoes, amylo maize and retrograded starch, whereas the C pattern, which is a mixed pattern of A and B, is found in mixtures of corn, potato and legume starches. The structural element of the B pattern is a double helix packed in an anti-parallel arrangement, and hexagonal mode. The central channel is filled with water. The A pattern (which occurs in rice) is very similar, except that the central channel is occupied by another double helix which is predicted to be lipid, and water molecules are distributed between the double helices.
Starch, and particularly its linear unbranched amylose fraction, has the capacity to form complexes with many low molecular-weight organic compounds (Rutschmann et al., 1989). According to Belitz and Grosch (1999), many molecules such as iodine, monoacylglycerides, phenols, arylhalogenides, n-butanol, t-butanol and cyclohexane form inclusion (clathrate) compounds with amylose molecules. However, the amylopectin portion of starch shows little tendency to form complexes (Godshall and Solms, 1992). According to Escher et al. (1999), the formation of complexes between aroma compounds and amylopectin had no effect on the retention or release of the compounds. The helix diameter of the amylose conforms to a certain extent to the size of the enclosed guest molecules; it varies from 1.37 to 1.62 nm (1 nm = 10\(^{-9}\) m). The helix is internally hydrophobic, and the enclosed guest has also to be lipophilic in nature. The enclosed molecule contributes significantly to the stability of a given conformation. Reineccius (1991) has further reported that partially hydrolyzed starches, dextrins, maltodextrins and glucose syrup are less effective complexing agents because the helical structure is degraded.

Proteins, the second most abundant solid components, contribute 7.3-8.3% of milled rice grains. Cagampang et al. (1966) found that, of the total rice proteins, water-soluble proteins (albumins) contribute approximately 3.8-8.8%, salt-soluble proteins (globulins) 9.6-10.8%, alcohol-soluble proteins (prolamins) 2.6-3.3%, and alkali-soluble proteins (glutelins) 66.1-78.0%. Rice glutelin proteins, substantially the largest protein fraction, are thought to occur in the protein bodies (the bulk of the storage proteins) which are distributed in the rice endosperm (Harris and Juliano, 1977). Scanning microscopy has shown that the protein bodies occupy the space
between the endosperm starch granules (Damadjati et al., 1982 cited in Damadjati, 1983). Glutelin, which incorporates hydrophobic bonding and disulfide linkages, is a high molecular mass molecule, and is only soluble in aqueous systems for pHs below 3 and above 10 (Juliano, 1985b). Earlier studies, which dealt with the interaction between protein and volatile compounds in the solution phase, showed that volatile compounds (benzyl alcohol, n-hexanol) can bind to protein. However, proteins showed no consistent effects with regard to the strength of their binding with aroma compounds, and in addition there was no aroma compound that was preferentially bound by all proteins (Franzen and Kinsella, 1974). The aroma-protein binding is complicated, since there are a number of factors that can affect the interactions, such as the type of proteins, the functional groups of the volatile compounds, temperature, pH and the presence of moisture and lipids (Franzen and Kinsella, 1974; Solms, 1985; Voilley et al., 1991). For example, Franzen and Kinsella (1974) studied the effect of moisture and lipids on aroma-protein binding, and found that the volatility of an aroma compound in an aqueous system was reduced by the presence of water in the mixture, while the addition of corn oil to the mixture reduced the binding between the aroma compound and protein, as a result of aroma solubility in the oil. In the earlier research on the aroma-protein binding, most researchers agreed that the binding was mostly dominated by hydrophobic interactions (Franzen and Kinsella, 1974; King and Solms, 1979; Voilley et al., 1991).

It was expected that fats, which contribute only 0.4-0.6% to milled rice, have only a minor effect on the aroma binding to milled rice due to the small amounts that are present. However, we have considered and included fats in the list of the potential components in milled rice for the aroma compounds to bind to.
Based on the description above, there is a potential for aroma compounds to bind to rice. White milled rice which consists of majority of starch (90%) and proteins (7.3-8.3%), and has a porous structure, facilitates the binding process. The organic compounds potentially bind to components such as the helical amylose, and hence the physicochemical properties of the compounds, molecular dimensions, and functional groups may play an important role in the binding process. The principal aim of this thesis was to study the binding and release of aromas by rice, with the longer-term goal of producing new aromatised rice products. It is very important for the injected aroma compound to be present in the rice grains, especially the rice cores, as the aromas will take a longer time to migrate towards the rice surface and be lost to the air. To address these issues, a range of techniques such as fluorescence microscopy, SPME (solid-phase microextraction) coupled GC-MS, FT-IR spectroscopy and NMR spectroscopy were used to study the presence and distribution of the aroma compounds in aromatised rice grains. A number of these methods successfully demonstrated the presence of injected aroma compounds in rice, including the cores of the rice grains. A detailed discussion of these experiments is presented in Chapter 2. A number of volatile compounds with different functional groups and various physicochemical properties were used as model aroma compounds. Experiments were carried out to evaluate the stabilities and strengths of the interactions between these aroma compounds and rice, and to ascertain the physicochemical factors that affect these interactions. These experiments are reported in Chapters 3, 4 and 5. The aroma compound in the aromatised rice was expected to stay in the grains during storage and be released during eating. The remaining aroma compounds in the cooked rice were also to be
perceivable by the human olfactory system. Experiments on these aspects of the project are presented in Chapter 6.

In conclusion, white milled rice, which consists mostly of starch, has the potential to be an absorbent for aroma compounds. Earlier studies have shown that carbon dioxide gas and compressed carbon dioxide (liquid and supercritical) can penetrate readily into milled rice due to the porosity of the grains. The potential absorbency and porous structure of rice offer advantages for the aromatising process. Nevertheless, the aromatisation of rice raises a number of issues such as the extent to which the injected aroma compounds can penetrate into the grain, the components in rice that play a major role in the binding process, the effect of the pore dimensions of rice grains and the physicochemical properties of the injected aroma compounds on the aroma binding to rice, the stability of the aromas in the grains during storage and, finally, whether the injected aroma compound is perceivable after the aromatised rice has been cooked. These issues, including the experimental methods used to determine them, are reported and discussed in the following chapters of this thesis.
Chapter 2

Development of Some Methods for Imparting Aromas into Raw Rice

2.1. Introduction

The initial aim of this project was to develop one or more methods for imparting aromas into raw rice without changing its physical appearance. Raw rice, especially white milled rice, is easily cracked during any treatments in which water or its solutions are involved. Therefore, the first step in developing an imparting method was to identify an aroma carrier which could carry aromas into the core of a rice grain without causing fissures in the grains. It was expected that if the aroma compound could reach the rice core, then it would be retained for a longer time. This is because the aroma compound would presumably take a longer time to migrate towards the surface of the rice grain before being lost to the air.

Carbon dioxide has been widely used in the food and cosmetic industries, since it is non-toxic and relatively inert; for example, supercritical fluid carbon dioxide has been used for improving peanut butter quality (Santerre et al., 1994), and in extraction of fragrances from plants (Moyler, 1993). Carbon dioxide can have different physical states, which depend on temperature and pressure (see Figure 1.8). As illustrated in this figure, there are solid, liquid, gas and supercritical fluid (SCF) states of carbon dioxide. Carbon dioxide gas and liquid carbon dioxide were used as aroma carriers in this research, and air was also used as a control. The conventional method of using gum arabic to coat the rice with an aroma compound was also tried. The appearance of raw rice grains did not change significantly when they were
flushed with SCF or liquid carbon dioxide during aromatising. However, SCF carbon dioxide was not chosen as the preferred aroma carrier in this study since the construction of the equipment was more expensive than that required for liquid carbon dioxide, owing to the higher pressures and temperatures required for producing SCF carbon dioxide, and liquid carbon dioxide was found to perform well. This study of imparting aroma compounds into raw rice aimed to develop new techniques for producing aromatised rice that could be economically and practically applied by the food manufacturing industry in Australia and overseas, especially in countries where the staple food is rice, such as Indonesia.

Other possible techniques of imparting aromas into rice, such as sprinkling aroma solutions and pure liquid aroma compounds onto rice, were intentionally not used in this research project. Sprinkling aroma solutions on rice grains was found to cause fissures, owing to rapid moisture re-adsorption by rice grains (Kunze, 1979); fissures can rapidly appear with any treatments that involve water. Sprinkling pure liquid aroma onto rice grains did not cause fissures, however this technique gave an uneven distribution of pure liquid aroma on rice grains if only a small amount of liquid was used. On the other hand, too much pure liquid aroma produced saturated rice grains, which tended to stick to each other. Ultimately the most effective way of producing homogenous samples of aromatised rice involved a volatile solvent, liquid carbon dioxide.

A preliminary study that identified an efficient aroma carrier for imparting aroma compounds into raw rice is described in Section 2.3 of this chapter. This section also reports various methods of imparting aromas into rice, as well as studies
of the retention of various aroma compounds in rice, and the optimum degree of milling of rice required for producing aromatised rice. The methods of extraction and quantification of aroma concentrations in rice (Section 2.4.2), and the detection of injected aroma compounds in the cores of aromatised rice grains using methanol extraction coupled with gas chromatography (GC), fluorescence microscopy, solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) spectroscopy, and Fourier Transform Infrared (FT-IR) spectroscopy (Section 2.4.3) are also discussed.

Many of the experiments that were carried out in this preliminary study had no replicates since they were only trials. If an experiment gave an encouraging result then it was studied more intensively and replicated. The preferred methods of imparting aromas into rice were identified by comparing a number of carriers that could carry aroma compounds into rice grains without significantly changing the original appearance of the white milled rice. The aroma carriers that were chosen were air, carbon dioxide gas and liquid carbon dioxide. The other technique of imparting aromas into rice by coating the grains with gum arabic was not intensively studied since it was found to cause fissures.

Air was used as an aroma carrier in this study (1) to find out whether rice grains readily absorb the aroma vapours, and (2) as a control, for comparison with the other aroma carriers. In studying these methods of imparting aromas into rice, different quantities of rice and concentrations of aroma compounds, as well as aroma compounds with different functional groups, were used to determine the effect of these factors on the aroma absorption and retention by rice. Headspace gas
chromatography and methanol extraction coupled with gas chromatography were used to determine the concentrations of aroma compounds in the aromatised rice. These methods were chosen as they involve no sample grinding that can cause aroma loss.

Choosing the optimum degree of milling of rice as an absorbent for the injected aroma compound may be important, since it might be expected to affect the amount of aroma compound that can be absorbed. "Degree of milling" refers to the amount of bran and polish that is removed from brown rice during the milling process; it is usually expressed as a percentage. Different degrees of milling yield samples of rice with different chemical compositions (see Section 1.3). One of the chemical components that was suspected to have a role in retaining the aroma compounds was lipid. Lipid is used industrially in coatings for food products since it offers the benefit of retarding aroma and water loss (Baldwin et al., 1997). The effect of degree of milling of rice on the retention of aroma compounds in rice is discussed in Section 2.4.1.

To determine whether an injected aroma compound can be carried by the aroma carrier into the cores of rice grains, the techniques of methanol extraction coupled with GC, fluorescence microscopy, SPME coupled with GC-MS, NMR spectroscopy and FT-IR spectroscopy were used. FT-IR spectroscopy was also used to measure the rate of aroma loss from rice cores. Absolute concentrations of aroma compounds in rice cores were not determined, owing to the difficulty of obtaining uniform sizes for the sliced aromatised rice samples; hence this section only
considers whether or not the injected aroma compounds reached the rice cores in detectable amounts.

2.2. Materials

The rice used in this research was white milled Australian non-fragrant rice (Doongara variety) at a degree of milling of approximately 15 percent. It was harvested in mid 1997 and supplied by Rice Growers Australia (Leeton, NSW). The rice was packed in a plastic bag and stored in a cold room (4°C); samples were equilibrated at room temperature (25°C) overnight before being used. In some parts of this research, rice with several different degrees of milling of rice was used. The rice was prepared by milling brown rice (Doongara variety, as above) to the degree specified in Figure 2.23 using a Satake grain testing mill (type TM, class 05, no. 5.54006) at the Satake Engineering Company (Revesby, NSW). The sources and purities of the aromas and other compounds used are listed in Table 2.1. All of these materials were used without any further purification.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Purity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-cyclodextrin</td>
<td>99% (HPLC grade)</td>
<td>Sigma</td>
</tr>
<tr>
<td>β-cyclodextrin</td>
<td>H₂O content 8 mol/mol</td>
<td>Sigma</td>
</tr>
<tr>
<td>γ-octalactone</td>
<td>97+%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>98% (GC grade)</td>
<td>Sigma</td>
</tr>
<tr>
<td>2-acetyl pyridine</td>
<td>99+%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>2-hexanone</td>
<td>98%</td>
<td>Fluka AG, Bucks SG</td>
</tr>
<tr>
<td>2-phenyl ethanol</td>
<td>98% (GC grade)</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>99%</td>
<td>Sigma</td>
</tr>
<tr>
<td>Amylopectin</td>
<td>From corn</td>
<td>Sigma</td>
</tr>
<tr>
<td>Amylose</td>
<td>From corn (practical grade, 70%)</td>
<td>Sigma</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>99+%</td>
<td>Aldrich</td>
</tr>
</tbody>
</table>

Table 2.1. Source and purity of materials used.

(continued)
Table 2.1 (continued).

<table>
<thead>
<tr>
<th>Carbon dioxide gas</th>
<th>Industrial grade</th>
<th>BOC gases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamaldehyde</td>
<td>98+%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>Laboratory Reagent</td>
<td>Hopkin &amp; Williams</td>
</tr>
<tr>
<td>Eugenol</td>
<td>99%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Gum arabic</td>
<td></td>
<td>TIC Gum</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>99%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>99+% mixture <em>cis</em> and <em>trans</em></td>
<td>Aldrich</td>
</tr>
<tr>
<td>Methanol</td>
<td>99.8% (Analytical Reagent)</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>98+%</td>
<td>Aldrich</td>
</tr>
<tr>
<td><em>n</em>-Decane</td>
<td>Minimum assay (GLC) 98%</td>
<td>BDH</td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>High purity</td>
<td>BOC Gases</td>
</tr>
<tr>
<td>Pyridine</td>
<td>99+%</td>
<td>Sigma</td>
</tr>
<tr>
<td>Toluene</td>
<td>99.5%</td>
<td>Sigma</td>
</tr>
</tbody>
</table>

2.3. Experimental Methods

2.3.1. Air as Aroma Carrier

The procedure for imparting aroma compounds using air as an aroma carrier was as follows. 1 to 5 grams of rice was placed in a 20-mL glass headspace vial. The vial was sealed with an aluminium crimp seal and a teflon coated septum. An amount of aroma compound (0.5 or 1 μL) was injected using a syringe through the septum on top of the vial, and deposited onto the inner wall of the vial. The aroma compound was injected onto the inner wall to prevent the liquid from being absorbed directly by the rice before the liquid evaporated. The vial was seated in a headspace sampler bath at 35°C and the headspace gas was analysed after 20, 40, 60, 80, 100 and 120 minutes. The headspace sampler was connected to a gas chromatograph, and the concentration of aroma vapour in the vial was measured by gas chromatography.

A Hewlett Packard Model 19395A headspace unit was used. The conditions of analysis were as follows: valve/loop and headspace sampler bath temperatures
55°C and 35°C, respectively; both nitrogen carrier gas and auxiliary pressures 0.85 bar. Auxiliary pressure is the gas pressure that is supplied to the headspace vial for pressurizing the headspace sample and for subsequent venting of the sample loop. The headspace vial was pressurized for 10 seconds and vented for 16 seconds, then the headspace gas sample (3 mL) was injected into the gas chromatograph. After the headspace sample was pressurized for 10 seconds, the sample gas from the headspace sample went to the sample loop for venting to the atmosphere; as a consequence, the pressure in the headspace vial was then equal to atmospheric pressure. During the 16 seconds venting, the carrier nitrogen gas carried a sample of the residual sample gas in the sample loop to the gas chromatograph. The gas chromatograph was a Hewlett Packard Model 5890A, which was equipped with an SGE BP 5 column (2.5 m × 0.22 mm × 1.0 μm) and a flame ionization detector (FID). The inlet and detector were at the same temperature (200°C) and the nitrogen carrier gas was used at 7 kPa with a split ratio of 40:1. The initial temperature of the oven was 120°C; it was maintained at this temperature for 1 minute then programmed to rise at 10°C/minute to 230°C. The data were then collected and processed using Hewlett Packard ChemStation CORE software (version A.03.02). The concentration of aroma vapour in the vial headspace can be used to quantify the extent to which the aroma compound was absorbed by the rice, as discussed in Section 2.4.1.

The effect of headspace vial volume on the concentration of aroma vapour in the vial was also determined. It was carried out by placing glass beads (total volume 2, 4 or 6 mL) in a headspace vial, which was sealed with an aluminium crimp seal and teflon septum. An amount of aroma compound (0.5 or 1 μL) was injected into the vial and deposited onto the inner wall. The vial was then seated in a headspace
sampler bath at 35°C, and the concentration of aroma vapour was measured after 20, 40, 60, 80, 100 and 120 minutes using GC, with the same conditions as described for the headspace rice samples.

2.3.2. Carbon Dioxide Gas as Aroma Carrier

Aroma compounds were imparted into rice using carbon dioxide gas as an aroma carrier, using the following procedure. 1 to 5 grams of rice was placed in a 20-mL headspace vial. The mouth of the vial was covered with cotton wool, which was tightly held in place with thumb pressure. Carbon dioxide gas was passed into the vial for 5 minutes in order to displace the air. The cotton wool was removed and the vial was quickly sealed with an aluminium crimp seal and teflon septum. 0.4 μL of aroma compound was injected using a syringe through the septum of the seal onto the inner wall of the vial. The vial was put in the headspace bath at 35°C, and the headspace gas was sampled after 30, 60, 90, 120, 150 and 180 minutes. The concentration of aroma vapour in the vial was measured by GC using the method described in Section 2.3.1.

The volume of injected aroma compounds in Section 2.3.2 (0.4 μL) was less than the volume that was used in Section 2.3.1 (0.5 or 1 μL) to make sure that the amount of aroma vapour in the headspace vial did not exceed the capacity of the rice samples to absorb it. Longer equilibration times were used in Section 2.3.2 to give more time for the injected liquid aroma compound to evaporate and be absorbed by the rice. The minimum equilibration time that is suggested for headspace sampling is 20 minutes (Hewlett Packard Model 19395A Headspace Sampler Operating and Service Manual, 1988). The principal aim of the experiments in Sections 2.3.1 and
2.3.2 was to compare the ability of air and carbon dioxide gas to carry aroma compounds into rice grains; hence a control (air in headspace vial; no carbon dioxide gas) was also carried out under the same conditions as the treatment for carbon dioxide gas. A limitation of this method was that each time the headspace was sampled, substantial amounts of nitrogen gas were used to pressurise the vials, and therefore the air and carbon dioxide contents of the headspace gas would decrease with successive sampling. The comparisons of aroma retention in rice for air and carbon dioxide gas as aroma carriers are discussed in Section 2.4.1.

2.3.3. Liquid Carbon Dioxide as Aroma Carrier

A schematic diagram of the equipment that was used to impart aromas into raw rice using liquid carbon dioxide ($CO_2$) is given in Figure 2.1. This equipment consists of two main parts which (1) liquefy carbon dioxide, and (2) impart aromas into the rice. There are two sections in the cylinder ($D_1$ and $D_2$); the top section ($D_1$) is for the nitrogen gas, and the bottom section ($D_2$) is for carbon dioxide gas. The two sections in the cylinder are separated by a movable steel disc (piston). The position of the piston in the cylinder can be detected by a magnet which is attached to the outside of the cylinder. When the piston moves in the cylinder, the magnet follows it. Before the equipment was operated, the two sections of the cylinder contained carbon dioxide and nitrogen gas at atmospheric pressure. This condition was reached by opening valves $V_1$ and $V_2$ and allowing excess gas to vent until the pressure gauges $P_2$ and $P_3$, which are attached to the ends of the cylinder, both read 0 MPa. Then all of the valves $V_1 - V_4$ were closed.
The CO₂ selector was switched towards the vertical "up" position and the carbon dioxide cylinder valve, C₁, was opened to fill the cylinder to a maximum pressure of 9 MPa. C₁ was turned off, and the CO₂ selector was switched to the horizontal "left" position. The pressure-control valve, P₄, attached to the nitrogen-gas cylinder was set to 11 MPa (this is the highest pressure that the components of the equipment were designed to withstand). The nitrogen cylinder valve was opened; nitrogen gas flowed into the top of the cylinder (D₁) and compressed the carbon dioxide gas in the lower part of the cylinder (D₂). At room temperature (below the critical temperature of CO₂, 31.06°C) the carbon dioxide is a liquid at these pressures.

After the liquid carbon dioxide was ready, approximately 2 grams of rice was placed in the chamber. An amount of aroma compound (2, 4, 6, 8, 10 or 15 µL) was injected onto an 8-mm diameter filter paper (Whatman 41), which was also put into the chamber (on the liquid-CO₂ inlet). The chamber was tightly sealed. The CO₂ selector was switched to the "down" position and valve V₄ was opened slowly, allowing the liquid carbon dioxide to flow into the chamber, while valve V₅ was opened for approximately 2 seconds in order to displace air from the rice chamber. After 5 minutes, the CO₂ selector was shut off (horizontal "right" position), then the CO₂ bleed valve V₃ was gradually opened in order to release the pressure in the chamber. Finally, the rice was removed from the chamber for analysis. During the 5 minutes equilibration time, valve C₂ might be opened for 1 second in order to maintain the pressure of CO₂ (P₁) at about 8 MPa, under which condition the carbon dioxide was a mixture of gas and liquid phases (see Figure 1.8).
Figure 2.1. Schematic diagram of the equipment for imparting aromas into rice using liquid carbon dioxide as aroma carrier.
The optimum equilibration time for the rice sample in the chamber was 5 minutes. Longer equilibration times did not significantly increase the absorption of aroma compound into the rice, and in addition they tended to cause the appearance of "white stripes" on the rice. Details of the effect of equilibration time on the appearance of the rice are discussed in Section 2.4.1. The optimum volume of injected aroma compound in the rice chamber is also discussed in Section 2.4.1.

Levels of retained aroma in the rice were measured by (1) headspace GC; and (2) methanol extraction followed by GC analysis. In the headspace GC method, the rice sample was placed in a 20-mL headspace vial and immersed in the headspace bath at 85°C for 30 minutes to release the aroma compound, which was then measured by GC. The conditions of the GC were as described in Section 2.3.1.

In the methanol extraction method, after the aromatized rice was taken out from the chamber, it was put in a 20-mL screw-cap vial and 3 mL of methanol was added. This mixture was left overnight (approximately 12 hours) and then continuously stirred for 2 hours, during which time a standard \( n \)-decane solution was prepared as an internal standard. An amount of \( n \)-decane (10-20 mg) was weighed into a 50-mL volumetric flask. Methanol was added up to the mark, and the mixture was shaken gently and left at room temperature (25°C) for at least 30 minutes. 1 mL of this internal standard was pipetted into a clean 20-mL screw cap vial, and 1 mL of the rice sample solution mixture was added. The resulting mixture (2 mL) was centrifuged at 4000 rpm for 20 minutes in order to get a clear solution. 1 \( \mu \)L of this solution was injected into the gas chromatograph and the concentration of aroma
compound was determined. The quantification of the aroma compound was achieved as follows:

\[
D = \frac{A \times B \times RRF}{C}
\]

\[
RRF = \frac{\text{known concentration of aroma (mg/L)}}{\text{GC peak area of aroma}} \times \frac{\text{peak area of IS}}{\text{concentration of IS (mg/L)}}
\]

\[A = \text{peak area of aroma compound in 2 mL of a 1:1 mixture of sample solution and internal standard;}
\]

\[B = \text{concentration of internal standard in 2 mL of the 1:1 mixture;}
\]

\[C = \text{peak area of internal standard in 2 mL of the 1:1 mixture;}
\]

\[D = \text{concentration (mg/L) of aroma compound in the 1:1 mixture;}
\]

\[IS = \text{internal standard;}
\]

\[RRF = \text{Relative Response Factor.}
\]

\[D \text{ (mg/L) is the concentration of aroma compound in the mixture of rice sample solution and internal standard. Hence, the concentration of aroma compound in 2 mL of the resulting mixture is } D \times 0.002. \text{ This value is equal to the mass of aroma compound in 1 mL methanol in the rice sample solution. As previously described, 3 mL methanol was used for soaking 2 g of rice, therefore the mass of aroma compound in 2 g of rice sample was } D \times 0.002 \times 3. \text{ The concentration of aroma compound in a 100-g rice sample is therefore:}
\]
Concentration of aroma compound (mg/100 g raw rice) =

\[
\frac{0.6 \times A \times B \times RRF}{C \times \text{mass of rice (g)}}
\]

Methanol extraction coupled with gas chromatography was found to be a better aroma extraction method than other methods such as the headspace GC method used in this study (Pratama et al., 1999). No heat is applied to the rice, nor is sample grinding required, which can cause aroma loss from the rice. Overnight soaking and 2 hours stirring in methanol were enough to break down the rice grains, allowing the aroma compound to be released into the methanol. An evaluation of the reliability and efficiency of methanol extraction is described in Section 2.4.2.

2.4. Results and Discussion

2.4.1. Methods of Imparting Aromas into Raw Rice

Preliminary experiments were carried out to assess the suitability of air as an aroma carrier, that is, a medium that could be used to impart an aroma compound into rice. It was not expected that air would be an efficient aroma carrier, but it was important to study this basic method, for comparison with the more sophisticated methods involving carbon dioxide. These experiments were also useful for developing some experimental methods that were used later in the project.

Our initial interest was in the aroma compound 2-acetyl-1-pyrroline, which is present in many popular, high-value fragrant rices. As explained in Section 1.3, this compound is relatively unstable and difficult to work with. Accordingly, we studied a range of different model compounds of varying molecular structure and volatility,
as summarised in Table 2.2. These compounds were not chosen for their pleasant aromas, and indeed some were later discarded owing to safety hazards associated with their use. However, they had a range of useful structural features, related to those of 2-acetyl-1-pyrrolidine, that may be involved with binding to rice.

It was expected that three factors would be important in these preliminary experiments: (1) the extent to which the aroma compound evaporated (governed by its equilibrium vapour pressure and, to a lesser extent, the rate at which it reached that pressure), (2) the rate at which the rice absorbed the aroma compound, and (3) the equilibrium vapour pressure of the aromatised rice.

Table 2.2. Physicochemical characteristics of model compounds that were used.

<table>
<thead>
<tr>
<th>Names and molecular structures</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>• Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>• Carbonyl group</td>
</tr>
<tr>
<td></td>
<td>• (^{a})Boiling point = 202.4°C</td>
</tr>
<tr>
<td></td>
<td>• (^{a})Vapour pressure = 0.98 mmHg (at 35°C)</td>
</tr>
<tr>
<td></td>
<td>• (^{b})Density = 1.039 g mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td>• Molar mass = 120 g mol(^{-1})</td>
</tr>
<tr>
<td><img src="image" alt="Acetophenone Structure" /></td>
<td></td>
</tr>
<tr>
<td>2-Acetylpyridine</td>
<td>• Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>• Heterocyclic nitrogen</td>
</tr>
<tr>
<td></td>
<td>• Carbonyl group</td>
</tr>
<tr>
<td></td>
<td>• (^{b})Boiling point = 188-189°C</td>
</tr>
<tr>
<td></td>
<td>• (^{c})Vapour pressure = 1.43 mmHg (at 35°C)</td>
</tr>
<tr>
<td></td>
<td>• (^{b})Density = 1.080 g mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td>• Molar mass = 121 g mol(^{-1})</td>
</tr>
<tr>
<td><img src="image" alt="2-Acetylpyridine Structure" /></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
Table 2.2 (continued).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Phenylethanol</td>
<td>- Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>- Primary alcohol</td>
</tr>
<tr>
<td></td>
<td>- Boiling point = 245.3°C</td>
</tr>
<tr>
<td></td>
<td>- Vapour pressure = 0.21 mmHg (at 35°C)</td>
</tr>
<tr>
<td></td>
<td>- Density = 1.023 g mL⁻¹</td>
</tr>
<tr>
<td></td>
<td>- Molar mass = 122 g mol⁻¹</td>
</tr>
<tr>
<td>Toluene</td>
<td>- Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>- Methyl group</td>
</tr>
<tr>
<td></td>
<td>- Boiling point = 110.6°C</td>
</tr>
<tr>
<td></td>
<td>- Vapour pressure = 46.8 mmHg (at 35°C)</td>
</tr>
<tr>
<td></td>
<td>- Density = 0.865 g mL⁻¹</td>
</tr>
<tr>
<td></td>
<td>- Molar mass = 92 g mol⁻¹</td>
</tr>
<tr>
<td>2-Hexanone</td>
<td>- Carbonyl group</td>
</tr>
<tr>
<td></td>
<td>- Boiling point = 127.5°C</td>
</tr>
<tr>
<td></td>
<td>- Vapour pressure = 7.5 mmHg (at 35°C)</td>
</tr>
<tr>
<td></td>
<td>- Density = 0.812 g mL⁻¹</td>
</tr>
<tr>
<td></td>
<td>- Molar mass = 100 g mol⁻¹</td>
</tr>
</tbody>
</table>

Sources: (a) Jordan (1954); (b) Aldrich Australia (1998/1999); (c) calculated vapour pressure using Antoine equation (Grain, 1990).

The vapour pressures for toluene, 2-hexanone and pyridine at 35°C were readily obtained from the published vapour-pressure charts (Jordan, 1954), whereas the vapour pressures of acetophenone and 2-phenylethanol were extrapolated using the available vapour-pressure data (at 50-215°C for acetophenone; at 58-220°C for 2-phenylethanol). The extrapolations for the vapour pressures were carried out using the Clausius-Clapeyron equation (Grain, 1990), which relates the vapour pressure, \( P \), to temperature, \( T \), as follows:
\[ P = P^* e^{-c} \quad ; \quad C = \frac{\Delta H_{\text{vap}}}{R} \left( \frac{1}{T} - \frac{1}{T^*} \right) \]

Where \( \Delta H_{\text{vap}} \) is the molar enthalpy of vaporisation (assumed to be independent of temperature); \( R \) is the ideal gas constant; \( P^* \) is the vapour pressure at \( T^* \). By taking logarithms of both sides it can be shown that:

\[ \ln P = \left( \ln P^* + \frac{\Delta H_{\text{vap}}}{RT^*} \right) - \frac{\Delta H}{R} \frac{1}{T} \]

A graph of \( \ln P \) versus \( 1/T \) should therefore be linear, and may be used to find extrapolated values of \( P \) at 35°C. Figure 2.2 shows the graph for acetophenone. A straight line of best fit was drawn through the data set, and its linear regression equation was used to find the vapour pressure at 35°C (308 K) by substitution. The linear regression equation that was obtained from the data points in Figure 2.2 is

\[ \ln (P) = -5796.6 \left( \frac{1}{T} \right) + 18.8 \]; therefore the acetophenone vapour pressure at 35°C was found to be 0.98 mmHg.
Figure 2.2. Graph of log (vapour pressure) of acetophenone versus inverse temperature.

As there were no published data available on the 2-Apy vapour pressure, the vapour pressure at 35°C was calculated using the Antoine equation (Grain, 1990) as follows:

\[
\ln P_{vap} = \frac{\Delta H_{vap} (T_b - C_2)}{\Delta Z_b R (T_b')^2} \left[ \frac{1}{(T_b' - C_2)} - \frac{1}{(T - C_2)} \right]
\]

(1)

\[
\Delta H_{vap} = K_p \left[ 8.75 + R \ln(T_b') \right] (T_b')
\]

(2)
Where:  
\[ P_{\text{vap}} = \text{vapour pressure in atm at a given temperature in } K; \text{ where } 1 \text{ atm} = 760 \text{ mmHg}; \]

\[ \Delta H_{\text{vap}} = \text{molar enthalpy of vaporisation at boiling point (cal/mol); where } \Delta H_{\text{vap}} = 10230 \text{ cal/mol (calculated by using equation (2))}; \]

\[ K_F = \text{Kistiakovskii factor; where } K_F \text{ for 2-Apy was assumed to be 1.06 (Grain, 1990);} \]

\[ T_b = \text{boiling point (K); where } T_b \text{ for 2-Apy} = 188^\circ C \text{ or 461 K (Aldrich Australia, 1998/1999)}; \]

\[ \Delta Z_b = \text{compressibility factor at the normal boiling point; where } \Delta Z_b \text{ is taken to be 0.97 (Grain, 1990);} \]

\[ R = \text{ideal gas constant (1.9872 cal/mol K)}; \]

\[ T = \text{a given temperature (K); where } T = 308 \text{ K}; \]

\[ C_2 = \text{a constant which was calculated using Thomson's rule (Grain, 1990); where } C_2 = -18 + 0.19 (T_b). \]

By substituting all the values in equations (1) and (2), the vapour pressure for 2-Apy at 35\(^\circ\)C was found to be 1.43 mmHg.

Samples of rice (1-5 g) held in sealed 20-mL headspace vials were injected with 1 \( \mu \text{L} \) of aroma compound, as described in Section 2.3.1. Care was taken to ensure that the liquid aroma compound did not make direct contact with the rice. Samples of headspace gas were analysed after the sample had been at 35\(^\circ\)C for 20, 40, 60, 80, 100 and 120 minutes. A control was also run, with 1 \( \mu \text{L} \) of aroma compound but no rice. Once all of the aroma compound had evaporated, the level of
headspace aroma would depend on the ability of the rice to absorb it. The results for
the six aroma compounds are summarised in Figures 2.3 – 2.7.

If the vapours from the model compounds (0.5 and 1 μL) in the headspace
vials (at equilibrium vapour pressure and temperature 35°C) were assumed to be
ideal gases, then the vapour volumes for 0.5- and 1-μL samples of each compound
could be calculated using the ideal gas law. The purpose of calculating the vapour
volume was to determine whether there was enough compound for the equilibrium
vapour pressure of the liquid to be reached. For some of the compounds, it was
observed that not all of the liquid compounds evaporated in the vial at 35°C. This
could be seen by the left-over liquid compounds on the inner wall after 120 minutes.
An example of calculating the vapour volume from 1 μL of acetophenone using the
ideal gas law is as follows.

\[ PV = nRT \]

Where: 
- \( P \) = equilibrium vapour pressure of compound (Pa);
- \( V \) = volume of vapour from compound (m\(^3\)); where 1 m\(^3\) = 10\(^6\) mL;
- \( n \) = number of moles of the injected compound (mol);
- \( R \) = ideal gas constant (8.314 m\(^3\) Pa K\(^{-1}\) mol\(^{-1}\));
- \( T \) = temperature (K), where 0 °C = 273 K.

1 μL acetophenone had a mass of 1.04 × 10\(^{-3}\) g (mass = volume (mL) ×
density (g mL\(^{-1}\))), and contained 8.66 × 10\(^{-6}\) mol (mass divided by molar mass).
Hence the corresponding vapour volume at 35°C was 173 mL. Details of equilibrium
vapour pressures, densities and molar masses of the model compounds are listed in Table 2.2. The vapour volumes for 0.5- and 1-μL samples of the injected model compounds are summarised in Table 2.3.

**Table 2.3.** Vapour volumes from 0.5- and 1-μL samples of 2-phenylethanol, acetophenone, 2-acetylpypyridine, 2-hexanone, pyridine and toluene at 35°C.

<table>
<thead>
<tr>
<th>Model compounds</th>
<th>Volume of vapour (mL) from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 μL</td>
</tr>
<tr>
<td>2-Phenylethanol</td>
<td>384</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>87</td>
</tr>
<tr>
<td>2-Acetylpypyridine</td>
<td>60</td>
</tr>
<tr>
<td>2-Hexanone</td>
<td>10</td>
</tr>
<tr>
<td>Pyridine</td>
<td>3.7</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The vapour volumes for the model compounds (Table 2.3) were inversely proportional to their vapour pressures (see Table 2.2). The compounds that have lower vapour pressures produced more vapour volume than the compounds with the higher vapour pressures at the same temperature (35°C).
Figure 2.3. The concentrations of acetophenone left by rice in a 20-mL headspace vial after 20, 40, 60, 80, 100 and 120 minutes at 35°C (air in headspace vial).

Figure 2.3 shows the headspace concentration of acetophenone as a function of time after 1 µL of compound was added to 1-, 2-, 3-, 4- and 5-g portions of rice, with an empty headspace vial as a blank. Headspace concentrations of acetophenone were plotted as logarithm of peak area, and straight lines of best fit were drawn through each data set. The graphs were plotted as logarithm of peak area due to the large range of peak areas (50000 – 320000 area counts) for each data set. In addition, as discussed in Section 3.3, a linear graph was consistent with a first-order absorption process. By assuming the acetophenone vapour to be an ideal gas, the volume of vapour that could be formed from 1 µL acetophenone was calculated to be approximately 173 mL. The actual headspace volume for the 20-mL vial was approximately 21 mL so there was sufficient acetophenone vapour available in the vial to be absorbed by the rice.

63
The lines of best fit in Figure 2.3, except for the control, slightly decrease with equilibration time. This could indicate that the rice (1, 2, 3, 4, and 5 g) in the vial was gradually absorbing the vapour during the 120 minutes equilibration time, and the lines might be expected to plateau eventually upon reaching an equilibrium. On the other hand, the control gradually increases and apparently reaches an equilibrium. The final aroma concentration in the control was presumably the equilibrium vapour pressure. Even though some acetophenone was lost from the vial each time the headspace gas was sampled, enough liquid remained to replace it. The vapour volume from 1 μL acetophenone (173 mL) was sufficient to replace the lost vapour in the 20-mL vial (for the control). The progressively lower concentrations with increasing amounts of rice indicate that the aroma is being absorbed, and equilibrium vapour pressure is not maintained.

Figure 2.4 shows the logarithm (peak area) of 2-acetylpuridine (2-Apy) as a function of time after 1 μL of that compound was added to 1-, 2-, 3-, 4- and 5-g portions of rice, with an empty headspace vial as a blank (no rice). Unlike the lines of best fit in Figure 2.3, those in Figure 2.4 do not behave as consistently, although the aroma concentration in the control has similar behaviour to that of acetophenone. The lines of best fit for the 2-, 4-, and 5-g portions of rice tend to decline gradually indicating that the 2-acetylpuridine vapour that had been absorbed by the rice was not totally replaced. The line for the 3-g portion increases slowly, whereas the line for the 1-g portion increases more rapidly and reaches a constant value. It was inconsistent that these lines increased, whereas the lines for the 2- and 4-g portions decreased. The discrepancies for the 1- and 3-g samples could be due to the liquid
2-acetylpyridine being absorbed directly by the rice, despite precautions that aimed to prevent this, and then partly evaporating to reach an equilibrium concentration. The very low concentration of 2-acetylpyridine during the first 20 minutes for the 1-g portion of rice might be due to this effect, whereas the 3-g portion may not have absorbed as much as the 1-g sample did. However, the overall results in Figure 2.4 show that the concentrations of vapour in the headspace tend to decrease as the amount of rice in the vial increases, especially after 120 minutes.

Figure 2.4. The concentrations of 2-acetylpyridine left by rice in a 20-mL headspace vial after 20, 40, 60, 80, 100 and 120 minutes at 35°C (air in headspace vial).
The headspace data for 2-phenylethanol are presented in Figure 2.5. There were some scattered data for the control (40 and 60 minutes) but the data settled down to equilibrium values after 60 minutes. The lines of best fit for the 1-, 2-, 3- and 4-g portions of rice tend to be horizontal. The results were very different from the corresponding results in Figures 2.3 and 2.4. This might be due to the very large vapour volume (767 mL) from 1 μL of 2-phenylethanol. The rice presumably absorbs a significant amount of the vapour, since even for 1 g the level is below the control, and the concentrations decrease in the expected order: 1, 2, 3 then 4 g. The line of best fit for the 5-g sample gradually declined which might be due to the higher capacity for the 5 g to absorb vapour. The behaviour of the 5-g portion was similar to the corresponding results for acetophenone and 2-acetylpipridine, where not enough liquid remained to maintain the equilibrium vapour pressure.

Figures 2.3 to 2.5 indicate that with more rice in the headspace vial, more vapour was absorbed, as might have been expected. Accordingly we only trialled 1-and 5-g portions of rice for the next preliminary experiments on three more model compounds: toluene, 2-hexanone and pyridine. These compounds have a range of molecular structures (see Table 2.2) which might have an effect on their absorption by rice. Figure 2.6 shows the headspace concentrations of toluene, 2-hexanone and pyridine as functions of time after 1-μL samples of those compounds were added to 1- and 5-g portions of rice. Control experiments (no rice) were also carried out for each compound. The vapour concentrations were plotted in Figure 2.6 as for the earlier results. The lines of best fit in Figure 2.6 are colour coded: each model compound has a different colour.
Figure 2.5. The concentrations of 2-phenylethanol left by rice in a 20-mL headspace vial after 20, 40, 60, 80, 100 and 120 minutes at 35°C (air in headspace vial).

The lines of best fit for the controls slightly decreased, and these results contrasted with the controls in Figures 2.3, 2.4 and 2.5. However, the theoretical vapour volumes from 1 μL of toluene, pyridine and 2-hexanone (4, 7.3 and 21 mL, respectively) are much lower than those for the compounds that were used earlier; as a consequence, the removal of vapour from the controls for the headspace gas sample reduced the vapour concentrations in the vials. The lines of best fit for the 1- and 5-g samples of the three compounds were similar to their controls. All of the lines in Figure 2.6 gradually decreased, indicating that the equilibrium vapour pressure could not be maintained, owing to insufficient vapour in the vials. The ratios of the vapour concentrations for the 1-g portions of rice to the controls were approximately 0.9 for both toluene and pyridine, and 0.8 for 2-hexanone after 120 minutes. The
corresponding ratios for the 5-g portions were 0.7 for toluene, 0.6 for 2-hexanone and 0.3 for pyridine. The pyridine ratio for the 5-g portion was far below that for toluene and 2-hexanone, presumably because the saturated vapour volume from 1 µL of pyridine at 35°C (7.3 mL) could not be fully replaced by further evaporation.

**Figure 2.6.** The concentrations of toluene, 2-hexanone and pyridine left by rice in a 20-mL headspace vial after 20, 40, 60, 80, 100 and 120 minutes at 35°C (air in headspace vial).
Results for 0.5- and 1-μL portions of two model compounds (acetophenone and 2-phenylethanol) in the headspace vials were also compared, as shown in Figures 2.7a and b. The vapour concentrations were plotted as logarithms of peak areas in Figure 2.7a in order to be consistent with the earlier Figures, whereas non-logarithmic results are presented in Figure 2.7b, to compare the absolute peak areas. As expected, the bars in Figure 2.7b show insignificant differences between the headspace vapour concentrations of the controls (no rice) for 0.5- and 1-μL portions of acetophenone. The vapour volume from the 0.5-μL portion was enough to saturate the 20-mL vial; therefore an increased liquid volume (1 μL) did not affect the vapour concentration. However, the concentrations dropped to 0.54 (1 g rice) and 0.19 (5 g rice) of their original values for 1 μL of acetophenone, and 0.32 (1 g rice) and 0.10 (5 g rice) for 0.5 μL. In each case, less vapour was left in the headspace vial for the 0.5 μL sample of acetophenone. Clearly, rice readily absorbs acetophenone, but appreciable amounts of aroma vapour remain in equilibrium with the rice after 120 minutes. Our earlier studies suggested that the vapour concentrations eventually become constant. Similar results were also obtained for 2-phenylethanol, but the vapour concentrations were much lower than those of acetophenone, due in the first instance to its lower equilibrium vapour pressure.

The overall results in Figures 2.7 a and b show that: (1) increased volume of aroma compound did not significantly affect the vapour concentrations in the vials (control); and (2) larger injected volumes in the vials would give more opportunity for the absorbed vapour to be replaced; however, even 1 g of rice absorbed most of the vapour from 1 μL of each compound.
Figure 2.7a. Comparison of the logarithms of headspace vapour concentration for 0.5- and 1-μL samples of acetophenone and 2-phenylethanol at 35°C after 60 minutes equilibration time.

Figure 2.7b. Comparison of headspace vapour concentrations for 0.5- and 1-μL samples of acetophenone and 2-phenylethanol at 35°C after 60 minutes equilibration time.
In order to draw further general conclusions from the results that were shown in Figures 2.3 to 2.7b, a logarithmic plot of peak areas for the six compounds (1-µL samples) after 60 minutes is shown in Figure 2.8a. A non-logarithmic plot is shown in Figure 2.8b, to compare the absolute peak areas. The six model compounds were arranged according to their theoretical vapour pressures (see Table 2.2). Overall the compounds with higher vapour pressures had higher experimental vapour concentrations for the controls, except in the case of pyridine. This discrepancy was probably due to the lower sensitivity of the flame-ionization detector (FID) of the GC to pyridine than to the other compounds. The detector responds variably to different compounds and the response diminishes for compounds that contain nitrogen (in this case pyridine) (Lochmüller, 1986). In addition, no internal standard was used as we did not intend to quantify the vapour concentration in the headspace. Hence the peak areas obtained for pyridine (as also for other compounds) were not corrected for the detector response factor. The case worsens with a compound that is outside the optimum registration capacity of the GC detector. The logarithmic plot disguises the extent to which the aroma concentrations dropped in the presence of the rice samples, but in all cases the results show that rice is a relatively good absorbent for aroma vapours. This is most encouraging, given the overall aims of this project. Figure 2.8b indicates that factors such as molecular structure and functional groups have an effect on the absorption, although these preliminary studies did not allow these factors to be fully evaluated. The compounds with an aromatic ring that includes either a carbonyl or hydroxyl group showed stronger binding to rice. This was indicated by larger decreases for the headspace vapour concentrations after rice samples were introduced into the vials. For example, the vapour concentration for toluene (an aromatic ring with neither a carbonyl nor hydroxyl group) decreased by
only 47% from the original concentration 60 minutes after the addition of 5 g of rice.
Under the same conditions, 2-phenylethanol (an aromatic ring with a hydroxyl
group) decreased by 85%, while acetophenone and 2-acetylpyridine (both possesing
an aromatic ring with a carbonyl group) decreased by approximately 82%. These
preliminary experiments for the six compounds were very encouraging, and allowed
us to proceed to more detailed studies of the effects of molecular structures and
functional groups on the absorption and loss of model aroma compounds from rice.
Some of these issues will be introduced later in this section, and more detailed
experiments are reported in Chapters 3, 4 and 5.

Figure 2.8a. Comparisons of the logarithms of headspace vapour concentration for
1-µL samples of 2-phenylethanol, acetophenone, 2-acetylpyridine,
2-hexanone, pyridine and toluene at 35°C after 60 minutes equilibration
time.
Figure 2.8b. Comparisons of the headspace vapour concentration for 1-μL samples of 2-phenylethanol, acetophenone, 2-acetylpyridine, 2-hexanone, pyridine and toluene at 35°C after 60 minutes equilibration time.

The effect of headspace volume on the vapour concentrations formed by the injected model compounds was also studied. The study aimed to determine whether the headspace volume had any effect on the vapour concentration. Earlier experiments showed that the mass of rice affected the vapour concentrations in the vials. The headspace volume decreases with the increased mass of rice, and hence we aimed to confirm whether the decreased vapour concentration after the introduction of rice was a result of the vapour absorption by rice or the decreased headspace volume of the vial. As expected, the headspace volume had no effect on the formation of vapour from the liquid, and the decreased vapour concentration was due to absorption by the rice. The headspace volume of acetophenone was used as a
representative model compound. The headspace volume was varied by filling the vials with glass beads. Three different volumes of glass beads (2, 4 or 6 mL) were placed in the vials, with two different initial acetophenone concentrations (0.5 and 1 μL), and a blank was also carried out (no glass beads). The headspace vapour concentrations are plotted in Figure 2.9. The vapour concentrations for all eight samples stay within a fairly narrow range ($10^{5.45} - 10^{5.60}$ area counts), indicating that headspace volume as well as the injected acetophenone volume did not have any systematic effect on the vapour concentrations; these experiments also give an indication of the magnitude of the experimental errors for this aspect of the analysis.

![Graph showing the effect of headspace-vial volume on the vapour concentration of acetophenone at 35°C.](image)

**Figure 2.9.** The effect of headspace-vial volume on the vapour concentration of acetophenone at 35°C.
As shown in Figures 2.7a and b, the injected liquid volumes did not significantly affect the vapour concentrations for the controls (no rice), provided the vapour volume was enough to occupy the headspace. The theoretical vapour volumes for 0.5- and 1-μL samples of liquid acetophenone at 35°C were 87 and 173 mL, respectively, so the 0.5-μL liquid portion was sufficient to reach the saturated vapour pressure. As mentioned above, the data in Figure 2.9 show some scatter, for example the 2- and 4-mL glass-beads with 1 μL of liquid. Similar behaviour was observed for the 2-Apy control (see Figure 2.4). In summary, headspace volume did not significantly affect the vapour concentrations formed by the injected model compounds, provided sufficient liquid was present.

As stated earlier in this section, air was not expected to be an efficient aroma carrier, but we aimed to see whether rice grains could absorb some model aroma compounds, and air was the simplest vehicle for these aromas. This preliminary study gave sufficiently encouraging results for us to proceed to the next experiment, that is, using carbon dioxide gas as aroma carrier. The conclusions that were drawn from the results in Figures 2.3 to 2.9 are that (1) rice grains readily absorb a range of organic vapours; (2) some compounds were absorbed more readily than others, which might be due the effect of their molecular structures and vapour pressures; (3) the capacity of the rice grains to absorb the vapour depends on the amount of rice and the concentration of vapour that is available in the vial; (4) the headspace volume did not affect the vapour concentration; and (5) the vapour absorption by rice was relatively fast for some compounds, as the vapour concentrations were far below the controls after the first 20 minutes of equilibration.
Preliminary experiments were carried out on the suitability of carbon dioxide gas as an aroma carrier. Samples of rice (1 and 5 g) were placed in 20-mL headspace vials, and 0.4-μL portions of the model compounds (2-Apy and 2-hexanone) were injected after carbon dioxide gas was passed into the vial for 5 minutes in order to displace the air, as described in Section 2.2.2. As stated earlier, 0.4 μL of liquid aroma compound was used instead of 0.5 μL, to make sure that the amount of vapour in the headspace was well below the capacity of the rice samples to absorb it. The theoretical vapour volumes for 0.4-μL samples of 2-Apy and 2-hexanone were 48 and 8.3 mL, respectively, and the headspace volume was approximately 21 mL. In addition, the injection of 0.4-μL portions of liquid was easier and more accurate than 0.5-μL using an 1-μL syringe, since each graduation corresponds to 0.2 μL. 2-Apy and 2-hexanone were chosen as model compounds as the molecular structure of 2-Apy is very close to that of 2-acetyl-1-pyrroline, the major aroma compound in most of the fragrant rices, while 2-hexanone is very different in structure (see Table 2.2). We note at this point that vapour pressure may also affect the binding of an aroma to rice, as a function of time. An empty vial (no rice) was run as a control. The headspace concentration was analysed after each sample had been held at 35°C for 30, 60, 90, 120, 150 and 180 minutes. For comparison, experiments without carbon dioxide gas (i.e., with air in the vial) were carried out under the same conditions. The headspace gas concentrations in all of the vials were plotted as logarithm of peak areas, as for the earlier results, and they are shown in Figures 2.10 and 2.11. As noted before, nitrogen flushing during the headspace analysis will progressively reduce the concentration of carbon dioxide in the vials, and hence the earlier data points will be more useful in these studies.
Figure 2.10 shows that the controls (no rice in vial) for both air and carbon dioxide gas overlap. This indicates that, as expected, carbon dioxide gas did not affect the formation of 2-Apy vapour in the headspace. These controls tend to reach a plateau, indicating that the removal of the vapour during headspace analysis did not significantly affect the concentrations. After 30 minutes the 1-g and 5-g rice samples gave higher 2-Apy concentrations in the presence of carbon dioxide, suggesting that less aroma had been absorbed by the rice. This difference disappeared at longer times, and after 180 minutes the rice samples (both 1 and 5 g) gave similar 2-Apy concentrations for air and carbon dioxide gas. Hence, using carbon dioxide gas as a carrier did not significantly increase the absorption of 2-Apy vapour by rice.

Figure 2.11 shows the 2-hexanone headspace concentrations, measured under the same conditions as for 2-Apy. In this case the levels for the controls slightly decreased with time, due to the lower vapour volume for 0.4 μL of 2-hexanone (8.3 mL) in comparison with that of 2-Apy (48 mL). After 180 minutes the concentrations with carbon dioxide were slightly lower, but in most cases the differences were not significant.
Figure 2.10. Comparison of headspace concentrations of 2-Apy at 35°C with air and carbon dioxide gas as aroma carriers.

These preliminary experiments on carbon dioxide gas (Figures 2.10 and 2.11) show that carbon dioxide gas does not give a significant increase in the vapour absorption by rice; there is, if anything, a decrease for 2-Apy. Hence, the next aroma carrier — liquid carbon dioxide — was tried.
The preliminary experiments using liquid carbon dioxide to impart an aroma compound into rice grains were carried out using the procedures described in Section 2.3.3, using 6 μL of 2-Apy. An injected volume of more than 10 μL produced “wet” aromatised rice but 6 μL was found to be satisfactory. Systematic trials were carried out to determine the optimum volume, as discussed below (see Figure 2.22). This experiment aimed to determine whether liquid carbon dioxide could carry a substantial amount of aroma compound into the rice grains. A control experiment (no liquid carbon dioxide) was also carried out under the same conditions as for the liquid carbon dioxide. The pressure in the rice chamber for the control was atmospheric, since neither carbon dioxide liquid nor nitrogen gas were used in the
process; only a 2-g rice sample and 6 µL of 2-Apy on a filter paper were placed in
the rice chamber (see Figure 2.1). The aromatised rice was removed from the
chamber after 5 minutes equilibration time for both the control and carbon dioxide
experiments, and placed on a open petri-dish at room temperature (21°C). The aroma
concentrations in the rice samples were measured using headspace gas
chromatography after 1, 3, 21 and 24 hours of exposure to the ambient air. These
times were chosen to study the stability of the aromatised rice after being processed
using liquid carbon dioxide as aroma carrier. The headspace sampler and gas
chromatograph were used to measure the aroma concentrations in rice under the same
conditions as described in Section 2.3.1, except that the headspace bath temperature
was increased to 85°C, to more completely release the 2-Apy from the rice samples
prior to GC analysis. Although not all of the 2-Apy could be released into the
headspace at this temperature, the technique was nevertheless able to assess the
suitability of liquid carbon dioxide as an aroma carrier. It was found in this
experiment that 85°C was the maximum temperature for the headspace bath to
produce a clear and distinct 2-Apy peak. The chromatogram of 2-Apy with rice
showed a strong 2-Apy peak and two other small peaks (see Figure 2.27). As the
headspace temperature increased further, these small peaks increased in size and
overlapped the 2-Apy peak. As a consequence it was difficult to measure the 2-Apy
peak area (see Figure 2.28). Further details of the aroma-compound extraction
techniques are discussed in Section 2.4.2.
Figure 2.12. Comparison of 2-Apy concentrations in 2-g aromatised rice samples that were processed with and without liquid carbon dioxide.

Figure 2.12 shows the concentration of 2-Apy in 2-g aromatised rice samples that were processed with and without liquid carbon dioxide, after standing in ambient air for 1, 3, 6, 21 and 24 hours. Error bars corresponding to maximum deviations from 3 replicates, as well as straight lines of best fit, were drawn through each set of data. The lines of best fit in Figure 2.12 show that the aromatised rice that was processed using liquid carbon dioxide had significantly higher levels of 2-Apy, and these levels decreased more slowly during 24 hours exposure time than the aromatised rice that was processed without liquid carbon dioxide. Nevertheless, these results confirm that (for small samples at least), air can be quite a good aroma carrier. Analysis of variance (ANOVA) from Statistica for Windows, Release 4.5 was used to analyse the data in Figure 2.12. The 2-Apy concentrations at different
exposure times were not significantly different (P=0.06) for rice processed using liquid carbon dioxide. However, there was a significant difference (P=0.00) for the aromatised rice processed without using liquid carbon dioxide (i.e., using air as an aroma carrier). There was no significant difference (P>0.05) between the aromatised rice that was processed with and without liquid carbon dioxide for the 1- and 3-hour exposure times, but there were significant differences (P<0.05) for the 5, 21 and 24-hour exposure times, indicating that the aroma compound was released at a slower rate from the aromatised rice that was processed with liquid carbon dioxide. There was a substantial amount of statistical error in the data, particularly for the 21- and 24-hour controls but the apparently slower rate of aroma loss for the control appears to be genuine. The faster aroma loss for the aromatised rice that was processed without liquid carbon dioxide might be due to the aroma vapour penetrating only the outer layers of the starchy endosperm (see rice structure in Figure 1.2), whereas the compound might be carried farther into the rice grains by the high-pressure liquid carbon dioxide, and as a consequence it would presumably take a longer time for the aroma compound to migrate towards the surface of the rice and be released. The methanol extraction method, SPME coupled with GC-MS, and FT-IR spectroscopy (see Section 2.4.3) showed that the injected aroma compounds were present in the cores of aromatised rice grains. It was speculated that the aroma compound that was in the rice cores would travel layer by layer through the starchy endosperm towards the rice surface to replace the lost aroma compound in order to reach (if possible) the equilibrium aroma-vapour concentration. On the other hand, there was significantly less replacement of the lost aroma compound if it had only penetrated the outer layers, and therefore the aroma loss was faster.
The experiment on the 2-Apy was encouraging since the aroma compound in the aromatised rice processed with liquid carbon dioxide was retained longer than in rice without liquid carbon dioxide. A similar and even more encouraging experiment was carried out using $\gamma$-octalactone ($C_8H_{14}O_2$; five-membered ring with a hydrocarbon side chain and a carbonyl group) as the aroma compound. 2-Apy is not a food flavorant, indeed it is toxic, and it was considered that in developing an efficient method of aromatising rice, it would be appropriate to use model aroma compounds that would render the aromatised rice safe for consumption. $\gamma$-octalactone, which occurs in coconut milk, has a pleasant coconut odour. The aroma compound was chosen because in Indonesia and other Asian countries coconut milk is sometimes added during the cooking of rice. The results of these experiments are shown in Figure 2.13, where error bars corresponding to maximum deviations from 3 replicates were drawn for each data point. For the rice that was processed with liquid carbon dioxide, the data points for the 1-, 3-, and 5-hour exposure times decreased sharply and then tended to level out until 24 hours had elapsed, whereas the data points for the control (without liquid carbon dioxide) were consistently much lower. Statistical analysis showed that the rice processed without liquid carbon dioxide (i.e., the control) showed no significant difference ($P=0.21$) in aroma levels during the 24 hours exposure to ambient air. In contrast, there were significant differences ($P=0.00$) for the rice processed using liquid carbon dioxide. $\gamma$-octalactone concentrations were much higher for the liquid carbon dioxide samples for up to five hours exposure, but there was no significant difference ($P=0.31$) between the control and liquid carbon dioxide for the 21- and 24-hour exposure times. This shows that liquid carbon dioxide is a much better way of imparting $\gamma$-octalactone than air alone. $\gamma$-octalactone has a pleasant odour, but it was not a
satisfactory aroma compound for this experiment owing to its rapid rate of loss from the rice; therefore it was not used for the remainder of the experiments. The rapid loss of the compound from rice, as mentioned earlier, is apparently due to its molecular structure, which contains a carbonyl group but no aromatic ring. The molecular structure of γ-octalactone shares similarities with that of hexyl acetate which was also used as model aroma compound in this project. The results that were obtained from experiments with hexyl acetate (see Figure 2.22, and also Sections 5.3.1 and 5.3.2) were consistent with the results for γ-octalactone. Although the results in Figure 2.13 showed a rapid loss of γ-octalactone at longer exposure times, the liquid carbon dioxide was nevertheless able to carry a substantial amount of the aroma compound into the rice, and performed very much better than air as a carrier.

![Graph](image)

**Figure 2.13.** Comparison of the extractable γ-octalactone from 2-g aromatised rice samples that were processed with and without liquid carbon dioxide.
The general conclusion that can be drawn from the results shown in Figures 2.12 and 2.13 is that the use of liquid carbon dioxide to impart aroma compounds into raw rice represents an effective method of aromatisation. This method would be especially valuable in industrial scale production due to the large quantity of rice that could be processed in short periods of time. Although the increase in aroma concentration in aromatised rice processed by using liquid carbon dioxide is not always large, as shown in Figure 2.12, it would take longer for a large amount of rice to absorb the aroma vapour if air were used as the aroma carrier since the formation of aroma vapour for these compounds is slow at room temperature (25°C). This is especially true for γ-octalactone. Although it is possible to speed up the release of the aroma vapour by heating the aroma compound during aromatisation of a large quantity of rice, this has the potential to crack the rice due to moisture loss as a result of heating. These preliminary experiments using liquid carbon dioxide gave promising results, since it appeared that aroma compounds might stay longer in the rice under these circumstances. Therefore further experiments were carried out to study the physical and chemical characteristics of aromatised rice that had been processed using liquid carbon dioxide. In the first instance, studies were carried out on the effect of immersion time in liquid carbon dioxide on the appearance of rice grains and their retention of aroma compounds, as well as the optimum degree of milling of rice for aroma absorption.

Concentrations of absorbed aroma compound were studied as a function of equilibration time in liquid carbon dioxide, and the results are presented in Figure 2.14. Rice samples (2 g) were placed in the absorption chamber with 6 μL of liquid hexyl acetate, as described in Section 2.3.3. The aroma concentrations in the rice
samples were measured after 1, 3, 5, 7, 9, and 15 minutes equilibration time in liquid carbon dioxide. Hexyl acetate was chosen as an aroma compound that is readily absorbed by rice. The concentrations of hexyl acetate in aromatised rice grains were plotted as mg/100 g rice, and error bars corresponding to maximum deviations from 3 replicates were drawn. Methanol extraction coupled with gas chromatography was used to measure the aroma concentration instead of headspace sampler gas chromatography, since it was found that the headspace sampler was less effective in extracting the injected aroma compound from the aromatised rice grains (see Section 2.4.2). Headspace GC analysis was used to monitor the aroma loss as a function of time in the previous experiments (see Figures 2.12 and 2.13), as it is a non-destructive method. However methanol extraction coupled with gas chromatography is potentially a more accurate method. Analysis of variance on the data in Figure 2.14 was carried out using Statistica for Windows, Release 4.5. The results showed that there was no significant difference (P=0.00) among the equilibration times that were used, indicating that the aroma compound penetrated the rice very rapidly. This would be important if the process were to be used commercially. Although statistical analysis showed that there was no significant effect of equilibration time on aroma concentration, the equilibration time was, however, found to affect the physical appearance of the rice.
Figure 2.14. The effect of equilibration time on the retention of hexyl acetate in rice.

Rice grains are mostly consumed as whole steamed rice. The physical appearance of rice, such as the colour or presence of cracks in the rice grains, can affect its marketability (Velupillai and Pandey, 1990; Juliano, 1992). Most rice consumers prefer white milled rice without fissures and clumps. As stated in Section 2.1, the initial aim of this project was to develop one or more methods for imparting aromas into raw rice without changing its physical appearance. Hence, the appearance of the rice grains after being processed with liquid carbon dioxide was important. The effect of equilibration time in liquid carbon dioxide on the appearance of rice grains is shown photographically in Figures 2.15 to 2.21. There was approximately 2 g of rice (110 grains) for each treatment (equilibration time), and a typical grain from each bulk sample was photographed.
Figures 2.15 to 2.21 show photographs of rice after 5, 6, 10, 30 and 60 minutes in liquid carbon dioxide, using the procedure as described in Section 2.3.3, but without any aroma compound. All rice grains had the same degree of milling (15%). Photographs of a rice grain that had not been processed in liquid carbon dioxide (as a control; Figure 2.15), as well as a rice grain that showed fissures after being coated with gum arabic (Figure 2.21) are also presented. The photographs were taken under a stereo microscope (SZH-ILLK No. 107022, Olympus Optical Co. Ltd., Japan) at a magnification of 45 times.

Figure 2.15 (the control) shows a rice grain at 15% degree of milling, and Figure 2.16 shows a similar grain after 5 minutes in liquid carbon dioxide. These grains show no significant differences, and in particular there were no scratches or white stripes on the surface of the rice after 5 minutes immersion. Figure 2.17 shows a photograph of a rice grain after 6 minutes in liquid carbon dioxide, showing well defined white stripes on the surface of the grain. These white stripes increased in size at longer equilibration times, as shown in Figures 2.18, 2.19, and 2.20. The white stripes, which felt like very fine powder when rubbed between the fingers, indicated that the high-pressure liquid carbon dioxide had damaged the surface of the rice. These white stripes disappeared if the rice was rubbed between the hands. Five minutes equilibration time in liquid carbon dioxide was chosen as the optimum for imparting aromas into rice grains in this research since the appearance of the original rice did not change significantly; in addition, longer equilibration times did not give higher aroma concentrations in aromatised rice (see Figure 2.14).
Figure 2.21 presents a photograph of a rice grain after being coated with a 5% gum arabic solution. Gum arabic is an effective flavour encapsulating material since it provides very good retention of volatile compounds even during the drying process (Leahy et al., 1983). It was therefore tested as a coating material for the aromatised rice. This experiment aimed to determine whether there were any significant physical changes in the coated-aromatised rice. The rice was sprayed with the gum arabic solution and dried in air at room temperature (21°C) overnight. It can be seen that fissures occurred in the grain, which was easily broken when it was held; therefore coating with gum arabic was not further studied. This result was consistent with the observation in Section 1.3 that water-based treatments tend to cause rice grains to crack.

Figure 2.15. A rice grain at approximately 15% degree of milling.
Figure 2.16. A rice grain after 5 minutes in liquid carbon dioxide.

Figure 2.17. A rice grain after 6 minutes in liquid carbon dioxide.
Figure 2.18. A rice grain after 10 minutes in liquid carbon dioxide.

Figure 2.19. A rice grain after 30 minutes in liquid carbon dioxide.
Figure 2.20. A rice grain after 1 hour in liquid carbon dioxide.

Figure 2.21. A rice grain after coating with gum arabic.
Aroma concentrations in aromatised rice were determined as a function of the volume of injected aroma compound, and the results are presented in Figure 2.22. Three aroma compounds (hexyl acetate, 2-phenylethanol and 1-hexanol) were introduced into rice samples using 2, 4, 6, 8, 10, 15 and 20 µL portions in liquid carbon dioxide. The compounds were chosen due to their molecular structures; 2-phenylethanol and 1-hexanol have hydroxyl groups whereas hexyl acetate does not. Unlike 2-phenylethanol (see Table 2.2), 1-hexanol does not have an aromatic ring. An additional aim at this stage of the experiment was to determine whether there was a significant difference in absorption for aroma molecules with and without hydroxyl groups, and whether the presence of an aromatic ring affected aroma absorption. The procedure that was used was described in Section 2.3.3. The aroma compounds in the aromatised rice samples were extracted and quantified using methanol extraction coupled with gas chromatography. The mean aroma concentrations for each compound and sample volume (five replicates) were plotted as mg/100 g rice and error bars corresponding to the standard deviation were drawn through each data point.

Analysis of the data in Figure 2.22 was carried out using the same statistical package as described for Figures 2.12 to 2.14. Statistical analysis showed that the aroma concentrations changed significantly (P=0.00) as the volume of the injected aroma compounds increased. As expected, these results confirmed that there was in all cases an increase in aroma concentration for the aromatised rice as the volume of injected aroma compound increased. It had been expected that the rice grains would reach an equilibrium aroma concentration, at which point they would not absorb any additional aroma. This point had not been reached when 20 µL of aroma compound
had been added, but it was found that although an increase in volume of injected aroma compound increased the aroma concentration in the rice, at higher concentrations the grains took on a wet appearance. This wet appearance was clearly seen after the addition of 15 μL of liquid aroma compound. The wet appearance might be the result of the grains reaching the limit of the amount of compound they could absorb in 5 minutes. When this threshold is reached, the aroma compound sticks to the surface of the grains, causing them to appear wet and form clumps. The statistical analysis also showed that the aroma levels were not significantly different (P>0.05) for hexyl acetate and 1-hexanol for the various injected volumes. However, although both 2-phenylethanol and 1-hexanol have hydroxyl groups, statistical analysis showed that they were significantly different (P<0.05) for all of the injected volumes except for the 2-μL portion of liquid. These results suggested that the combination of a hydroxyl group and an aromatic ring gives improved absorption by rice. This was further examined in Chapter 5.

The relationship between degree of milling and the aroma concentration in aromatised rice was also studied. Seven degrees of milling of rice (0, 2, 3, 5, 8, 12 and 15%) and three aroma compounds (eugenol, cinnamaldehyde and cinnamyl alcohol) were used. All of these aroma compounds were prepared using 6 μL of aroma compound using the procedures as described in Section 2.3.3. The reasons for choosing these three aroma compounds, which are related to naturally occuring aromas, are explained later in this section. It should be noted that all three molecules contain aromatic rings and either carbonyl or hydroxyl groups. The average concentrations of extractable aroma compound in aromatised rice from 3 replicates (mg/100 g rice) and error bars corresponding to the standard deviations are shown in
Figure 2.23. The mean levels of aroma compound in aromatised rice appeared to increase with an increase in degree of milling of rice, then plateau for the 8%, 12% and 15% degrees of milling. It was speculated that the increase in aroma content with increased degree of milling might be due to the removal of the outer layers of the rice during milling, which could produce a porous surface on the rice grains; this porous condition could increase the aroma absorption. However, an analysis of variance showed that the degree of milling caused no significant difference in the concentrations of eugenol (P=0.00), cinnamyl alcohol (P=0.00), and cinnamaldehyde (P=0.02) in the aromatised rice. A 15% degree of milling of rice was chosen for the rest of the experiments in this research since this degree of milling gave a white shiny appearance, which is preferred by most rice consumers, and satisfactory aroma absorption. Thus our remaining experiments were carried out using a commercially acceptable grade of rice. We note in passing that cinnamaldehyde (a carbonyl compound) was absorbed less well than eugenol and cinnamyl alcohol (hydroxyl compounds).

These preliminary experiments using liquid carbon dioxide to produce aromatised rice gave encouraging results. It was found that (1) liquid carbon dioxide did not cause significant changes in the rice appearance after 5 minutes equilibration time; and (2) the aroma compound appeared to remain longer in the aromatised rice than was the case when air or carbon dioxide gas were used as carriers. Hence, liquid carbon dioxide was used to impart aroma compounds into rice grains, using the procedures that were described in Section 2.3.3. A 15% degree of milling for the rice, and an injected aroma volume of no more than 10 μL were used for the rest of the experiments in this research.
Figure 2.22. The aroma concentrations in aromatised rice as a function of the volume of injected aroma compound.

Figure 2.23. The relationship between degree of milling of rice and aroma concentration in aromatised rice.
The model compounds that were used in the preliminary experiments (see Table 2.2) were not used in the next series of experiments, since they did not have pleasant odours, and some of them (notably 2-acetylpyridine) were hazardous. Eugenol, cinnamyl alcohol, isoeugenol, methyl eugenol and cinnamaldehyde were selected as model aroma compounds for the next experiments, owing to their functional groups and odours. Eugenol (clove) was selected first due to its pleasant odour and its molecular structure, which included an aromatic phenyl group and a hydroxyl group. As noted earlier, these may lead to good absorption by rice. The other compounds listed were selected because their molecular structures resemble that of eugenol (see Table 2.4), but with systematic differences. Isoeugenol, which exists in ylang-ylang oil, is an isomer of eugenol differing only in the placement of a double bond in the sidechain. In methyl eugenol the hydroxyl group is replaced by a methoxy, which should exhibit weaker hydrogen bonding. Cinnamyl alcohol is a simpler molecule with an aliphatic hydroxyl group, rather than an aromatic (phenolic) hydroxyl. Cinnamaldehyde is a carbonyl analogue of cinnamyl alcohol. Both of these compounds are found in cinnamon oil. We hoped that we might use this series of compounds to identify the functional groups and physicochemical properties in aroma compounds that cause improved binding to rice.

The next experiments in this section were preliminary attempts to determine the capacity of these aroma compounds to bind to rice. More detailed and quantitative binding experiments are discussed in Chapters 4 and 5. The structures and physicochemical properties of the five model aroma compounds are summarised in Table 2.4.
The vapour pressures for eugenol, isoeugenol, cinnamaldehyde and cinnamyl alcohol were extrapolated using the available vapour-pressure data (Jordan, 1954) at 78.4-253.5°C for eugenol, 86.3-267.5°C for isoeugenol, 60-260°C for cinnamaldehyde, and 72.6-250°C for cinnamyl alcohol. The extrapolation for each compound was carried out using the Clausius-Clapeyron equation (Grain, 1990) as described earlier in this section for acetophenone. As there were no published data available for methyl eugenol, the vapour pressure at 25°C was calculated using the Antoine equation (Grain, 1990) as described for 2-Apy.

Table 2.4. Structures and physicochemical properties of eugenol, isoeugenol, methyl eugenol, cinnamaldehyde and cinnamyl alcohol.

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<td>Eugenol</td>
<td>• Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>• One aromatic hydroxy group</td>
</tr>
<tr>
<td></td>
<td>• One aromatic methoxy group</td>
</tr>
<tr>
<td></td>
<td>• Boiling point = 253.5°C</td>
</tr>
<tr>
<td></td>
<td>• Vapour pressure = 0.028 mmHg (25°C)</td>
</tr>
<tr>
<td></td>
<td>• Density = 1.07 g mL⁻¹</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Compound</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoeugenol</td>
<td>- Aromatic ring&lt;br&gt;- Isomer of eugenol&lt;br&gt;- One aromatic hydroxy group&lt;br&gt;- One aromatic methoxy group&lt;br&gt;- (^a)Boiling point = 267.5°C&lt;br&gt;- (^a)Vapour pressure = 0.019 mmHg (25°C)&lt;br&gt;- (^b)Density = 1.082 g mL(^{-1})</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>- Aromatic ring&lt;br&gt;- Two aromatic methoxy groups&lt;br&gt;- (^b)Boiling point = 228.2°C&lt;br&gt;- (^c)Vapour pressure = 0.091 mmHg (25°C)&lt;br&gt;- (^b)Density = 1.034 g mL(^{-1})</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>- Aliphatic aldehyde group&lt;br&gt;- Aromatic ring&lt;br&gt;- (^a)Boiling point = 251.0°C&lt;br&gt;- (^a)Vapour pressure = 0.051 mmHg (25°C)&lt;br&gt;- (^b)Density = 1.050 g mL(^{-1})</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>- Aromatic ring&lt;br&gt;- One aliphatic hydroxy group&lt;br&gt;- (^a)Boiling point = 250.0°C&lt;br&gt;- (^a)Vapour pressure = 0.048 mmHg (25°C)&lt;br&gt;- (^b)Density = 1.044 g mL(^{-1})</td>
</tr>
</tbody>
</table>

Sources: (a) Jordan (1954); (b) Aldrich Australia (1998/1999); (c) calculated vapour pressure using Antoine equation (Grain, 1990).
The initial aim of these binding experiments was to determine how suitable these five aroma compounds were for being imparted into rice. This was done by injecting 6 μL of each aroma compound, as described in Section 2.3.3. Each aroma compound was treated separately. The aroma compounds in the aromatised rice were measured using methanol extraction coupled with gas chromatography. Levels of extractable eugenol, cinnamyl alcohol, isoeugenol, methyl eugenol and cinnamaldehyde are shown in Figure 2.24. There were 3 replicates for each aroma compound, and error bars corresponding to maximum deviations were also drawn for each set of data. The data were analysed using an analysis of variance carried out with *Statistica for Windows, Release 4.5*. This showed that there were no significant differences (P=0.15) for the levels of the five aroma compounds in aromatised rice. The concentration of extractable eugenol in aromatised rice was approximately 160 mg/100 g rice, corresponding to 3.2 mg in a 2-g rice sample. 6 μL of eugenol was used for a 2-g rice sample, which corresponds to 6.4 mg eugenol in the sample. Thus, approximately half (50±10)% of the injected eugenol was absorbed by the rice during the 5-minute absorption time, and values of (41±8)%, (34±10)%, (35±8)%, and (33±7)% were determined for cinnamyl alcohol, cinnamaldehyde, isoeugenol and methyl eugenol, respectively. The actual aroma contents would have been slightly higher, as the extraction method was not quite quantitative (see below). These figures confirm that rice is an affective absorber for these compounds.
Figure 2.24. Masses of extractable eugenol, cinnamyl alcohol, isoeugenol, methyl eugenol and cinnamaldehyde in aromatised rice after being freshly processed.

The results in Figure 2.24 show that the five aroma compounds are absorbed equally well when they are the sole aroma present. A competitive binding experiment was carried out, during which 6 µL of each compound was injected at the same time. This experiment aimed to determine which (if any) of these five aroma compounds was absorbed more readily by the rice during the 5-minutes equilibration time in liquid carbon dioxide. Given that a total of 30 µL of aroma had been added to the rice, it was possible that there would be competition for binding sites at such a high total concentration. The levels of extractable cinnamyl alcohol, cinnamaldehyde, eugenol, isoeugenol and methyl eugenol are shown in Figure 2.25, together with error bars corresponding to maximum deviations from 3 replicates. Again, the levels of extractable aroma compounds were measured using methanol
extraction coupled with gas chromatography. An analysis of variance showed that there were no significant differences (P=0.53) between the concentrations of the five aroma compounds in aromatised rice, and hence these compounds have an equal opportunity of penetrating into the grains in the presence of the high-pressure liquid carbon dioxide. Using the calculation for the mass of the injected eugenol as described for Figure 2.24, it was found that methyl eugenol (38±13%) and eugenol (38±18%) absorbed the highest percentages of the injected aroma, followed by iso-eugenol (31±6%), cinnamaldehyde (26±9%) and cinnamyl alcohol (24±11%).

**Figure 2.25.** The concentrations of extractable eugenol, cinnamyl alcohol, iso-eugenol, methyl eugenol and cinnamaldehyde in aromatised rice after 6 μL of each aroma compound was simultaneously introduced into rice samples.
In aromatised foods, the stability of the aroma compounds during storage is important. It is not worth producing an aromatised food if the aroma is too easily lost; therefore, experiments on the stability of eugenol, cinnamyl alcohol, isoeugenol, methyl eugenol, and cinnamaldehyde in aromatised rice were carried out. 6-μL samples of these compounds were used, following the procedure described in Section 2.3.3. The freshly aromatised rice grains were placed in open petri-dishes at room temperature (21°C). The concentrations of aroma compounds in the rice samples, which were reported as mg/100 g rice, were measured after 1, 3, 5, 24, and 72 hours (Figure 2.26). Methanol extraction coupled with gas chromatography was used to quantify the aroma concentrations. No replicate was carried out because this experiment was only designed to establish whether the aroma compounds were still present in the rice samples after they had been exposed to the open air for up to three days. Further experiments to measure the rate of aroma loss for these five aroma compounds are discussed in detail in Chapter 3. Unlike the concentrations of eugenol, isoeugenol, methyl eugenol and cinnamyl alcohol, the concentration of cinnamaldehyde after 72 hours in an open petri-dish was almost zero. This means that cinnamaldehyde was more weakly bound to the rice, and therefore it was easily lost during the 72 hours exposure time. Small amounts of eugenol, isoeugenol, methyl eugenol and cinnamyl alcohol remained in the rice samples, although they had been exposed to the open air for 72 hours. Further, it was still possible to recognise the distinctive odours of eugenol and cinnamaldehyde, but not isoeugenol, cinnamyl alcohol and methyl eugenol, which originally had no distinctive smells.
Figure 2.26. The concentrations of eugenol, isoegenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde during 72 hours exposure time in open petri-dishes at room temperature (21°C).

These preliminary experiments encouraged us to proceed to a further experiment. As shown above, approximately half of the injected aroma compound was absorbed by the rice grains, and some of this remained in the rice after being exposed to the open air for 72 hours. In addition, eugenol (clove) and cinnamaldehyde (cinnamon), which are used in cooking, gave pleasant odours and could potentially be used for commercial products. The other aroma compounds — cinnamyl alcohol, isoegenol and methyl eugenol — did not have such distinctive odours. However, they were still used in the following experiments because of their molecular structures. The interest was in determining, for example, whether the hydroxy group (-OH) or the methoxy group (-OCH₃), or related physicochemical properties led to stronger binding of the aroma compounds to the rice.
The results in Figure 2.24 show that approximately half of the injected aroma compound could be absorbed by rice grains; however, it was not yet known whether the aroma compounds were transported by the liquid carbon dioxide into the rice cores, as we proposed in Section 2.4.1. Therefore, the techniques of fluorescence microscopy, nuclear magnetic resonance (NMR) spectroscopy, SPME-gas chromatography, Fourier Transform Infrared (FT-IR) spectroscopy, and methanol extraction coupled with gas chromatography were used to test for the presence of the injected aroma compounds in the cores of aromatised rice grains. The results that were obtained using these techniques are discussed in Section 2.4.3, while the experiments that found the most practical technique for extracting aroma compounds from aromatised rice are discussed in Section 2.4.2, as follows.

### 2.4.2. Aroma-Extraction Methods for Aromatised Rice

There are two steps involved in the determination of aroma compounds in rice: aroma extraction and aroma analysis. The extracted aroma compounds are used in the identification and quantification of aromas. The extraction method for an aroma volatile can significantly affect the results of an aroma analysis. An aroma-extraction method that uses high temperatures can break down some compounds, with the result that the identified or quantified compounds are not the original compounds. A variety of methods has been used for extracting aroma compounds from rice: steam distillation (Fujimaki et al., 1977; Yajima et al., 1978; Yajima et al., 1979; Tava and Bocchi, 1999); Tenax gas-chromatography trapping (Fujio et al., 1991; Kato et al., 1983); Likens-Nickerson simultaneous distillation-extraction (Buttery et al., 1983b; Paule and Powers, 1989; Laksanalamai and Ilangantileke, 1990), etc. Methanol extraction coupled with gas chromatography was used to test for the presence of the injected aroma compounds in the cores of aromatised rice grains. The results that were obtained using these techniques are discussed in Section 2.4.3, while the experiments that found the most practical technique for extracting aroma compounds from aromatised rice are discussed in Section 2.4.2, as follows.
1993; Widjaja et al., 1996a, b). Each of these aroma-extraction methods has its advantages and disadvantages. Some factors that should be taken into account when choosing a method include sample size, number of replicates, and the thermal stability and volatility of the aroma compound. For aroma analysis, gas chromatography (GC) and GC-mass spectrometry (GC-MS) are the most common modern methods that are used to quantify and identify the aroma compounds in rice.

To measure the concentrations of aroma compounds in aromatised rice, we required a method that could extract most of the injected aroma compounds, with minimal loss. In general we used five replicates for each treatment in this study, especially for the kinetic studies (Chapter 3), in which the aroma compound was extracted from a rice sample every hour for 12 hours; therefore a simple and rapid method was essential.

Aroma-extraction methods such as steam distillation and Likens-Nickerson simultaneous distillation were not used because they were too time-consuming. Tenax gas-chromatography was quicker, but it was not used due to difficulties in the quantification. Headspace sampling and methanol extraction were evaluated as aroma-extraction methods, and gas chromatography was used to quantify the concentrations of the extracted aroma compounds. Both methods were relatively simple; all five replicates could be carried out simultaneously for the methanol extraction, or sampled automatically by the headspace sampling method.

A Hewlett Packard Model 19395A headspace unit was used to extract the injected aroma compounds from aromatised rice. The sampling was similar to that
described in Section 2.3.1, but the headspace-sampler bath temperature was increased to 85°C. The higher temperature allowed more of the injected aroma compound in the rice sample to be released into the headspace. A Hewlett Packard Model 5890A gas chromatograph equipped with an SGE BP 5 column (2.5 m × 0.22 mm × 1.0 μL) and a flame ionization detector (FID) was used to measure the concentration of extracted aroma compound. The conditions of the gas chromatograph were described in Section 2.3.1. The headspace concentration of aroma compound was expressed as peak area count.

The rice samples that were used for headspace sampling were prepared using the procedures as described in Section 2.3.1, but with 5 μL of 2-acetylpyridine (2-Apy) and 1 g of rice. A headspace vial with 1 g rice (no aroma), and also a vial with only 0.5 μL of 2-Apy were prepared as controls. This experiment was carried out at an earlier stage of the study when carbon dioxide gas was being tested as an aroma carrier (Section 2.3.1). 2-Apy was used at that time since its molecular structure resembled that of 2-acetyl-1-pyrroline, the major aroma compound in most fragrant rices. Figure 2.27 shows a typical chromatogram of 2-Apy at 85°C. Only one peak (2-Apy) is seen in the chromatogram, at a retention time of 3.08 minutes. Figure 2.28 shows a typical chromatogram of 2-Apy with 1 g of rice in a headspace vial at 85°C. It shows a strong 2-Apy peak, and two other small peaks which may be from the volatile compounds that originally existed in the rice (see, for example, Widjaja et al., 1996a; Yajima et al., 1978); however, the peak for 2-Apy is still very clear and distinct.
Figure 2.27. A typical chromatogram of 2-acetylpyridine (2-Apy) at a headspace-bath temperature of 85°C.

Figure 2.28. A typical chromatogram of 2-acetylpyridine and 1-g rice sample at a headspace-bath temperature of 85°C.
Figure 2.29 shows a typical chromatogram of 2-Apy and 1 g of rice in a headspace vial after the headspace-bath temperature was increased to 115°C. It was expected that by increasing the temperature, more 2-Apy from the aromatised rice could be released into the headspace. Unfortunately, with the increased temperature, other large peaks appeared in the chromatogram; consequently, it was difficult to measure accurately the peak area for 2-Apy, since some of the peaks overlapped each other. The new peaks might be from the volatile compounds that exist naturally in rice grains, or from the breakdown of 2-Apy at the higher temperature (115°C). The results from the headspace sampler were not encouraging since the 2-Apy was not totally extracted from the rice sample at 85°C, as indicated by the larger 2-Apy peak when the temperature was increased to 115°C (see Figures 2.28 and 2.29). Hence, the headspace sampling method was not used further in this study, and another method — methanol extraction — was tried instead.

The methanol extraction procedures were described in Section 2.3.3. The rice samples were not ground before being soaked in the methanol solution, as aroma compounds could be lost during grinding. In the first extraction that was attempted, we found that soaking the rice in 3 mL of methanol for 2 hours was not enough to soften the rice grains. The rice could not be broken down into fine granules during the 2 hours stirring time, and we suspected that some of the injected aroma compounds might still in the rice. Hence, the soaking time was increased to about 24 hours (i.e., overnight). Overnight soaking gave rice that was soft enough to be broken down into fine granules during the 2-hours stirring time, giving a cloudy suspension after stirring. It was assumed that the smaller particle size would lead to more efficient aroma extraction. Levels of extracted aroma compound were
expressed as mg/100 g rice (see Section 2.3.3). A summary of the methanol extraction method is shown as a flow-chart diagram in Figure 2.30.

**Figure 2.29.** A typical chromatogram of 2-acetylpyridine and a 1-g rice sample at a headspace-bath temperature of 115°C.
Figure 2.30. A flow chart diagram for aroma extraction using methanol as a solvent.
Figure 2.31. The cinnamaldehyde, cinnamyl alcohol, eugenol, methyl eugenol and isoeugenol concentrations in rice, for three successive extractions using methanol/GC analysis.

Figure 2.31 shows the histogram of cinnamaldehyde, cinnamyl alcohol, eugenol, methyl eugenol and isoeugenol concentrations that were extracted using from one to three successive extractions using the procedures described in Figure 2.30, but with 2-hours soaking time for each extraction. This means that after the rice samples were extracted for the first time, the samples were collected and extracted again using the same procedures as for the first extraction. No replicates were used in this experiment since the aim was to show whether there were still some aroma compounds left after the successive extractions. The samples in this experiment were prepared using the procedures described in Section 2.3.3, with 6 µL.
of each aroma compound (separate treatments). Figure 2.31 shows that some
extractable aroma compounds remained in the samples after the first extraction, but
very little after the second extraction. Clearly, a soaking time of 2 hours was not
enough to extract all of the aroma compounds from the rice samples. As has been
noted, this may be related to the relatively large particle size of the rice samples.
However, overnight soaking followed by a 2-hour stirring period yielded much finer
particles, which should allow the aroma compounds to be released readily. We
concluded that, while not giving total extraction of the sample, it was a time-effective
and acceptably efficient method of extraction.

These experiments (Figures 2.27-2.31) showed that methanol extraction gave
better results than headspace sampling. Headspace sampling was a simpler
procedure, but it was not an efficient method at a headspace bath temperature of
85°C. However, if the bath temperature were increased to improve its efficiency,
additional aroma volatiles were released into the headspace. Methanol extraction
gave better results since no heat was involved and the aroma compounds were readily
released into the methanol. This method was not complicated, and many samples
could be analysed at the same time. For example, in the kinetic measurements for
eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde (see
Section 3.2) the aroma compounds were extracted from rice samples every hour for
12 hours, with 5 replicates for each measurement. With the methanol-extraction
method, each replicate could be placed in a separate 20-mL screw-cap headspace
vial, and 3 mL of methanol was added to each vial. All five vials could be stirred
and then centrifuged at the same time, then analysed using gas chromatography.
2.4.3. The Detection of Aroma Compounds in Aromatised Rice

Several different experiments were carried out in an attempt to determine whether liquid carbon dioxide can carry aroma compounds into the cores of rice grains. This was considered to be important, since it could reduce the rate of aroma loss. It was hypothesised that aroma compounds in the rice cores would take a longer time to migrate towards the surface of rice grains, before being lost to the air.

The presence and distribution of aroma compounds in rice grains were studied by fluorescence microscopy (Olympus BX 60, Olympus Optical Co., Ltd., Japan), solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) (Varian 3800 gas chromatograph, Saturn 2000 ion trap spectrometer), Fourier Transform Infrared (FT-IR) spectroscopy (Perkin-Elmer system 2000) and nuclear magnetic resonance (NMR) spectroscopy (Varian Unity Plus 300 MHz). Some of these methods successfully showed the presence of injected aroma compounds in rice, but others were unsuccessful for a variety of reasons. Problems arose because the rice grains could not be thinly sliced owing to their hardness and brittleness. They tended to break into powder if they were thinly sliced.

Attempts were made to detect the injected aroma compounds in rice using fluorescence microscopy. This method had the potential advantages of high sensitivity (useful for low aroma concentrations) and the capacity to show how aroma compounds are distributed in rice grains. Aromatised rice grains were sliced as thinly as possible across the long axis of the grain using a very sharp razor blade. Only slices from the middles of the grains were used. The sliced samples were
placed on glass microscope slides, and observed under a fluorescence microscope that was equipped with a BP (Band Pass) excitation filter with a wavelength range of 330-385 nm. It was hoped that this would excite fluorescence from eugenol and cinnamaldehyde, which were used as model aroma compounds. These compounds were selected since the results from Figures 2.24 and 2.25 showed that eugenol was the most effectively absorbed aroma compound and cinnamaldehyde least effectively absorbed of the five aroma compounds (isoeugenol, methyl eugenol, cinnamyl alcohol, eugenol and cinnamaldehyde). It was hoped that fluorescence microscopy would show different images for the rice samples with and without the aroma compounds. This could, in principle, show the distribution of the aroma compounds in cross-sectional slices of aromatised rice grains. The aromatised rice samples were prepared using the procedures as described in Section 2.3.3, but with 10 μL of liquid aroma compound. Sliced-rice samples without any injected aroma compound, and thin smears of each model compound on microscope slides, were prepared as controls. The fluorescence images were photographed with a 3CCD (charge coupled device) digital camera (MTI) at a magnification of 10 times; the images were saved as computer files, and printed as shown in Figures 2.32a to 2.32e.

Figures 2.32a and b show the fluorescence images of samples of pure eugenol and cinnamaldehyde, and Figure 2.32c shows an image of unaromatised rice. All three samples gave similar blue fluorescence upon excitation at 330-385 nm. Figures 2.32d and 2.32e show the images of aromatised rice grains that had been processed using eugenol and cinnamaldehyde as model compounds, respectively. (Note: the background of the image in Figure 2.32d is lighter owing to the presence of transmitted light. More typically a dark background was applied to enhance the
contrast of the fluorescence images so they could be more clearly observed. However, both of the images in Figures 2.32d and 2.32e show the typical blue appearance of the unaromatised rice sample, and the presence of the aroma compounds caused no discernible change. To address this problem, further experiments using a UV lamp (Camag-Muttenz-Schweiz) were carried out to find out whether a change of excitation wavelength could give perceptibly different responses for the model aroma compounds and rice. Aromatised and non-aromatised rice grains were placed under the UV lamp, and the results showed that for both 254 and 366-nm excitation the different samples gave indistinguishable responses. It was considered that it was not worth proceeding with further experiments of this type, as the fluorescence microscope that was available could not detect these aroma compounds in aromatised rice, as any aroma fluorescence was dominated by background emission.

SPME (solid phase microextraction) coupled with GC-MS was used to show the presence of the aroma compounds in rice cores. SPME is an extraction method that uses adsorbent material to extract a trace amount of organic compounds from a sample. The adsorbent material has a strong affinity towards organic compounds and therefore it can retain and concentrate those compounds from an aqueous or gaseous sample. The adsorbent material coats a fibre that is incorporated into a holder (syringe). There are two steps in the SPME method: (1) the equilibration between the organic compounds in a sample matrix and the coated fibre of the syringe, and (2) the transfer of the fibre that has adsorbed the organic compounds to an analytical instrument for analysis (Pawliszyn, 1997). In the current experiment, the SPME extraction was performed by inserting and exposing the fibre in the headspace of a
sealed headspace vial that was filled with aromatised rice cores. The fibre was then desorbed into an injection port of the gas chromatography-mass spectrometry (GC-MS) system.

The aromatised rice was prepared using the procedures described in Section 2.3.3, with 10 μL of eugenol as the model aroma compound. Five rice grains from the aromatised samples were peeled evenly (i.e., the surface of the rice was shaved off using a sharp razor blade). Then a third of each peeled-rice grain was cut from both ends to obtain the rice cores. A control (non-aromatised rice core) was also run in order to provide a comparison with the result of the aromatised rice cores. The samples (5 rice cores) were placed in a 100-μL insert which was in turn placed into a 2-mL screw-cap vial. The screw-cap incorporated a septum. The samples were allowed to equilibrate for 30 minutes prior to SPME sampling. An SPME manual holder (Supelco) equipped with a 1-cm polydimethylsiloxane (PDMS)/carboxen fibre was used to trap the volatile compounds from the samples. The analysis was performed using a Varian 3800 gas chromatograph which was connected to a Saturn 2000 ion-trap mass spectrometer (1 scan/second, 20 μA emission current). The column of the GC was a Chromapack CP Wax 52 CB (30 m × 0.25 mm × 0.25 μm). The SPME fibre was desorbed into the injection port of the GC-MS at a temperature of 220°C and allowed to stay for 10 minutes. The temperature of the GC oven was initially 60°C and it was increased at 3°C/minute until 240°C. It was held at this temperature for 5 minutes, resulting a total running time of 65 minutes. The flow rate of helium in the GC was kept constant at a pressure of 10 psi. Two replicates were applied in this experiment. The chromatograms of the eugenol-aromatised rice cores and non-aromatised rice cores are shown in Figure 2.33. The strong peak for
eugenol was clearly seen in the first chromatogram at a retention time of 37 minutes, and although there were weak broadened bands in this region of the second chromatogram, there was a clear difference between the two chromatograms. Based on these results, it was concluded that SPME was successful in showing the presence of the injected eugenol in the rice cores of the aromatised rice. This was a significant finding for this project since the liquid carbon dioxide was successful in delivering the injected aroma compounds into the rice cores. Hence, it is expected that the aromas can be retained longer in the aromatised grains before being lost to the open air.

The third method that was used in an attempt to detect the injected aroma compounds in rice cores was FT-IR spectroscopy. Samples of aromatised rice were prepared using the procedures as described in Section 2.3.3, with eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde as model aroma compounds (10 µL for each compound). The freshly prepared aromatised rice grains (2 g, consisting of about 110 grains) were evenly peeled using a very sharp razor blade. The average dimensions of the peeled grains were about 90% of the unpeeled grains. The peeled-rice grains were cut into halves, and these halved kernels were placed onto a horizontal attenuated total reflectance (ATR) crystal plate (zinc selenide coated crystal) with the cut sections facing downwards for analysis.
Figure 2.32. Images of eugenol, cinnamaldehyde and rice under a fluorescence microscope at an excitation wavelength of 330-385 nm. * Please see note in text.
Figure 2.33. Typical chromatograms of eugenol-aromatised rice and non-aromatised rice using SPME coupled with GC-MS.

The infrared beam path of the ATR crystal plate in the FT-IR spectrometer is illustrated in Figure 2.34. The samples can be placed either on top of or under the crystal plate. The infrared beam enters the ATR crystal, which is transparent to infrared radiation. The beam is progressively reflected along the crystal owing to total internal reflection at the surface. Each reflection creates an evanescent wave
either at the top or bottom of the ATR crystal. For each reflection, a finite amount of radiation can pass into the sample.

The input and output spectra of the infrared radiation would, in principle, be the same if there were no absorption by the samples placed either on or under the ATR crystal plate. On the other hand, if the samples contained substances that absorbed infrared radiation, the amount of the radiation that passed through the output end of the crystal would be reduced, and the spectrometer would record the infrared absorption spectrum of the sample.

![Diagram of ATR crystal plate](image)

**Figure 2.34.** The infrared beam path for the ATR crystal plate.

Standard spectra were recorded of the pure liquid model compounds, by thinly smearing them onto the crystal plate. Absorption spectra were run at wavenumbers between 600 and 5200 cm\(^{-1}\). Approximately 150 halved kernels were placed on the crystal plate from the 2-g rice sample; some of the original kernels had broken during the peeling and cutting. The concentrations of aroma compounds in
the rice kernels were not quantified since there was some aroma loss during peeling and cutting, and the halved kernels were not of uniform size. However, it was possible to use FT-IR spectroscopy to measure the rate of aroma loss from rice cores by following changes in the FT-IR absorbance.

Figure 2.35 shows the FT-IR absorption spectrum of rice (control), while the spectra of aromatised rice samples containing eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde are shown in Figures 2.36 to 2.40. These Figures report spectra between 700 and 1300 cm\(^{-1}\), as they contain characteristic absorption bands, and are free from interference due to ambient water vapour and carbon dioxide. Figures 2.36 to 2.40 show spectra for the model compounds in rice after 0, 30, and 60 minutes exposure on the crystal plate, except for cinnamyl alcohol, where the exposure times were 0, 15, and 30 minutes, owing to the weakness of the cinnamyl alcohol spectrum in terms of the absolute absorbance compared to other spectra. All of the Figures show clear similarities between the spectra of the aromatised rice samples and the pure aroma compounds. This indicates that the rice cores contained the aroma compounds, and confirmed the suitability of liquid carbon dioxide as an aroma carrier. The spectra also showed that some injected aroma compounds were lost during the exposure time, indicated by the smaller absorbances with longer exposure times. This was particularly the case for cinnamaldehyde. Quantitative kinetic studies of aroma loss were carried out, as discussed in Chapter 3.
Figure 2.35. FT-IR absorbance spectrum of rice sample (no injected aroma compound).
Figure 2.36. FT-IR absorbance spectra of pure liquid eugenol and eugenol-aromatised rice.
Figure 2.37. FT-IR absorbance spectra of pure liquid isoeugenol and isoeugenol-aromatised rice.
Figure 2.38. FT-IR absorbance spectra of pure liquid methyl eugenol and methyl eugenol-aromatised rice.
Figure 2.39. FT-IR absorbance spectra of pure liquid cinnamyl alcohol and cinnamyl alcohol-aromatised rice.
Figure 2.40. FT-IR absorbance spectra of pure liquid cinnamaldehyde and cinnamaldehyde-aromatised rice.
Attempts were made to detect the presence and distribution of injected aroma compounds in rice samples using nuclear magnetic resonance (NMR) spectroscopy. The use of NMR has been extensively reported for studies of water and fat distributions in food products; for example the water distribution in rice grains (Ruan et al., 1997; Takeuchi et al., 1997), water diffusivity in rice starch/water mixtures (Gomi et al., 1998), and lipid distribution in low moisture bread (Roudaut et al., 1998). In addition, NMR has also been used for measuring water content in rice nondestructively (Cho and Chung, 1997). The measurement of both water and lipid distributions in a system was based on the principle that the chemical shifts (i.e., frequencies) of the NMR signals are determined by the protons (hydrogen atoms) in the samples. The protons could be the hydrogens in water or fat or other chemical compounds that contain hydrogen. In principle, each chemically distinct proton has a distinctive chemical shift or pattern of shifts (multiplet structure is common in NMR spectra), although overlaps are common, and are worse in solid samples owing to broadening of the lines in the spectra. The NMR spectrometer that was used in this experiment used a spin echo multi slice (SEMS) pulse sequence to obtain the NMR images. It is possible to use chemical-shift selective imaging to distinguish the distributions of different chemical compounds in a system, for example the distributions of water, fat, protein and eugenol in rice; however, it was found that the NMR facilities available for this experiment were unable to produce selective images of this type; the images that were obtained can be understood to provide a map of the distribution of all chemical components in rice that contained hydrogen. More specifically, it was speculated that the images mapped the water distribution in rice due to the small amount of injected eugenol, with contributions also from other rice components such as fat and protein.
The rice sample was prepared as described in Section 2.3.3, using 10 μL of eugenol. Pure liquid eugenol and water were also run as controls. The NMR images for the rice sample, pure liquid eugenol and water are presented in Figures 2.41 and 2.42.

![NMR images for some rice grains.](image)

**Figure 2.41.** The NMR images for some rice grains.
Figure 2.42. The NMR images for: (a) pure liquid eugenol and (b) water.

The experiment was carried out by placing rice grains into an NMR sample tube (5 mm inner diameter) with the long axes of the grains parallel to the tube. The diameter of a rice grain was approximately 1.5 mm; therefore there might be two or three rice grains in the sample tube. The experiment for pure eugenol was carried out by filling an NMR tube with pure liquid eugenol, and another tube filled with water was run for a comparison. It took about 11 hours to obtain one image. The size of the pixel for the image was about 65 μm x 100 μm. The image in Figure 2.41 does not show the distribution of eugenol or water in rice separately, but it is likely that the darker areas indicate higher concentrations of water than the lighter areas. Takeuchi et al. (1997) studied the change of moisture distribution in a rice grain during boiling (after boiling for 6, 12, 14 and 25 minutes) as observed by NMR imaging as shown in Figure 2.43. The lighter areas in the images are associated with regions of higher water concentration. It was clear that there was much more water
distributed in the boiled rice after 25 minutes boiling time than in the uncooked or partly cooked rice. The images showed that for short cooking times the water distribution in the rice grain was mainly in the surface regions. This demonstrates that NMR imaging has the potential to reveal the distribution of a proton-containing substance in rice. However, the amounts of aroma in aromatised rice are low and cannot be distinguished from water, given our current instrumentation.

Source: Takeuchi et al. (1997).

**Figure 2.43.** The NMR images of the moisture distribution in a partly boiled rice grain.
Of the various experimental methods that were used to study the presence of the injected aroma compounds in rice grains, SPME coupled with GC-MS, and FT-IR spectroscopy successfully demonstrated the presence of the injected aroma compounds in rice cores.

2.5. Conclusion

The initial aim of this project was to develop one or more methods for imparting aromas into raw rice without significantly changing its physical appearance. This was achieved using liquid carbon dioxide as an aroma carrier. White milled rice is a unique grain to work with, and it is easily cracked during any treatments in which water or its solutions are involved. For this reason, the first step of this project was to establish a method that did not cause any fissures in rice. The aromatising process that used liquid carbon dioxide as carrier needed a high pressure of 8 MPa and 5 minutes equilibration time to obtain optimum quality for the aromatised rice. Five minutes equilibration period produces insignificant physical changes (see Figures 2.15 and 2.16), and better aroma retention (see Figures 2.12 and 2.13) in aromatised rice than the other methods used in this project, such as using air and carbon dioxide gas as carriers, coating with gum Arabic, or sprinkling with liquid aroma compound.

We were initially interested in using the aroma compound 2-acetyl-1-pyrroline, which is present in many popular, high-value fragrant rices. However, this compound is relatively unstable and difficult to work with, and does not appear to be present in uncooked fragrant rice. Accordingly, a range of different model
compounds of varying molecular structures and volatilities, as summarised in Table 2.2, was used; for example, acetophenone, 2-acetylpypridine, 2-phenylethanol, toluene and 2-hexanone. These compounds were not chosen for their pleasant aromas, and indeed some were later discarded owing to safety hazards associated with their use. However, they had a range of useful structural features, related to those of 2-acetyl-1-pyrroline, that may be involved with binding to rice. Those aroma compounds that were mentioned above were not used in the next series of experiments, but were replaced by eugenol, cinnamyl alcohol, isoeugenol, methyl eugenol and cinnamaldehyde. These compounds were chosen due to their odours and functional groups (see Table 2.4). For example, eugenol (cloves) was first selected due to its pleasant odour, and the presence of an aromatic ring and a hydroxyl group. Later studies, reported in Chapter 5, evaluate the effects that the functional groups in aroma compounds have on interactions with rice components.

A number of preliminary experiments were carried out to establish the optimum conditions for aromatising rice using liquid carbon dioxide, including the optimum equilibration period for rice in liquid carbon dioxide, the optimum injected aroma volume, and the degree of milling of the rice. An equilibration period of 5 minutes for rice in liquid carbon dioxide gave acceptable aromatisation and did not produce observable (and hence undesirable) physical changes to the original rice.

Increased volumes of injected aroma compound caused an increase in aroma absorption. However, for injected volumes over 10 μL the grains took on a visibly wet appearance, which might be the result of grains reaching the limit of the amount
of compound they could absorb in 5 minutes. Aroma volumes of up to 10 μL were satisfactory for aromatising 2-g samples of rice.

Choosing the optimum degree of milling of rice as an absorbent for the injected aroma compound is important, since it affects the amount of aroma compounds that can be absorbed, and also the appearance and consumer perceptions of the rice. It was speculated that lipid could increase the aroma absorption. Lipid associates with the outer layers of rice grains, therefore a number of degrees of milling were evaluated. The results showed that the degree of milling of rice did not significantly affect aroma absorption. A 15% degree of milling of rice was used in this project because it offered a white appearance which was preferable for most rice consumers.

The presence and distribution of aroma compounds in rice grains were studied by fluorescence microscopy, solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS), Fourier Transform Infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The SPME and FT-IR methods successfully showed the presence of the injected aroma compounds in the rice cores, but the other methods were unsuccessful for a variety of reasons. These results established that liquid carbon dioxide allows the injected aroma compounds to penetrate the rice. It was hypothesised that the aroma compounds in the rice cores would take longer to migrate towards the surface of the rice grains and be lost to the air.
The determination of aroma concentrations in aromatised rice involved aroma extraction and aroma analysis. Two methods were evaluated: methanol extraction coupled with gas chromatography, and headspace gas chromatography. These methods were chosen as neither required a complicated process of sample preparation which could cause aroma loss during the extraction. Furthermore, they were less time consuming than other methods such as steam distillation and Likens-Nickerson simultaneous distillation. Since five replicates were used for most studies, a relatively rapid method was required. It was found that methanol extraction coupled with gas chromatography offered better results as it does not involve the application of heat that could cause aroma loss or degradation during sample preparation, and was more effective at extracting the aroma compounds. Overnight soaking of the samples in methanol solution, followed by stirring for 2 hours was found to be acceptably (though not completely) effective at extracting the injected aroma compounds.

The final aim of our experimental program was to obtain the best possible aroma retention in the aromatised rice. Ideally, aroma compounds should not be easily lost during storage, but should be readily released during preparation or chewing of the cooked aromatised rice, so that rice consumers can detect them. To achieve this aim, experiments were carried out to determine the reasons why some aroma compounds are more easily lost than others. These included experiments to detect the presence of aroma compounds in the rice cores (Section 2.4.3), measure the rates of aroma loss (Chapter 3) and aroma diffusivities (Chapter 4), the development of rice-flour chromatography columns, the measurement of complexing indexes for aroma compounds and amylose (Chapter 5), and the measurement of
injected aroma compounds in cooked aromatised rice (Chapter 6). The results of these experiments were correlated with each other to establish, among other conclusions, factors that affect the extent to which certain aroma compounds are retained by rice.

Factors such as the vapour pressures (see Section 3.4), physicochemical properties of the injected compounds (see Section 4.4.2) and the pore dimensions of whole rice grains and rice flour (see Sections 3.4 and 5.3.1) were considered as possible factors that could affect aroma retention. The results and discussions of these experiments can be seen in Chapters 3 to 6.
Chapter 3

Kinetic Study of Aroma Loss in Aromatised Raw Rice

3.1. Introduction

Aroma compounds, which generally have very low concentrations in food products, usually have no particular nutritive value. However, they have attracted the attention of a number of researchers, since aroma is important to product acceptability. Aromas, which are volatile compounds, tend to be labile and susceptible to thermal processing. One aspect of food-product acceptability is the desire of consumers for the release of aromas prior to eating or during masticating. The loss of aroma compounds during storage of raw rice is undesirable, but on the other hand, aroma release from cooked rice is desirable in order to give a pleasant odour before and during eating.

As mentioned in Section 2.4.1, it is not worth producing aromatised food if the aroma is too easily lost. Preliminary experiments on the stability of aromatised rice containing eugenol, isoeugenol, methyl eugenol, cinnamaldehyde and cinnamyl alcohol have been described in Section 2.4.1. Experiments providing detailed measurements and analysis of the rate of aroma loss from uncooked rice for each of the aroma compounds are presented in this chapter, and experiments on the release of aromas by cooked rice will be discussed in Chapter 6.
The major volatile compounds that contribute to the aroma of rice have been identified and quantified by a number of researchers, as reviewed in Section 1.3. Yajima *et al.* (1978, 1979), Tsugita *et al.* (1980), and Tava and Bocchi (1999) studied the aroma volatile compounds that were released by cooked rice, while Fujio *et al.* (1991) observed the changes in the aroma of fresh and stored cooked rice during warm-keeping. However, none of these published papers reported the rate of aroma loss by uncooked rice, for example, during storage. A study of the kinetics of aroma loss by raw aromatised rice may provide valuable information to the food manufacturer about packaging and potential shelf life of the product. The rate of aroma loss can also be used to evaluate the strength and modes of binding of different aroma compounds to rice. Based on the relative rates of aroma loss for the aroma model compounds that were used, we can assess the various factors that may affect the retention of these compounds. These results can be used for identifying other aroma compounds with different odours that are likely to be retained well in rice, producing a diversity of aromatised rice products. Of course, the right choice of aroma compound to be injected into the rice also depends on its sensory characteristics.

This chapter discusses the kinetics of the loss of eugenol, isoeugenol, methyl eugenol, cinnamaldehyde and cinnamyl alcohol from raw aromatised rice. Factors that might affect the rate of loss, such as vapour pressure, are also discussed to evaluate the strength and mode of the binding between the injected aroma compounds and rice.
3.2. Materials and Methods

Eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde were used as model aroma compounds due to their pleasant odours and range of functional groups, as mentioned in Section 2.4.1. 10-μL samples of each aroma compound in liquid carbon dioxide were used for aromatising 2-g rice samples using the procedures described in Section 2.3.3. 10 μL was assumed to be the maximum injected volume to be used for processing the rice, since higher volumes produced “wet” samples of aromatised rice. The maximum injected volume was used in these experiments, as the aromatised rice was exposed to the open air at 21°C for four days, and it was expected that much of the aroma compounds would be lost under these conditions. Aroma levels were measured every hour for 12 hours exposure time, then every 12 hours until 96 hours had been reached. There were five replicates for each measurement. The aroma concentrations in the rice samples were measured using methanol extraction coupled with gas chromatography (GC), as described in Section 2.3.3.

Freshly prepared 2-g samples of aromatised rice were placed in open petri-dishes at room temperature (21°C). Aroma concentrations were measured as described above, or until the aroma compound could no longer be detected by the methanol extraction/GC analysis.
3.3. **Analysis of the Rate of Aroma Loss**

The general equation describing the rate of loss of a food component (*e.g.* an aroma) during a period of time can be expressed as follows (Labuza and Riboh, 1982):

\[
- \frac{dC}{dt} = k \ C^n
\]  

(1)

Where:  
\( C \) = a component concentration; an aroma in these experiments, expressed as mg/100 g rice;  
\( t \) = time; namely the exposure time (hours) of the aromatised rice to the open air at room temperature (21°C);  
- \( dC/dt \) = rate of change of aroma concentration with the exposure time;  
\( k \) = a pseudo-rate constant;  
\( n \) = reaction order.

If \( n = 0 \) (zero-order reaction), equation (1) collapses to:

\[
- \frac{dC}{dt} = k
\]  

(2)

The integral of equation (2) yields:

\[
C - C_0 = k(t - t_0)
\]  

(3)

Where: \( C_0 \) = the concentration at time zero \( (t_0) \).
Equation (3) indicates that there is a linear relation between concentration and time, where the rate constant is the slope of the straight line. If \( n = 1 \) (first-order reaction), equation (1) becomes:

\[
- \frac{dC}{dt} = k \ C
\]

and the integral of equation (4) yields:

\[
\ln C = \ln C_0 - k(t - t_0)
\]

A plot of the logarithm of the concentration versus time yields a straight line, where the rate constant is the absolute value of the slope of the straight line. In contrast to the zero-order reaction, the rate of a first-order reaction depends on the concentration of the reactant. Many reactions follow either zero or first-order kinetics (Labuza and Riboh, 1982), and first-order kinetics adequately describe the degradation of a component in many food systems (Saguy and Karel, 1980); for example thiamine degradation by heat (Mulley et al., 1975), drip loss, cooking loss and colour degradation in frozen ground beef during storage (Bhattacharya, 1989).

We expected that, to a first approximation, the rate of aroma loss would follow first-order kinetics, since it seemed likely that the rate of the aroma loss would depend on the aroma concentration in the aromatised rice. This hypothesis was based on our observations of moisture loss from food systems. Larger concentrations of moisture in food result in longer times for all the moisture to be lost to the surroundings, for example during drying. We note in passing that moisture loss from
a food matrix is often accompanied by the loss of other components, such as aroma compounds. If there were substantial amounts of aroma compound in a rice grain, once the aroma compound that occupied the outer layer was lost to the air, it could in principle be replaced readily since there was enough aroma compound in the rice to replace the lost aroma. With smaller amounts of compound, less would be available to migrate towards the surface to replace the lost compound.

The injected aroma compound was found to be in the rice cores after the 5-minute equilibrating period in liquid carbon dioxide (see Section 2.4.3). Unfortunately, we were unable to determine whether or not the aroma compound was distributed evenly in the rice grain. However, the distribution of the compound in rice was assumed to be homogenous for the sake of the aroma diffusion study (Chapter 4). During the aromatising process, the injected aroma compounds could bind to components of the rice, then the compounds would be lost from the aromatised rice through the rice surface and pores. The dynamic exchange of the aroma molecules between the rice and its surroundings would result in a decrease in aroma concentration in the rice until the concentrations of the two reached an equilibrium. Due to the exposure of the aromatised rice to the open air, and the loss of the released aroma vapour, this equilibrium cannot be reached, and the rice continuously loses its aroma. The outward movement of the aroma molecules depends on their diffusion coefficient and strength of the binding to starch. The physicochemical properties of the aroma molecules which might affect aroma diffusion and the strength of binding are discussed in Section 4.4.2.
3.4. Results and Discussion

Kinetic studies were carried out to determine the kinetic orders and rate constants of the aroma loss from aromatised rice containing eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde. The freshly prepared samples were exposed to ambient air at 21°C, and their aroma concentrations were measured over periods of up to four days. Preliminary studies showed that some aroma compounds could still be detected by methanol/GC analysis after 72 hours (see Figure 2.26). We intended to carry out further measurements after a longer period of time, namely 96 hours, but some aroma compounds were no longer detectable using methanol extraction/GC analysis after this period. For example, methyl eugenol and cinnamaldehyde were barely detectable after 48 and 10 hours exposure time, respectively, whereas eugenol, cinnamyl alcohol and isoeugenol-aromatised samples were still detectable after the full 96 hours. We note that the undetectability of an aroma compound did not indicate that it was completely absent from the sample. Other factors that could have affected the detection of an aroma include the effectiveness of the aroma extraction technique, and the sensitivity of the method of aroma analysis. The GC detection limit depends, among other factors, on temperature, split ratio, gas flow rate and the type of column and aroma compound. However, to obtain data points that were comparable, the gas chromatograph was maintained under the same conditions throughout the experiments for all aroma compounds, and conditions were not varied in an attempt to increase sensitivity for individual samples.

Each of the aroma concentrations reported in this kinetic study was the mean of five replicates. Each sample was analysed immediately after each exposure time
(up to 96 hours) had elapsed to avoid further aroma loss afterwards. These were
time-consuming experiments, but replication was essential, given the unavoidable
scatter in the experimental data. Fortunately, the methanol extraction coupled with
the gas chromatography method, as illustrated in Figure 2.30, was relatively simple,
and therefore the five replicates could be analysed at the same time.

All results of the kinetic measurements were plotted as logarithms of the
aroma concentrations (mg/100 g rice) against the exposure times. As discussed
earlier, these graphs should be linear for first-order aroma loss. Graphs of aroma
concentrations against time were also plotted, and used in the evaluation of the
kinetic order. Lines of best fit were drawn through each set of data, where
appropriate, and error bars corresponding to standard deviations from five replicates
were also drawn.

The logarithmic plots for eugenol (Figure 3.2), isoeugenol (Figure 3.4),
cinnamyl alcohol (Figure 3.6), methyl eugenol (Figure 3.8) and cinnamaldehyde
(Figure 3.10) produced reasonably straight lines, indicating that the aroma loss from
the aromatised rice, as expected, followed first-order kinetics to a fair approximation,
as formulated in equation (2) in Section 3.3. The determination of the reaction order
was carried out by evaluating both logarithmic and non-logarithmic plots of the
aroma concentrations against the exposure times. For example, the plot of eugenol
concentration against time (Figure 3.1) is curved, confirming that aroma loss is not a
zero-order process.
For eugenol, the logarithmic graph as shown in Figure 3.2 yielded two linear regions: a sharp decline during the first 24 hours, and a slower decline thereafter. This is different from other plots, as it clearly shows two different rates of eugenol loss, with the faster rate of loss occurring during the first 24 hours exposure (first-order rate constant = 0.055 ± 0.008 hr⁻¹) and the slower rate after 24 hours (rate constant = 0.0035 ± 0.0013 hr⁻¹). These two different rates of eugenol loss indicate that there might be two binding modes (i.e., two ways in which the aroma can bind to the rice). So far, we do not fully understand what caused the two distinctive binding modes for eugenol. We speculated that there were factors, such as vapour pressure and the physicochemical properties of the aroma compounds (see Section 4.4.2), which might affect the eugenol loss from rice. The possible relationship between vapour pressures and rate constants is discussed later in this section.

Figure 3.1. Eugenol concentration in aromatised rice during 96 hours exposure to ambient air.
Figure 3.2. First-order kinetic plot of eugenol loss from rice.

10 µL of liquid eugenol (equivalent to a mass of 10.7 mg, see Section 2.3.3 for the calculation) was used to aromatise a 2-g rice sample, which corresponds to 535 mg of eugenol in 100 g rice. As shown in Figure 3.1, there was 257 mg of eugenol in 100 g of aromatised rice before it was exposed to the air, indicating that approximately 50% of the injected eugenol was absorbed by rice. The eugenol retention was reduced to half (120 mg/100 g rice) after being exposed to air for one hour. The concentration at 24 hours exposure was 9 mg/100 g, after which it decreased slowly until it reached 5 mg/100 g at the end of the kinetic study (96 hours).
Figure 3.3. Isoeugenol concentration in aromatised rice during 96 hours exposure to ambient air.

Figure 3.4. First-order kinetic plot of isoeugenol loss from rice.
Figures 3.3 and 3.4 show the non-logarithmic and logarithmic plots of isoeugenol loss, respectively. The data points in Figure 3.3 produce a curve, while the data points in Figure 3.4 fit a straight line. For the reasons described earlier for the loss of eugenol from aromatised rice, it was established that the isoeugenol loss is a first-order process. Unlike the graph in Figure 3.2, only one binding mode was positively identified for isoeugenol in rice, as the rate of aroma loss was relatively constant throughout the 96 hours exposure (rate constant $= 0.0137 \pm 0.0009$ hr$^{-1}$). The possibility that there may be a second binding mode is evaluated in Section 4.4.1. Using the same procedures for calculating the amount of eugenol loss as described above, it was found that approximately 541 mg of liquid isoeugenol was available for absorption by a 100-g rice sample during the aromatising process. As shown in Figure 3.3, 100 g of aromatised rice contained approximately 154 mg isoeugenol before it was exposed to the air which means that 29% of the injected isoeugenol was absorbed. Although the ability of the rice to absorb isoeugenol was lower than for eugenol, the rate of isoeugenol loss was lower during the first 24 hours; the rate constant of $0.0137 \pm 0.0009$ hr$^{-1}$ compares with $0.055 \pm 0.008$ hr$^{-1}$ for eugenol. After 96 hours only 8 mg/100 g remained; this amount was slightly higher than the amount of eugenol (approximately 5 mg/100 g). The rate constants of the five model aroma compounds and the capability of rice to absorb the compounds are summarised in Table 3.1. Although isoeugenol and eugenol are isomers, the rates of their losses were significantly different and we speculated that factors such as vapour pressure may contribute to this difference, as discussed later in this section.

Figures 3.5 and 3.6 show the non-logarithmic and logarithmic plots of cinnamyl alcohol loss. The graphs were similar to those for isoeugenol and the
aroma loss also followed first-order kinetics (rate constant = 0.0163 ± 0.0014 hr⁻¹). Although five replicates were used in the experiments, some scattered data occurred during the first 12 hours, as shown in Figure 3.5. However, the overall trends for aroma loss were clear. Cinnamyl alcohol loss appeared to be relatively slow, and there is tentative evidence for two binding modes, as for eugenol. However, since only one data point was available for the measurements after 72 hours, this result is not conclusive, and we did not plot two straight lines as was done for eugenol in Figure 3.2. If the last data point at 96 hours exposure were ignored, the rate constant for cinnamyl alcohol loss would be 0.0203 ± 0.0015 hr⁻¹, which was slightly higher than the value for 96 hour period. Using the same procedures as for eugenol and isoeugenol, it was found that 30% of the injected liquid cinnamyl alcohol was absorbed by rice during the 5-minute aromatising process. This initial concentration was reduced to 70% of the original value (50 mg/100 g rice) after 24 hours. The concentration of cinnamyl alcohol that was detectable by GC after 96 hours was approximately 8 mg/100 g rice; similar to the value for isoeugenol.

Table 3.1. Rate constants and percentages of eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde that were absorbed by rice.

<table>
<thead>
<tr>
<th>Aroma compounds</th>
<th>Rate constant for aroma loss (hr⁻¹)</th>
<th>Amount of aroma that was absorbed from its initial concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>0.055 ± 0.008* and 0.0035 ± 0.0013**</td>
<td>48</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>0.0137 ± 0.0009</td>
<td>29</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>0.033 ± 0.004</td>
<td>31</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>0.0163 ± 0.0014</td>
<td>33</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>0.206 ± 0.014</td>
<td>38</td>
</tr>
</tbody>
</table>

* during the first 24 hours of exposure;
** after 24 hours exposure.
Figure 3.5. Cinnamyl alcohol concentration in aromatised rice during 96 hours exposure to ambient air.

Figure 3.6. First-order kinetic plot of cinnamyl alcohol loss from rice.
The non-logarithmic and logarithmic plots of methyl eugenol and cinnamaldehyde loss are shown in Figures 3.7 to 3.10. Unlike the earlier cases, both of the aroma compounds were undetectable by GC after 96 hours exposure. Measurements for methyl eugenol ceased at 48 hours while cinnamaldehyde measurements ceased at 10 hours. Although the aromas could not be detected by GC, it did not mean that their concentrations were zero. As discussed earlier in this section, there are limitations on the ability of the methanol extraction and GC methods to extract and detect the aromas. In cases where no peak for an aroma compound appeared in the chromatogram, this was taken as an indication that the concentration was below the detection limit. The loss of methyl eugenol and cinnamaldehyde followed first-order kinetics as did the other compounds.

The rate constant for methyl eugenol loss was found to be $0.033 \pm 0.004 \, \text{hr}^{-1}$. The ability of rice to absorb methyl eugenol was similar to that of cinnamyl alcohol: about 30% of the injected liquid aroma concentration. 9 mg/100 g rice remained after 24 hours (the same as for eugenol), but only 4 mg/100 g was retained after 48 hours — less than for eugenol, cinnamyl alcohol and isoeugenol, over the same period of time. The results indicate that methyl eugenol did not bind better to rice than the other compounds. In contrast to eugenol, there was no evidence for a second mode of aroma binding. This does not rule out the possibility of such a mode, but aroma levels were below detection limits for the later stages of aroma loss.
**Figure 3.7.** Methyl eugenol concentration in aromatised rice during 96 hours exposure to ambient air.

**Figure 3.8.** First-order kinetic plot of methyl eugenol loss from rice.
Figure 3.9. Cinnamaldehyde concentration in aromatised rice during 96 hours exposure to ambient air.

Figure 3.10. First-order kinetic plot of cinnamaldehyde loss from rice.
As shown in Figure 3.10, cinnamaldehyde was undetectable by gas chromatography after 10 hours exposure. The rate constant for aroma loss was 0.206 ± 0.014 hr⁻¹, which was higher than the other rate constants in these experiments. The cinnamaldehyde concentrations were similar after 8 and 10 hours of exposure, but the 12-hour value was taken to be zero; we could not draw two convincingly linear regions as we did for the eugenol. When data points for the cinnamaldehyde loss were plotted only up to 8 hours exposure, a rate constant of 0.232 ± 0.008 hr⁻¹ was obtained.

These kinetic studies show that the rate constants for aroma loss vary with the aroma compounds that were used. It was hoped that it would be possible to evaluate the factors that affected the rate of loss of particular aroma compounds; these factors included vapour pressure and other physicochemical properties such as polarities, log $P$ (partition coefficient between $n$-octanol and water), molecular weight and the functional groups of the aroma compounds, as well as the pore volume and area of the rice grains. As discussed in Section 4.4.2, amylose was initially thought to be the most likely component in rice starch for the aroma compounds to bind to; this view was based on earlier studies by other workers. Therefore, we tried to aromatise amylose and amyllopectin powder using the procedure as described in Section 2.3.3, to evaluate the strength of the interactions between the aromas and the different types of starch. Unfortunately, these experiments were unsuccessful because the amylose and amyllopectin powders were very difficult to work with. They were easily dispersed by the high-pressure liquid carbon dioxide, and tended to stick to the inner wall of the chamber (see Figure 2.1). As a consequence, it was very difficult to collect satisfactory samples for use in aroma analysis. To circumvent these
difficulties, amylose, amylopectin and rice-flour gas chromatography columns were developed to evaluate the degree of the interaction between the injected aroma compounds and rice components, as discussed in Section 5.3.1.

As indicated above, attempts were made to correlate the rate constants for aroma loss with the vapour pressures of the aromas. We first expected that the aroma compounds with higher vapour pressures would be more rapidly lost. However, as discussed below, the results did not demonstrate that this was the case. The vapour pressures of eugenol, isoeugenol, cinnamyl alcohol and cinnamaldehyde at 21°C were extrapolated from the available experimental vapour-pressure data (Jordan, 1954), using the Clausius-Clapeyron equation (Grain, 1990) as described in Section 2.4.1. As there are no published data for the vapour pressure of methyl eugenol at 21°C, it was calculated using the Antoine equation (Grain, 1990) (see Section 2.4.1), and the parameters for methyl eugenol as listed in Table 2.4. The vapour pressures of eugenol, isoeugenol, cinnamyl alcohol and cinnamaldehyde were calculated using the same equation in order to establish the accuracy of the method by comparing the calculated and experimental vapour pressures. The results are summarised in Table 3.1, with the values showing that the calculated values were 30-50% lower than experiment. The difference (%) between the calculated and experimental methyl eugenol vapour pressures was taken to be the mean of the values for eugenol and isoeugenol (approximately 38%). Therefore, the experimental vapour pressure of methyl eugenol was estimated to be 100/62 of its calculated vapour pressure (0.065 mmHg), resulting in 0.105 mmHg. The calculated vapour pressure of methyl eugenol was much higher than that of the other compounds. Based on the equations that were used for calculating the vapour pressures (see Section 2.4.1), it can be seen
that the boiling point of methyl eugenol, which was lower than that of the other compounds tested, led to the higher vapour pressure.

The vapour pressures from Table 3.2 were graphed to show the relationship between the rate constants of aroma loss and the corresponding vapour pressures, as shown in Figure 3.11. The error bars for the rate constants correspond to one standard deviation. The rate constant for eugenol loss during the first 24 hours was used, rather than the much lower value for the 24 to 96 hours period. Figure 3.6 shows that aroma compounds with higher vapour pressures do not always have a higher rate constant for aroma loss; indeed the correlation is very poor. For example, cinnamyl alcohol had a higher vapour pressure but a lower rate constant than eugenol. These results indicate that there are other important factors that affect the rates of aroma loss from the aromatised rice.

**Table 3.2.** Calculated and extrapolated experimental vapour pressures of eugenol, isoeugenol, cinnamaldehyde, cinnamyl alcohol and methyl eugenol at 21°C.

<table>
<thead>
<tr>
<th>Aroma compounds</th>
<th>a Calculated vapour pressure at 21°C (mm Hg)</th>
<th>b Extrapolated experimental vapour pressure at 21°C (mm Hg)</th>
<th>Difference between a and b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>0.015</td>
<td>0.022</td>
<td>32</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>0.007</td>
<td>0.013</td>
<td>46</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>0.018</td>
<td>0.037</td>
<td>51</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>0.019</td>
<td>0.034</td>
<td>44</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>0.065</td>
<td>d 0.105</td>
<td>c 38</td>
</tr>
</tbody>
</table>

a The calculated vapour pressures used the Antoine equation (Grain, 1990).

b The extrapolated vapour pressures of the experimental vapour pressures that were obtained from Jordan (1954). The extrapolation used the Clausius-Clapeyron equation (Grain, 1990).
Table 3.2 continued.

c The difference was assumed to be the mean of the eugenol and isoeugenol differences.

d Calculated as 100/62 of 0.065 mm Hg.

![Graph showing rate constant for aroma loss and vapour pressure for various substances.]

* 0-24 hours exposure time

Figure 3.11. The relationship between rate constants for aroma loss of cinnamaldehyde, eugenol, methyl eugenol, cinnamyl alcohol and isoeugenol and the corresponding vapour pressures.

Other experiments were carried out to determine the factors that might affect the rate constant of the aroma loss from rice. These included the measurement of rice-pore volumes (see below), gas-chromatographic studies using a rice-flour column (see Section 5.3.1), and studies of complexes between amyllose and aroma compounds using UV-visible absorption spectrophotometry (see Section 5.3.2). As mentioned in Section 1.4, 80% of a rice grain consists of starch and might therefore
provide a matrix for aroma compounds to bind to. The possible physicochemical
characteristics that may influence the binding between aroma compounds and rice
starch, and hence the rate of aroma loss, are evaluated in Section 4.4.2. These
include molecular weight, polarity, hydrophilic-lipophilic index, and potential to
form hydrogen bonds (see Table 4.2). These properties are compared with the
experimental results in this project, namely the rate constants for aroma loss, the
retention times of the aroma compounds in rice-flour and starch gas-chromatography
columns, and complexing indexes for amylose and the aroma compounds. Some
possible binding sites between amylose and aroma compounds are proposed, as
reported in Section 4.4.2.

The measurement of the rice-pore volumes and entrance diameters was
conducted for both whole rice grains and rice flour (see Section 5.3.1) using a
mercury Micromeritics poresizer 9310 (Particle and Surface Sciences, Gosford,
NSW). The rice samples were heated to 60°C and exposed to flowing helium gas for
72 hours to remove any contaminants and reduce inherent moisture prior to analysis.
The poresizer operates on the principle that liquid mercury, being essentially non-
wetting, will not penetrate rice pores by capillary action unless forced to do so by
applied pressure. The relationship between applied pressure and pore diameter is
described by the Washburn equation (equation 6) (Hiemenz and Rajagopalan, 1997).
Methods that use the principle of the penetration of liquids (such as liquid mercury)
into porous solids can be applied in a variety of ways, such as in the measurement of
pore dimensions (Danino and Marmur, 1994: Marmur and Cohen, 1997). In the
present study we are interested in the distribution of pore sizes of a solid rice grain.
\[ D = \frac{-4 \gamma \cos \theta}{P} \]  

(6)

Where:  
\( D \) = pore diameter (\( \mu \)m);  
\( \gamma \) = mercury surface tension (dynes/cm);  
\( \theta \) = contact angle (degrees); angle between liquid mercury and rice grain  
(130\(^\circ\)) (Particle and Surface Sciences, Gosford, NSW);  
\( P \) = applied pressure (psi).

There were 45 applied pressures in the range from 0.6 to 30000 psi, with gradual increments of the applied pressure. For data-analysis purposes the pores in the rice grains were assumed to be cylindrical, although they have been shown to be irregular (Rani and Bhattacharya, 1997). Mathematical modelling of irregular pore shape is very difficult, so in liquid mercury porosimetry, pore shapes for a solid matrix are generally taken to be cylindrical (Hiemenz and Rajagopalan, 1997), noting that the mathematical model (i.e., the Washburn equation) is straightforward when this assumption is used. The intrusion of mercury into a cylindrical model pore void requires the application of a pressure that is inversely proportional to the diameter of the pore opening (see equation (6) above). Incremental pressure increases will force the mercury to fill progressively smaller pore diameters and, when these are plotted against the pore diameters, will characterise the porosity of the sample.

These experiments aimed to determine whether or not the rates of aroma loss from aromatised rice correlated with the relationship between the molecular
dimensions of the aroma compounds and the pore volumes and diameters of the rice. Because whole rice grains were used in the kinetic studies, the whole-grain rice-pore data were used for comparison. Rice-flour pore volumes and entrance diameters were used to determine whether there was a correlation between these quantities and the retention times of aroma compounds in a rice-flour gas-chromatography column, as discussed in Section 5.3.1.

The smallest measurable pore diameter for whole rice grains was $6 \times 10^{-3} \mu m$ (6 nm) and the largest pore diameter was 300 $\mu m$. The cumulative pore volume plot for whole rice grains (Figure 3.12) shows that a large proportion of the total pore volume derived from the pores with a small diameter. The cumulative percentage pore volume can be seen in Figure 3.13, plotted as a percentage against pore diameter. The graph shows that only 10% of the total pore volume is given by pores with diameters of more than 60 $\mu m$. To determine whether the compounds could fit into the pore entrances, and also because most of the total pore volume derived from pores with a small diameter, we compared the smallest measurable pore diameter in a whole rice grain to the molecular dimensions of the injected aroma compounds.
Figure 3.12. Cumulative pore volume for whole rice grains.

Figure 3.13. Cumulative percentage pore volume for whole rice grains.
WindowChem software (Molecular Modelling Pro, revision 2.4) was used to calculate the molecular dimensions of eugenol, isoeugenol, methyl eugenol, cinnamaldehyde and cinnamyl alcohol. Molecular structures, as shown in Table 2.4, were used as input geometries, which were then optimised by minimising the strain energy in the molecules, to obtain stable conformations. The molecular dimensions were calculated using the dimensions function, which is based on Van der Waals radii. The dimensions are presented in Table 3.3.

**Table 3.3.** Calculated molecular dimensions of eugenol, isoeugenol, methyl eugenol, cinnamaldehyde and cinnamyl alcohol.

<table>
<thead>
<tr>
<th>Aroma compounds</th>
<th>Dimensions (nm)</th>
<th>*Maximum and minimum dimensions (nm)</th>
</tr>
</thead>
</table>
| Eugenol          | Width = 0.85  
                  Length = 1.10  
                  Depth = 0.51 | Maximum = 1.10  
                  Minimum = 0.48 |
| Isoeugenol       | Width = 0.85  
                  Length = 1.13  
                  Depth = 0.54 | Maximum = 1.13  
                  Minimum = 0.47 |
| Methyl eugenol   | Width = 0.85  
                  Length = 1.24  
                  Depth = 0.46 | Maximum = 1.26  
                  Minimum = 0.46 |
| Cinnamaldehyde   | Width = 0.61  
                  Length = 1.07  
                  Depth = 0.45 | Maximum = 1.07  
                  Minimum = 0.36 |
| Cinnamyl alcohol | Width = 0.64  
                  Length = 1.02  
                  Depth = 0.38 | Maximum = 1.02  
                  Minimum = 0.36 |

*Maximum and minimum dimensions were determined relative to the cartesian axes for the molecules.
All five compounds have similar dimensions, with methyl eugenol being slightly longer, and cinnamaldehyde and cinnamyl alcohol slightly shorter. The maximum dimension of these five aroma compounds was approximately 1.3 nm, whereas the minimum measurable pore-entrance diameter was 6 nm (i.e., five times larger). We cannot rule out the possibility of smaller pores that might discriminate between these molecules on the basis of size, but it appears likely that none of the aroma compounds used in the kinetic experiments had any difficulty entering the large number of available and measurable pores of the whole rice grains. Furthermore, the molecular dimensions of the model aroma compounds are calculated to be quite similar, so discrimination between them is more likely to result from their substantially different physiochemical properties than size selection.

The natural surface condition of rice grains has the potential to influence the interaction between the aroma compounds and rice components during the aromatisation process. The surface of the whole rice grains is porous with a distribution of different pore-entrance diameters (see Figures 3.12 and 3.13). The milling process, which involves the removal of the outer bran, causes the white milled rice to have a rough surface that facilitates the penetration of the grains by the injected aroma compounds. In the current project, the penetration was enhanced by the high-pressure liquid carbon dioxide which was used as a carrier. Hence, the injected aroma compounds that had potential to interact with the rice components could penetrate the grains.

The general conclusions that can be drawn from the results shown in Figures 3.1 to 3.6 are that (1) the rate constants of aroma losses (first-order reaction) vary
among the aroma compounds that were used, with cinnamaldehyde having the highest rate constant followed by eugenol (<24 hours exposure time), methyl eugenol, cinnamyl alcohol and isoeugenol, and (2) there were other factors than vapour pressures and pore sizes of whole grains that affected the rate constants for aroma loss from rice, such as the physicochemical properties of the aroma compounds which are discussed in Section 4.4.2.

3.5. Conclusion

The results of these time-consuming experiments on the kinetics of aroma loss for five model aroma compounds gave rate constants (see Table 3.1) that varied from 0.0035 ± 0.0013 hr⁻¹ for eugenol (after 24 hours exposure) to 0.206 ± 0.014 hr⁻¹ for cinnamaldehyde. These values can be used to evaluate the strength of the binding between the aroma compounds and rice. Larger values indicate a faster rate of aroma loss from aromatised rice, and hence weaker binding to the rice. An interesting aspect of this study was the clear evidence of two aroma-binding modes for eugenol, and suggestions that the same may apply for other aromas.

Based on the poor correlation between the vapour pressures and the rates of aroma loss, it was inferred that other factors also affect the rate of loss of aroma compounds. The pore dimensions of the whole rice grains were measured to determine whether they have an influence on aroma retention. The results showed that none of the aroma compounds were larger than the measurable pore entrance diameters, and the molecular dimensions of the aroma compounds were so similar that it seemed unlikely that they could be discriminated between by any smaller pores. Further experiments were carried out to determine the other factors that could
affect the rate of aroma loss: measurements of the rate of aroma diffusion within aromatised rice, and a discussion of possible binding sites between the aroma compounds and rice starch, are presented in Chapter 4, while Chapter 5 reports a series of experiments that were carried out with amylose, amylopectin and rice-flour gas chromatography columns, as well as studies of aroma compound and amylose complexes.
Chapter 4

Aroma Diffusion in Raw Aromatised Rice

4.1. Introduction

Aromatised foods are complex systems containing volatile compounds and non-volatile components that consist mainly of carbohydrate, water, protein and lipid. These major components can interact with and affect the activity of the volatile compounds in various ways, such as by entrapment, the formation of hydrogen bonds, and physical adsorption through hydrophobic bonds (Maier, 1970; Solms et al., 1973; Voilley et al., 1990). Aromatised food is also faced with the problem of aroma loss by diffusion, especially during storage or packing. This diffusion can occur over a short period of time, for example, during the “waiting period” for packing after the rice is processed. Although concentrations of aroma compounds are generally very low within a given food product, and are usually of no particular nutritive value, nevertheless they have major effects in influencing consumer choice and acceptance of foods.

There are two different problems in producing aromatised food. Firstly, during processing, packing or storage the aroma should be retained as much as possible. At this stage, additional components may be added, for example, modified starch to hold the aroma. Secondly, the food produced should release the aroma during serving and chewing in order that the aroma be detected by consumers. The challenge for food manufacturers, therefore, is first to develop aromatised food that remains stable until
consumed, and second to ensure that the aroma released during eating is perceivable by the consumer.

Aromatised rice is an example of an aroma-added product. The added aroma compounds in the raw rice should be retained as much as possible until the rice is consumed. In the present study, we optimised the aromatisation process as well as the amount of added materials in order to obtain an end product that was as close in physical appearance to the original raw rice as possible (which coincides with the initial aim of this project as mentioned in Section 2.1).

Raw milled rice was used in the preparation of the aromatised rice. As discussed in Section 3.4, the injected model aroma compounds had no difficulty entering the pores of rice grains, and due to the high-pressure liquid carbon dioxide used during the aromatisation process, the aroma compounds penetrated readily into the grains, as confirmed using SPME coupled with GC-MS and FT-IR spectroscopy (see Section 2.4.2). Furthermore, the aroma compounds that have advantageous physicochemical properties for the chemical interaction with rice, perhaps through interactions involving phenyl groups or the formation of hydrogen bond, will stay longer in the rice. However, as volatile compounds, the aromas can be readily lost to the air through diffusion and evaporation, where the loss can be enhanced due to the porous rice surface. All injected model aroma compounds have a similar opportunity to be lost to the air, but those that have weak binding to the rice are expected to diffuse at a higher rate. This chapter evaluates the diffusion rates for the model aroma compounds within a rice grain. In the simplest case, the rate of aroma diffusion (effective diffusivity) to the rice surface can be modelled using a
mathematical model as described below. It was expected that the effective
diffusivity values would be useful in so far as they reflect the binding strength
between different model aroma compounds and rice.

According to Lewis (1990), diffusion can be defined as the motions of particles
into their surroundings. Diffusion occurs from regions with higher concentrations to
regions of lower concentrations. The term *diffusivity* refers to the predisposition of
substances (in this case, aroma compounds) to diffuse. Studies of diffusivity have
been reported for rice, soybeans, peanuts and corn (Becker and Sallans, 1955; Fan
*et al.* 1961; Suarez *et al.* 1981; Chinnan and Young, 1977; Muthukumarappan and
Gunasekaran, 1994a, b, c; Steffe and Singh. 1980a, b, c). All of these studies
focussed on moisture diffusion in grains during drying, soaking or tempering.

In previous diffusion studies (Becker and Sallans, 1955; Steffe and Singh,
1980b, c; Aguerre *et al.*, 1982; Banaszek and Siebenmorgen, 1990; Lu and
Siebenmorgen, 1992), the moisture movement in grains was usually assumed to
occur by diffusion, and Fick’s Law was used to compute the effective diffusivities.
In any study of diffusion (e.g. liquid or vapour), the choice of appropriate boundary
conditions and grain shape for the theoretical model are very important. This is
because the aim in a diffusion study is to obtain as close a fit as possible between the
calculated and experimental data.

In diffusion, substances can diffuse into or out of the matrix. For example,
Banaszek and Siebenmorgen (1990) studied the moisture adsorption of rough rice to
show the rates of moisture diffusion into the rice grains, while Aguerre *et al.* (1982)
carried out experiments on drying rough rice grains to show the diffusion of moisture out of the grains. Most of the published literature, whether the moisture diffuses into or out from the grains, uses Fick's second Law as the basis for a mathematical model.

As mentioned above, the choice of the diffusion type (liquid or vapour) is important for obtaining a close match between the calculated and experimental data, although a combination of liquid and vapour diffusion can be applied where different types of diffusion dominate at different drying stages (Steffe and Singh, 1980b). As discussed by Chinnan and Young (1977), it is not clear whether the vapour or liquid concentration gradient is the true driving force in the transfer of moisture during drying grains, and the best solution to this problem is to make a comparison between the results of experiments based on vapour and liquid diffusion, respectively. These researchers carried out experiments on drying peanut pods, and found that both liquid and vapour models gave reasonably good fits to the experimental data. However, they found that vapour diffusion gave a better overall fit to the experimental data during the total drying period, whereas liquid diffusion yielded a better fit than vapour diffusion during the later stages of the drying period.

In our experiments, the aromatised rice was processed using liquid carbon dioxide at a pressure of 8 MPa for 5 minutes with an addition of pure liquid aroma compound (see Section 2.3.3). The rice was then removed from the chamber and placed on open petri dishes for aroma analysis. In this aromatisation process at least two diffusion processes occurred: firstly, during immersion in the liquid carbon dioxide, which carried the aroma compounds into the rice, and secondly, when the rice grains were placed on the petri dishes, and the aroma compounds diffused
towards the surfaces of the grains. The liquid carbon dioxide was assumed to evaporate totally during the "release" of the pressure when the samples were about to be taken out from the chamber, but the aroma compound remained in the rice grains, as shown in Section 2.4.3. In our diffusion studies we were primarily interested in the second diffusion process, which is related to the shelf life of the product, and the nature of the aroma-rice binding, as well as being accessible to experimental study. Unfortunately, we could not determine whether the aroma compound in the rice acted more like a gas or a liquid when it diffused, since we were not certain about how it was bound to the rice. In addition, unlike the data points for the peanut-pod drying study of Chinnan and Young (1977), which gave consistently smooth lines of best fit, the data for our aroma concentrations were scattered, and it was difficult to distinguish which of the vapour or liquid models gave the best fit to the experimental data. The diffusion of the aroma compound from a rice core towards the surface was assumed to be similar to the diffusion of moisture in grains during drying (mostly assumed by earlier workers to be liquid diffusion) as studied during the drying of rough rice grains (Aguerre et al. 1982); wheat kernels (Becker and Sallan, 1955); soybeans, wheat, whole peanuts and rough rice (Suarez et al., 1981). The liquid and vapour diffusion equations have different mathematical forms, but give similar results for moisture diffusion, as shown by Chinnan and Young (1977). We assumed that the diffusion of the aroma compounds in the rice grains towards the surface was a process of liquid diffusion, noting that the liquid diffusion model is mathematically straightforward, and we found that it gave a satisfactory fit to our experimental data. Our simple model of aroma diffusion in a rice grain is illustrated in Figure 4.1. We were unable (see chapter 2) to determine the distribution of aroma compounds within aromatised rice grains, but it was clear that the compounds had penetrated the grains,
and were able to diffuse through the grains on a timescale of from hours to days. Thus, as a working approximation, we assumed that the compounds were evenly distributed through the grains.

![Injected aroma compound](image)

A = rice core       B = rice surface

**Figure 4.1.** The diffusion of an aroma from a rice core towards the surface of the grain (assuming: rice grains are spherical; the distribution of aroma compound in the grain is homogenous).

Another factor that can affect the accuracy of diffusivity calculations is grain shape. Rice and other grains do not have simple shapes, such as spherical, cylindrical or slab-like, but irregular shapes that are difficult to model mathematically. In theoretical modelling, grains are mostly assumed to have the closest simple shape. In rice, for example, this simple shape is usually assumed to be spherical (Aguerre et al., 1982; Steffe and Singh, 1980a; Tutuncu and Labuza, 1996), while peanuts are mostly assumed to be cylindrical. Rice grains could also be assumed to be cylindrical, as stated by Engels et al. (1986), who modelled water diffusion during long-grain rice soaking.
In our study we used the conclusions of Suarez et al. (1981), who studied the effects of shape on a simple diffusion analysis. They compared two possible shapes to find out which gave the better fit to the experimental data. Figure 4.1 shows plots of predicted and experimental moisture ratios \(m^*\) against \(Dt/r^2\) (where \(D = \) effective diffusivity, \(t = \) time and \(r = \) radius of grain) for cylindrical and spherical models during drying of rice at 40.5°C. The graph that assumed rice grains to be spherical gave a better fit to the experimental data than the cylindrical model. Suarez et al. (1981) further reported that the intercept value or standard deviation from the predicted linear regression could be used to characterise the closest simple shape of a grain. This was done for cylindrical and spherical shapes, respectively. Graphs of \(\log m^*\) against \(Dt/r^2\) (see Figure 4.2) showed that the use of cylindrical and spherical shapes in the analysis yielded almost no observable differences between the predicted lines and their corresponding experimental data, suggesting that either shape gave satisfactory results. In published articles on moisture diffusion in rice grains, most researchers assumed that rice grains were spherical, where the mathematical model for a sphere is simpler than that for a cylinder. The model for cylindrical grains requires the use of Bessel functions (Engels et al., 1986; Crank, 1998), which make calculations significantly more difficult, with only a small (if any) improvement to the model. Therefore, we followed the precedent set by most researchers in this area and assumed that the rice grains in our experiments were satisfactorily modelled by spheres.

In summary, based on previous studies of moisture diffusion in rice grains (Steffe and Singh, 1980b,c; Banaszek and Siebenmorgen, 1990; Lu and Siebenmorgen, 1992), we have assumed that aroma diffusion in aromatised rice from
the rice cores towards the surface may be described by liquid diffusion, and rice grains may be assumed to be spherical.

\[ \text{RICE, } 40.5^\circ \text{C} \]

\[ \text{predicted, (cylinder)} \]
\[ \text{experimental} \]
\[ m^* \]
\[ Dt/ \]

(a)

\[ \text{RICE, } 40.5^\circ \text{C} \]

\[ \text{predicted, (sphere)} \]
\[ \text{experimental} \]
\[ m^* \]
\[ Dt/ \]

(b)

(a) = cylindrical grains
m* = moisture ratio
(b) = spherical grains

**Figure 4.2.** Comparison of predicted and experimental data in drying rice at 40.5°C.

Source: Suarez et al. (1981).
4.2. Materials and Methods

Eugenol, isoeugenol, methyl eugenol, cinnamyl alcohol and cinnamaldehyde were used as model aroma compounds. Freshly prepared aromatised rice (see Section 2.3.3) was exposed to open air, and placed in a single layer on a petri dish at room temperature (21°C). The data used for the calculation of the diffusion coefficients of aromas were obtained from the kinetic measurements, as reported in Chapter 3.

4.3. The Diffusion Equation

The diffusion model was based on Fick’s second law, which may be written as follows (Crank, 1998).

\[
\frac{\partial C}{\partial t} = D \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right)
\]  

(1)

Where:

\( C \) = aroma concentration (mg/100 g rice);

\( D \) = aroma effective diffusivity (mm/hour);

\( r \) = radial coordinate (mm);

\( t \) = time variable (hour).
In our diffusion analysis for injected aroma compounds in aromatised rice, the following assumptions were made: (1) the mechanism of aroma movement is liquid diffusion; (2) the white rice grain (the starchy endosperm) was spherical in shape; (3) the components of the starchy endosperm were homogenous and the aroma had reached an equilibrium distribution in the rice; (4) the shrinkage or swelling of the aromatised rice was negligible during aroma loss; (5) the temperature remained constant during exposure of the aromatised rice to open air; (6) the radius of each rice grain was half of the width of the rice grain; (7) rice grains in a sample were of uniform size and shape. Under these conditions, Fick's second law of diffusion was used to fit the experimental data for aroma concentrations in rice after it was exposed to open air.

If the initial aroma concentration \( (C_0) \) in aromatised rice was homogenous throughout the spherical grains, and the aroma concentration at all points on the surface of the grains was \( C_i \) after exposure time \( t \), then boundary conditions (2), (3) and (4) apply. Equation (1) can then be solved by the separation of variables method to yield equation (5) (Crank, 1998):

\[
\frac{\partial C}{\partial r} = 0, \quad r = 0, \quad t \geq 0 \tag{2}
\]

\[
C = C_i, \quad r = r_i, \quad t > 0 \tag{3}
\]

\[
C = C_0, \quad 0 \leq r \leq r_i, \quad t = 0 \tag{4}
\]

\[
C^* = \frac{C_i}{C_0} = \frac{2r_i}{\pi r} \sum_{n=1}^{\infty} \frac{(-1)^n}{n} \sin \left( \frac{n \pi r}{r_i} \right) \exp \left( - \frac{D n^2 \pi^2 t}{r_i^2} \right) \tag{5}
\]
Where:

\[ C_t = \text{aroma concentration in rice after } t \text{ hour(s) exposure to air (mg/100g rice);} \]

\[ C_0 = \text{initial aroma concentration in rice (mg/100g rice);} \]

\[ C^* = \text{ratio of } C_t \text{ and } C_0; \]

\[ t = \text{exposure time (hours);} \]

\[ r_t = \text{radius of sphere (half of the mean width of rice grains in mm);} \]

\[ r_t = 0.762 \text{ mm);} \]

\[ D = \text{aroma diffusivity (mm}^2/\text{hour).} \]

By integrating equation (5) with respect to the radius, \( r_t \), the equation for the diffusion analysis is as follows (Tutuncu and Labuza, 1996):

\[
C^* = \frac{C_t}{C_0} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( -\frac{D n^2 \pi^2 t}{r_t^2} \right) \tag{6}
\]

which may be expanded to give

\[
C^* = \frac{6}{\pi^2} \exp \left[ -\frac{D \pi^2 t}{r_t^2} \right] + \frac{3}{2\pi^2} \exp \left[ -\frac{4D \pi^2 t}{r_t^2} \right] + \ldots \ldots \ldots \tag{7}
\]

Following the example of Tutuncu and Labuza (1996) we have neglected the second and higher terms of the series expansion, as the first term gave a satisfactory fit to the experimental results. Hence:
\[ C^* = \frac{6}{\pi^2} \exp \left( -D\pi^2 \frac{t}{r_i^2} \right) \] (8)

and

\[ \ln C^* = \left( -\frac{D\pi^2}{r_i^2} \right) t + \ln \left( \frac{6}{\pi^2} \right) \] (9)

For each compound, values of \( \ln C^* \) were plotted against exposure time (\( t \)) as shown in Figures 4.3-4.7. A linear least-squares regression was used to calculate the gradient (with standard deviation, \( SD \)) of each graph. These values were used to calculate \( D \) and its \( SD \) for each aroma compound, where:

\[ \text{gradient} = -\frac{D\pi^2}{r_i^2} \] (10)

4.4. Results and Discussion

The effective diffusivity of aroma compounds in aromatised rice was calculated from data obtained during at least 24 hours exposure time to open air, except for cinnamaldehyde which was lost more rapidly. The graphs of \( \ln C^* \) against exposure time for the five aroma compounds are shown in Figures 4.3 through 4.7. Least-squares straight lines of best fit are shown on these graphs, and the gradients were used to calculate effective diffusivities (\( D \)). The results are summarised in Table 4.1.
Table 4.1. Effective diffusivity of cinnamaldehyde, cinnamyl alcohol, eugenol, isoeugenol and methyl eugenol.

<table>
<thead>
<tr>
<th>Aroma Compounds</th>
<th>Effective Diffusivity ($D$) mm²/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamaldehyde (8 hours)</td>
<td>0.0318 ± 0.0009</td>
</tr>
<tr>
<td>Cinnamyl alcohol (48 hours)</td>
<td>0.00300 ± 0.00018</td>
</tr>
<tr>
<td>Eugenol (≤ 24 hours)</td>
<td>0.0068 ± 0.0009</td>
</tr>
<tr>
<td>Eugenol (24-96 hours)</td>
<td>0.00044 ± 0.00008</td>
</tr>
<tr>
<td>Isoeugenol (≤ 24 hours)</td>
<td>0.0036 ± 0.0004</td>
</tr>
<tr>
<td>Isoeugenol (1-96 hours)</td>
<td>0.00180 ± 0.00009</td>
</tr>
<tr>
<td>Methyl eugenol (24 hours)</td>
<td>0.0063 ± 0.0009</td>
</tr>
</tbody>
</table>

The discussion of the aroma diffusion results in this chapter is divided into two sections. The first section discusses the effective diffusivities of aromas in rice; in Chapter 5 these values are correlated with retention times of the aroma compounds in a rice-flour chromatography column. The second section discusses the relationship between physicochemical properties of the aroma compounds and their possibilities for binding to rice starch.
Figure 4.3. Graph of $\ln C^*$ versus exposure time for cinnamaldehyde.

Figure 4.4. Graph of $\ln C^*$ versus exposure time for cinnamyl alcohol.
Figure 4.5a. Graph of $\ln C^*$ versus exposure time ($\leq 24$ hours) for eugenol.

Figure 4.5b. Graph of $\ln C^*$ versus exposure time (24-96 hours) for eugenol.
Figure 4.6a. Graph of $\ln C^*$ versus exposure time ($\leq 24$ hours) for isoeugenol.

Figure 4.6b. Graph of $\ln C^*$ versus exposure time (1-96 hours) for isoeugenol.
Figure 4.7. Graph of $\ln C^*$ versus exposure time for methyl eugenol.

4.4.1. The Effective Diffusivity of Aromas in Rice

Figure 4.3 represents the linear regression fit of $\ln C^*$ for cinnamaldehyde in rice plotted against exposure time; the fit is clearly very good. The effective diffusivity of cinnamaldehyde was only calculated for 8 hours exposure time, since the graph levelled out, and the compound was undetectable by gas chromatography after 10 hours exposure (see Figure 4.8). We note that Figure 4.8 has the same general behaviour as Figure 3.10, so rather than drawing additional graphs of $\ln C^*$ versus time for the remaining aroma compounds, we will refer back to the comparable Figures in Section 3.4. The effective diffusivity of cinnamaldehyde was the highest of the aroma compounds used in this research, and this higher value indicates that it was not well retained in rice. This result correlates with experiments on gas chromatography using a rice-flour column, as discussed in Chapter 5.
Figure 4.4 shows a satisfactory line of best fit for cinnamyl alcohol (up to 48 hours), although the data points in the figure show a fair amount of scatter. As mentioned in Section 3.2, there were five replicates in each aroma measurement. Thus, as was the case for all of the model compounds, each diffusion data point on the graph derived from the means of 5 replicates. Some aroma diffusion plots show a significant amount of scatter, but we were still able to draw general conclusions about aroma diffusion in rice. The concentration of cinnamyl alcohol decreased slowly until 72 hours had elapsed, after which time it reached a plateau as shown in Figure 3.6. As discussed in Section 3.4, there is tentative evidence for two rates of aroma diffusion for cinnamyl alcohol. However, we were unable to calculate a reliable value for the second rate of aroma diffusion because there only one data point obtained after the 72 hour exposure period. More data points would be needed to yield more accurate results that might allow a better understanding of this aspect of the effective diffusivity.

Figures 4.5a and b show the corresponding graphs for eugenol. The loss of eugenol from rice was relatively rapid during the first 24 hours of exposure, but then slowed markedly. The effective diffusivity of eugenol in rice during the 24 hour exposure period was $0.0068 \pm 0.0009 \text{ mm}^2/\text{hour}$. This value is close to the value for methyl eugenol ($0.0063 \pm 0.0009 \text{ mm}^2/\text{hour}$). These results are discussed further in Chapter 5. The effective diffusivity after 24 hours exposure was $0.00044 \pm 0.00008 \text{ mm}^2/\text{hour}$, a much lower value. Although both eugenol ($\leq 24$ hours) and methyl eugenol had similar effective diffusivities, the odour of eugenol in the product was
stronger than methyl eugenol after 24 hours of exposure, due to their different odour thresholds.

Figures 4.6a and b show the diffusion data for isoeugenol. The diffusivity (0.0036 ± 0.0004 mm²/hour) over a 24-period was similar to that of cinnamyl alcohol during 48 hours exposure (0.00300 ± 0.00018 mm²/hour). The data show some scatter over the 96 hour exposure period (see Figure 3.4), and it is not clear whether there were two distinct rates of aroma diffusion for isoeugenol (0-24 hours, and 24-96 hours), or just one. A case could be made for a single effective diffusivity of 0.00182 ± 0.00006 mm²/hour, which covered the full 96-hour period. The plot of the full data set from 5 replicates for isoeugenol is shown in Figure 4.9. The data points were widely separated at the point of 48-hour exposure, leading to a very large standard deviation, and some ambiguity as to whether isoeugenol follows two rates of diffusion. To resolve this ambiguity would require a prohibitively large number of replicates, given the amount of analytical work involved, so this question remains open.

The plot for methyl eugenol is shown in Figure 4.7. The loss of methyl eugenol was rapid during the first 24 hours, then decreased slightly during the next 24 hours (Figure 4.11); again, as may be seen in Figure 3.8, there is tentative evidence for two different binding modes. Although the effective diffusivities of methyl eugenol (0.0063 ± 0.0009 mm²/hour) and eugenol (0.0068 ± 0.0011 mm²/hour) were relatively similar during the first 24 hours of exposure, eugenol was better retained in the longer term. Methyl eugenol was undetectable by GC after 48 hours exposure, whereas eugenol could still be detected after 96 hours. As discussed in Section 3.4,
approximately 50% of the initial volume of injected liquid eugenol was absorbed by rice, but only 30% for methyl eugenol, which may be partly responsible for the lower levels of methyl eugenol. However, there is a clear difference between eugenol and its methylated form in terms of its short-term and long-term capacities to bind to rice and diffuse through it.

Figure 4.8. Graph of $\ln C^*$ versus exposure time for cinnamaldehyde (maximum 10 hours).
Figure 4.9. Graph of $\ln C^*$ versus exposure time for isoeugenol (full data set of 5 replicates).

This study has shown that the five aroma compounds have a range of effective diffusivities which, to a first approximation, can be arranged in the order cinnamaldehyde $>>$ eugenol $\simeq$ methyl eugenol $>$ isoeugenol $\simeq$ cinnamyl alcohol. These can be used as indicators to determine which aroma compounds were better retained in the rice. There are a number of physicochemical properties of aroma compounds that may contribute to these different rates of diffusion, and these are discussed in Section 4.4.2, in relation to the different chemical components of rice grains.
4.4.2. Physicochemical Characteristics of Aromas that can Affect Their Binding to Rice Starch

As shown in Table 1.1, the major component of white milled rice is starch, which comprises more than 80% of the total mass, with the remainder consisting of protein, lipid, water and minerals. Starch, as well as gum arabic, maltodextrin, β-cyclodextrin, and gelatin have been used industrially as coating materials for encapsulating the volatile compounds in aromatised foods such as confectionery, and cake and biscuit mixes (Bhandari and D’Arcy, 1996; LaBell, 1993; Goubet et al., 1998; Leahy et al., 1983; Bakan, 1973). Polysaccharides such as starch are used due to their capabilities for retarding aroma loss during food processing and storage. Protein and lipids are also able to interact with volatile compounds (Solms et al., 1973; Franzen and Kinsella, 1974; Baldwin et al., 1997), but in the first instance we have studied the potential interactions between aroma compounds and starch, as the major constituent of rice, and one that can bind to aroma compounds. We note that, in Section 2.4.1, the degree of milling, which significantly affects the lipid content of rice, did not have a significant effect on aroma retention in the aromatised rice, and this suggests that lipids are not significant contributors to aroma binding. As listed in Table 1.1, proteins contribute 7.3-8.3% to milled rice compositions. We have found no published data on the interaction between gas-phase aroma compounds and rice proteins, but other studies that used different types of proteins might be potentially used to assess whether or not rice proteins can bind to volatile compounds. A number of studies that dealt with aroma-protein binding in the solution phase have been carried out, and these show that aroma-protein binding is a complicated process (see Section 1.3). However, most researchers agreed that the aroma-protein binding was mostly dominated by hydrophobic interactions. Based on the earlier findings
and the significant protein content of rice, we considered that there was also a possibility that protein in aromatised rice grains might interact with the injected aroma compounds, either through hydrophobic or hydrophilic interactions. We also considered the possibility that the presence of water might influence aroma binding, since water contributes approximately 11-13% of the mass of milled rice. These proposals are further evaluated by using the relative retention times for the aroma compounds in amylose, amyllopectin and rice-flour GC columns (see Section 5.3.1).

The interactions between volatile compounds and starch have been extensively investigated, for example by Solms et al. (1973); Godshall and Solms (1992); Hau et al. (1998); Plug and Haring, (1993); and Escher et al., (1999). The binding of volatile compounds to a matrix that is rich in starch, such as rice, is potentially a complex process, but it is likely that the formation of hydrogen bonds plays a significant role in the interaction (Maier, 1972). However, as discussed below, lipophilic interactions may also be important for some compounds. According to Solms et al. (1973) and other researchers mentioned above, the unbranched fraction of starch, amylose, appears to be capable of interacting with volatile compounds. The helical structure of amylose which has hydroxyl groups on the outside of the coil (Godshall and Solms, 1992) can form hydrogen bonds with the volatile compounds (see Section 4.4.2), and it is also conceivable that the side chains of the branched fraction of starch (amylopectin) could interact with the volatile compounds (Langourieux and Crouzet, 1994; Escher et al., 1999). However, Escher and colleagues stated that the complexation between volatile compounds and amylopectin is weaker than that which occurs with amylose, which suggests that amylopectin is
significantly less important to the absorption and release of the volatile compounds from the starch systems.

There are a number of physicochemical parameters that can affect the strength of the interactions between the molecules of an aroma compound and starch (Goubet et al., 1998). Strong binding interactions between aroma molecules and starch will reduce the rate at which the aroma compounds are lost. On the other hand, weak interactions will permit the aromas to diffuse out from the starch easily. A series of properties of aroma compounds, including molecular weight, polarity and hydrogen bonding (see Table 4.2) have been chosen as parameters in the discussion of the interactions between injected aroma compounds and rice starch because earlier workers have found that they influence the retention of some aroma compounds by systems that are rich in starch (Solms et al., 1973; Reineccius, 1988; Rosenberg et al., 1990; Le Thanh et al., 1992; Voilley, 1995). In addition, log $P$ ($n$-octanol/water partition coefficient) and HLB (hydrophilic-lipophilic balance) were chosen as they have the potential to assess the mode of binding to starch, especially the unbranched fraction (amylose). Amylose, which has a helical structure, is internally hydrophobic or lipophilic (Belitz and Grosch, 1999), while the outside of the coil contains hydroxyl groups which can form hydrogen bonds with the volatile compounds (Godshall and Solms, 1992); it is therefore hydrophilic. Lipophilic “guest” molecules (for example aroma compounds) can fit inside the amylose helices, provided their molecular dimensions lie within the range of the inner helical amylose diameters (1.37-1.62 nm) (Belitz and Grosch, 1999). Based on the calculated dimensions of the injected aroma compounds (see Table 3.2), all would fit inside the amylose helices. Log $P$ ($n$-octanol/water partition coefficient) is the ratio of the
concentrations of a compound in the octanol and water components, respectively, of a two-phase octanol/water system. Lyman (1990) found that measured log $P$ values lie in a range of $-3$ to 7 (organic compounds), with the lower values corresponding to a more lipophilic compound. Log $P$ was therefore important in determining whether injected aroma compounds were more likely to be enclosed in the lipophilic helical coils of amylose, or bound to the hydrophilic exterior of the coils through, for example, the formation of hydrogen bonds. HLB (hydrophilic-lipophilic balance) is another parameter that indicates the extent to which a compound is hydrophilic. It is obtained by dividing the molecular weight of the water-soluble portion of the molecule by the total molecular weight of the molecule and multiplying the results by 20. The larger the value of HLB, the more hydrophilic the molecule is. Polarity and hydrogen bonding values may also be related to the strength of the interaction of a compound with rice. High polarity and hydrogen bonding values for the compound may indicate stronger interactions. For example, cinnamaldehyde, which is known to bind weakly to rice, has the lowest polarity and hydrogen values among the five aroma compounds. Cinnamyl alcohol, eugenol and isoeugenol, however, have higher polarity and hydrogen bonding than cinnamaldehyde, and demonstrated stronger binding to rice, as indicated by their lower values of effective diffusivities (see Table 4.1). Other physicochemical properties such as dipole moment and percentage of hydrophilic surface were not included in these discussions as they were implicit in the chosen parameters.

Physicochemical parameters of the five model aroma compounds, cinnamaldehyde, cinnamyl alcohol, eugenol, isoeugenol and methyl eugenol were calculated using WindowChem Software (WindowChem Software Inc., California),
except for the \( n \)-octanol/water partition coefficients (\( \log P \)), which were calculated using \textit{KOWWIN for Windows} (version 1.65). Input geometries for the aroma compounds were constructed and optimized by minimizing their energies using \textit{ChemSite for Windows} (version 2.42; a component of the \textit{WindowChem} package), and then transferred to \textit{Molecular Modelling Pro} (revision 2.4; also a component of \textit{WindowChem}) and \textit{KOWWIN for Windows} to obtain their physicochemical parameters, as summarised in Table 4.2. The \textit{WindowChem} software package was chosen to compute these parameters, since the computational methods were based on recognised estimation methods (for example \textit{Handbook of Chemical Property Estimation Methods} by Lyman et al. (1990) and \textit{Handbook of Solubility Parameters and Other Cohesion Parameters} by Barton (1983)). The software package has been successfully used by other workers, for example by Griffin et al. (1999) for the determination of octanol/water partition coefficients for terpenoids.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Characteristics & Cinnamaldehyde & Cinnamyl alcohol & Eugenol (\( \leq 24 \) hrs) & Isoeugenol (\( \leq 24 \) hrs) & Methyl eugenol \\
\hline
\textbf{Molecular Structure (see Table 2.4)} & & & & & \\
Molecular weight (MW) (g mol\(^{-1}\)) & 132.16 & 134.18 & 164.20 & 164.20 & 178.23 \\
\textsuperscript{a}Polarity & 3.95 & 5.58 & 6.70 & 6.60 & 5.80 \\
\textsuperscript{b}log \( P \) & 1.82 & 1.87 & 2.73 & 2.65 & 3.03 \\
\hline
\end{tabular}
\caption{Calculated physicochemical characteristics of cinnamaldehyde, cinnamyl alcohol, eugenol, iso-eugenol and methyl eugenol.}
\end{table}
Table 4.2. continued.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cinnamaldehyde</th>
<th>Cinnamyl alcohol</th>
<th>Eugenol (≤24 hrs)</th>
<th>Isoeugenol (≤24 hrs)</th>
<th>Methyl eugenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>⁵HLB</td>
<td>4.39</td>
<td>4.33</td>
<td>8.41</td>
<td>8.41</td>
<td>8.98</td>
</tr>
<tr>
<td>⁶Hydrogen bonding</td>
<td>7.29</td>
<td>12.02</td>
<td>11.70</td>
<td>11.62</td>
<td>8.76</td>
</tr>
<tr>
<td>⁷Effective diffusivities (mm²/hour)</td>
<td>0.0318±</td>
<td>0.00300±</td>
<td>0.0068±</td>
<td>0.0036±</td>
<td>0.0063±</td>
</tr>
<tr>
<td></td>
<td>0.0009</td>
<td>0.00018</td>
<td>0.0009</td>
<td>0.0004</td>
<td>0.0009</td>
</tr>
<tr>
<td>⁸Vapour pressures at 21°C (mm Hg)</td>
<td>0.037</td>
<td>0.034</td>
<td>0.022</td>
<td>0.013</td>
<td>0.105</td>
</tr>
<tr>
<td>% Absorption</td>
<td>48</td>
<td>33</td>
<td>48</td>
<td>29</td>
<td>31</td>
</tr>
</tbody>
</table>

a Polarity (using Hansen parameters⁸) is a measurement of the molecular polarity. Higher values indicate that the molecule is more polar.

b log P is the partition coefficient between n-octanol and water. Higher log P values correspond to more lipophilic molecules. Log P was calculated using KOWWIN for Windows (version 1.65).

c HLB (hydrophilic-lipophilic balance) is a measurement of the proportion of a molecule’s mass that is hydrophilic (maximum value is 20).

d Hydrogen bonding (using Hansen parameters⁸) is a measurement of the tendency of a molecule to form hydrogen bonds. Higher hydrogen bonding values correspond to a greater capacity for the molecule to form hydrogen bonds.

e Diffusion coefficients (effective diffusivities) were calculated using Fick’s second Law (see section 4.3.1 and Table 4.1).

f Extrapolated vapour pressures of the experimental vapour pressures that were obtained from Jordan (1954), except for methyl eugenol (see detail in Table 3.2). The extrapolation used the Clausius-Clapeyron equation (Grain, 1990) (see Section 2.4.1).

g Hansen parameters are multicomponent cohesion parameters that were developed by Hansen (1967) based on the Hildebrand parameters. Each of the parameter components was determined empirically on the basis of many experimental observations. Hansen parameters can be described in terms of molecular parameters related to intermolecular forces and molecular sizes. For example, the hydrogen bonding (δₜ) of ethanol, benzaldehyde, acetic acid, cyclohexanol and cyclohexane are 19.4, 5.3, 13.5, 13.5 and 0, respectively.
As mentioned above, it is generally agreed that starch plays an important role in retaining aroma substances in many food matrices especially in starch-rich foods, although proteins and lipids may also contribute to the interaction. Numerous studies have shown that amylose is capable of forming inclusion complexes with volatile substances (Osman-Ismail et al., 1961; Osman-Ismail and Solms, 1973; Rutschman et al., 1989; Rutschman and Solms, 1990; Voilley et al., 1991; Nuessli et al., 1997; Kollengode and Hanna, 1997a, b; Escher et al., 1999), where the outer regions of the amylose coils are hydrophilic, while the insides are hydrophobic. As a consequence, hydrophobic volatile substances are well retained inside the helical structure of amylose (Godshall and Solms, 1992).

The molecular weight (MW) of aroma compounds can also affect their retention in starch (Reneiccius, 1988). The higher the molecular weight, the slower the rate of loss for similar types of molecule. This is known to be the case because Voilley (1995) found that the retention of isoamyl butyrate (MW=158 g mol⁻¹) was greater than that of ethyl butyrate (MW=116 g mol⁻¹) in glucose during encapsulation. This could be explained by the increased size of the higher molecular weight aroma compounds, which as a consequence could inhibit their ready migration to the matrix surface. Furthermore, higher molecular weight results in larger compounds which have greater contact with the matrix, and hence stronger binding. However, in the present study it is clear that molecular weight is not a dominant influence on effective diffusivities for the five model aroma compounds. For example, the molecular weights of cinnamaldehyde (MW=132 g mol⁻¹) and cinnamyl alcohol (MW=134 g mol⁻¹) are similar, but the effective diffusivity of cinnamaldehyde is approximately ten times greater than that of cinnamyl alcohol. Also, the molecular
weights of eugenol and isoeugenol are the same; but their diffusivities are significantly different. Thus molecular weight did not critically affect the retention of these aroma compounds in rice. Our results were in agreement with the findings by Maier (1972), Le Thanh et al. (1992), Voilley (1995), Kollengode and Hanna (1997a) that other characteristics also affect aroma retention in starch-rich matrices. However, they did not correlate the retention of aroma compounds with their polarities, log $P$ or HLB values. It seems that they mostly discussed the interaction between the compounds and starch with respect to the functional groups of the compounds, finding that alcohols (which have $-\text{OH}$ groups) are better retained by starch than compounds that have no hydroxyl groups, although they may have aldehyde and ketone groups.

As discussed above and in Section 3.4, it is clear that molecular weight and vapour pressure do not have a dominant effect on the effective diffusivities. An examination of the other parameters in Table 4.2 suggests that the situation may be complex. Cinnamaldehyde is clearly the exception in this group of molecules. It has by far the largest effective diffusivity, and consistently low values of polarity, log $P$, HLB and hydrogen bonding. In molecular terms, the absence of methoxy or hydroxy groups appears to be correlated with high diffusivity. At the other extreme, cinnamyl alcohol has the lowest effective diffusivity. Its log $P$ and HLB values are low, its polarity is relatively low, and its hydrogen bonding is the strongest of the five compounds. On this basis hydrogen bonding, due to the hydroxyl group on cinnamyl alcohol, is an important factor. The relationship between the effective diffusivities and log $P$, polarity and HLB values are plotted in Figures 4.10a, b and c. Based on these graphs it is plain that no single factor controls the diffusivities of these
compounds. For example, eugenol and isoegenol have very similar structures and physicochemical properties, but their mean effective diffusivities differ by 47%. Eugenol is lost more rapidly in the first instance, but then there is evidence of a second diffusion process, which presumably is due to a second mode of binding. It is also apparent that there is not a simple relationship between effective diffusivity and the percentage of absorption for an aroma compound. Cinnamaldehyde has the highest effective diffusivity and percentage of absorption, but the pattern is less clear for the other compounds. The weak binding of cinnamaldehyde to rice was confirmed by earlier experiments, as shown in Figure 2.22, in which hexyl acetate (neither hydroxyl nor methoxy group) showed a lower absorption in rice than compounds with hydroxyl groups (1-hexanol and 2-phenylethanol). Similar results were also obtained from the study of retention times in a rice-flour gas chromatography column (see Section 5.3.1).

At this stage, we can draw some general conclusions: (1) the capability of aroma compounds to form hydrogen bonds with starch offers the possibility of stronger interactions, (2) proteins, which lie outside the starch granules, might also interact with the injected aroma compounds either through hydrophobic or hydrophilic interactions, but hydrophilic interactions appear to dominate the aroma-rice binding, and (3) for these compounds, hydroxyl groups correlate with improved hydrogen bonding, but methoxy groups also promote binding. These conclusions are in agreement with those of Maier (1972), Le Thanh et al. (1992), Voilley (1995), and Kollengode and Hanna (1997a), as stated earlier, who also found that alcohols were better retained than aldehydes and ketones.
Figure 4.10a. The relationship between effective diffusivities and polarity.

Figure 4.10b. The relationship between effective diffusivities and HLB.
Figure 4.10c. The relationship between effective diffusivities and log P.

In order to study how and where the aroma compounds were bound to the starch, presumably through the formation of hydrogen bonds, we firstly explored some characteristics of starch, such as the molecular structure of the unbranched (amylose) and branched fractions (amylopectin), as well as their arrangement in a starch granule. This information was important for speculating as to the binding sites of the aroma compounds and the matrices.

Amylose, which is generally assumed to consist of linear polymers of glucose that are joined by α-1,4 glucosidic bonds, is structurally arranged in a helical conformation (Szejtli, 1971) (see Figure 1.9). Amylopectin consists of branched polymers with approximately 96% of α-1,4 glucosidic bonds and 4% of α-1,6
glucosidic bonds (Champagne, 1996) (see Figure 1.10). Common starch usually contains approximately 20-25% amylose with a low molecular weight (degree of polymerization 250-300) (Whistler and BeMiller, 1997) with the remainder consisting of amylopectin. According to Yanai et al. (1979), 90% of white milled rice consists of starch, with 30% amylose and 60% amylopectin.

As mentioned earlier in Section 4.4.1, it is conceivable that the amylopectin branches could interact with volatile compounds. However, Escher et al. (1999) found that the amylopectin interaction was weaker than that for amylose, and that it therefore did not affect the absorption or release of aroma compounds.

Aromatised rice is a complex system, and the relationship between amylose and amylopectin in a starch granule is not fully understood (Oates, 1997) since it is not easy to precisely locate the amylose in a starch system. However, amylose is assumed to exist within a starch granule as an entity that is largely separated from the amylopectin fraction (Belitz and Grosch, 1999). According to Blanshard (1987), amylose is located in bundles between amylopectin clusters, and randomly interspersed among the clusters in both amorphous and crystalline regions. Starch granules are arranged in concentric layers which vary greatly in size and shape. Rice-starch granules are the smallest among the common starches, ranging in size from 3 to 5 μm, and have a polyhedral shape (Bechtel and Pomeranz, 1978). A schematic diagram of the structure of a starch granule, showing the amorphous amylose and crystalline layers of amylopectin, is reproduced in Figure 4.11. According to Champagne (1996), the structural properties of amylose from rice are
similar to those of wheat and maize, with amylose being mainly found in the amorphous areas.

Figure 4.11. A schematic model of a starch granule that shows amorphous amylose and crystalline amylopectin domains.

Earlier studies, as described above, concluded that amylose is the matrix in starch that aroma compounds bind to, and binding occurs primarily through the formation of hydrogen bonds. However, amylopectin should also be able to form hydrogen bonds to aroma compounds, provided the compounds can reach and penetrate it. In rice it might be expected that aromas could penetrate the amorphous amylose domains more readily than the crystalline amylopectin. To study this further, and to obtain additional data on the binding of the aroma compounds to rice, we undertook a further series of experiments to determine whether the natural condition of the rice starch, including the structural organisation of the amylose and amylopectin, could affect the degree of interaction. The experiments were carried out by separating the aroma compounds by means of a rice-flour gas chromatography
column, with amylose and amylpectin GC columns for comparison. In these experiments, the water content of the matrices was reduced, which minimised the direct involvement of water in the binding. Our working hypothesis was that the aroma compounds that had stronger interactions with the rice particles in the columns would have longer retention times. Detailed results and discussion of the experiments are presented in Chapter 5.

4.5. Conclusion

Aromatised rice is a complex matrix that contains volatile components (such as the injected aroma compounds) and non-volatile components, of which starch constitutes the majority. As reported in Section 3.4, the rate of aroma loss from rice varies for different aroma compounds, and vapour pressure is not the only factor that affects the rate of loss of an aroma. Nor are the physical properties of the whole rice grains, such as the pore volume and entrance diameter, the principal factors affecting aroma absorption or loss. In this chapter effective diffusivity values were determined for the aroma compounds (Table 4.1), as one measure of the strength of binding to rice. Attempts to correlate these values with the structures and physicochemical properties of the compounds did not give clear-cut conclusions, but it appears that the formation of hydrogen bonds between the aroma compounds and rice starch, and/or interactions between the aroma compounds and proteins, are major reasons for the slower release of aroma compounds from aromatised rice.

Of the calculated effective diffusivities for the five model aroma compounds, it was found that cinnamaldehyde had the highest value, followed by eugenol, methyl eugenol, isoeugenol and cinnamyl alcohol. Higher effective diffusivities for an
aroma compound correspond to more rapid aroma loss. Regarding the ability to form hydrogen bonds between the aroma molecules and rice starch, the availability of –OH and –OCH₃ groups proved to be significant. These factors are studied further in Chapter 5.
Chapter 5

Interaction between Aroma Compounds and Rice — Further Experiments

5.1. Introduction

As described in Section 4.1, aromatised rice is a complex system that consists of volatile and non-volatile components. The volatile components include the injected aroma compounds which may be lost from the rice, either rapidly or slowly, while the major non-volatile component in rice is starch (see Section 1.3). The rate of aroma loss from rice has been studied and reported in Chapter 3 for some model aroma compounds. Chapter 4 discussed the potential factors that may affect the relative rates of loss of certain aroma compounds from aromatised rice, as well as some physicochemical properties of the aroma compounds that may result in strong interactions between aroma compounds and rice starch. A series of experiments was carried out to evaluate these potential factors and physicochemical properties (see Section 4.4.2). This chapter describes further experiments, including the development and use of rice-flour chromatography columns (Section 5.2.1), the measurement of complexing indexes for aroma compounds and amylose (Section 5.2.2), and the determination of pore sizes for rice flour (Section 5.3.1).

There are two kinds of interaction between volatile and non-volatile compounds. These are attraction (fixation of volatile compounds on a non-volatile substrate) and repulsion (release of the volatile compounds) (Le Thanh et al., 1992). Much research has been done on the interaction between aroma compounds and food.
components (Plug and Haring, 1993; Kollengode and Hanna, 1997a; Hau et al., 1998; Cayot et al., 1998; Goubet et al., 1998; Escher et al., 1999), but we have only found a small number of published papers that report on the interactions between aroma compounds and rice starch; for example, the formation of inclusion compounds between rice starch and flavour substances such as 1-hexanol, decanal, \( \beta \)-pinene, and limonene (Osman-Ismail and Solms, 1973). Most of these papers dealt with the effects of processing on the physicochemical properties of rice starch; for example, the effect of rice starch-lipid complexes on complexing index and viscosity (Guraya et al., 1997), and the effect of lipid and protein removal on starch gelatinisation in whole milled rice (Marshall et al., 1990). This chapter continues our discussions of the interactions between some model aroma compounds and raw milled rice. It is difficult to determine where and how the interactions occur. As mentioned in Section 2.4.3, we were unable to map the distribution of the injected aroma compounds in an aromatised rice grain, although we demonstrated that the compounds penetrate the rice cores. However, our subsequent experiments have aimed to determine which components of a rice grain might bind with the aroma compounds. A characteristic of the present study is that we have tried to avoid the use of solvents for studying aroma-starch binding, in an attempt to model the interactions between raw rice and aroma compounds. Solution-phase studies, which are more common in the literature, have more direct relevance to aroma binding during rice cooking. Hence, we developed a second method of studying the interactions in which no solvents were involved. This method was inspired by gas chromatography, in which a stationary phase is confined to a column, through which the mobile phase (carrier gas) passes. When an aroma is injected into a GC column, the vapour of the compound is immediately partitioned between the mobile and
stationary phases. The compound that interacts more strongly with the stationary phase requires longer periods of time to reach the end of the column. Since GC is a gas-phase method in which no solution is involved, the development of a GC column that uses rice as a stationary phase is, in principle, an ideal method of studying the interactions between rice and aroma compounds. Ideally, whole rice grains would be used as the stationary phase, but such a column would be most unsatisfactory from a chromatographic point of view. Hence powdered rice was used, as being representative of the composition of rice, and retaining most of the starch granules intact.

White milled rice is a starchy endosperm of which more than 80% is starch, with the other components being fat (0.3-0.5%), protein (5-10%), water (12%) and ash (0.4%) (Juliano, 1985a). Rice starch granules, which are polyhedral in shape, are the smallest (3-5 μm) of the common starches (Bechtel and Pomeranz, 1978). This may have an important influence on their absorption characteristics. A rice-flour GC column was developed to study the interaction between aroma compounds and rice particles. By running a series of aroma compounds through the column, it was hoped that we could distinguish between the rates at which they passed through. An aroma compound that was retained longer in the column would presumably bind more strongly to the rice. The rice-flour pore dimensions were also measured to determine whether they could affect the retention time of an aroma compound in the column. It was hoped that the development of the rice-flour column and the measurement of pore sizes would provide further information for establishing the reasons for the different rates of aroma loss for different injected aroma compounds.
The development of a rice-flour GC column is a novel technique. We have not so far found any prior studies that used rice flour as a stationary phase in a packed GC column. This might be due to the fact that such a column potentially has a short lifetime, as the relatively high temperature of the GC oven can damage the starch granules over a long period of exposure. We were aware of the potential for physicochemical changes in the starch, and therefore a set of preliminary experiments was undertaken on the rice flour to determine the optimum GC temperature for both the aroma compounds and rice flour, as discussed in Section 5.2.1. We note, however, that starch powder from maize or rice has been used as a stationary phase in a capillary GC column (Kaiser, 1968), where starch was used on its own and in conjunction with other substances such as carbon tetrachloride and polypropylene glycol, which were mixed together with the starch suspension prior to packing into the capillary column. This was, however, only a preliminary study and few chromatographic separations were reported. We also considered the possibility of developing a rice-starch HPLC column, but were unable to obtain a stable pressure in the column. Acetonitrile, water and ethanol were used as mobile phases, and we suspected that the water component caused the starch to swell, resulting in an increase in pressure in the column. The use of rice flour as a stationary phase in GC was assumed to be a reasonably suitable technique for evaluating the interaction between the aroma vapours and rice, since the rice flour was not given any chemical treatment (only size reduction by grinding and passing through a 125 μm sieve). This does not mean that the rice did not undergo some physical or chemical change while in the column, as the column temperatures required for acceptable retention times reduced the moisture content of the rice and eventually caused visible changes. However, care was taken to choose the optimum temperature that maintained the
natural characteristics of rice for as long as possible. In gas chromatography, the 
mobile phase is an inert gas and the aroma compounds are retained in the column for 
different durations owing to their interactions with a stationary phase. According to 
Poole and Poole (1991), the components distribute on the column in zones according 
to their ability to interact with the stationary phase. During their migration through 
the column, the components spend part of the time in the mobile phase and part in the 
stationary phase. The apportionment of the molecules between the stationary and 
mobile phases is reflected in the distribution coefficient \( K_r \) (Jennings et al., 1997). 
The analysis of the results from the rice-flour chromatography was based on the 
ratios of the retention times of the aroma compounds to that of an unretained solute 
in the column; this ratio was defined as the relative retention time. Further 
descriptions and a discussion of the retention times of the aroma compounds and the 
unretained solute are presented in Sections 5.2.1 and 5.3.1.

Section 4.4.2 described the proposition that amylose is the component of rice 
that has the greatest potential for binding with the injected aroma compounds. As in 
other starches, rice starch consists of two polymeric forms of glucose — amylose and 
amylopectin. The amylose and amylopectin are organised into a radially anisotropic, 
seemicrystalline structure in the starch granules (Nikuni, 1978; Lineback, 1984; see 
Figure 4.11). Pure amylose and amylopectin GC columns were also packed to 
compare the interaction between aroma compounds and these two polymeric types of 
glucose. A \( \beta \)-cyclodextrin GC column was also developed in order to compare its 
relative retention times with those for the same compounds in the amylose, 
amylopectin and rice-flour columns. \( \beta \)-cyclodextrin has been widely used in aroma 
encapsulation in the food industry due to its capacity to bind well with most aroma
compounds (Goubet et al., 1999; Tanada et al., 1997; Hedges et al., 1996). An additional reason for choosing to use a β-cyclodextrin column is that the molecules of most aroma compounds can fit into the molecular cavity of this compound (Shahidi and Han, 1993). It was expected that the relative retention times for the model aroma compounds in a β-cyclodextrin column would be higher than those of the amylose, amyllopectin and rice-flour columns since β-cyclodextrin is generally thought to possess a higher ability to entrap aroma compounds. The results from this experiment could be used to confirm whether the structural organisation of amylose and amyllopectin in rice flour is responsible for the substantially better retention of the aroma compounds in rice.

Amylose is known to form complexes with a variety of ligands such as fatty acids, emulsifiers and flavour substances (Nuessli et al., 1997), and a variety of solution-phase studies has been carried out. Rutschmann et al. (1989) studied the formation of inclusion complexes of starch with menthone, and Osman-Ismail et al. (1961) carried out experiments on complexes of amylose with surfactants and flavour substances. These workers agreed that the helical arrangement of the amylose molecules (see Figure 4.13) is responsible for the formation of inclusion complexes in all of the experiments which were carried out in the solution phase. Inclusion complexes, which are not the result of chemical interactions, result from the addition of another compound (“guest” molecule) that fits into the “host” molecule (Rutchmann and Solms, 1990; Osman-Ismail and Solms, 1973). Hence in the present work an analysis of the formation of complexes between amylose and aroma compounds was undertaken using iodine as the indicator. This aspect of the project aimed to extend the scope of the earlier solution-phase complexation studies to
include the model aroma compounds from this study. It also aimed to compare the results of aroma binding experiments in the gas and solution phases. The complexing index for each combination of aroma compound and amylose was calculated to show the extent of the formation of complexes. This can be observed through the intensity of the blue colour that appears when iodine is added to solutions of amylose and the aroma compounds, where the intensity is measured as an absorbance using a UV-visible spectrophotometer. Iodine forms a well-known blue complex with starch; the portion of the starch bound by an aroma compound will not bind iodine (Guraya et al., 1997) and therefore the intensity of the blue colour in the solution is lower than it would be in the absence of the aroma compound. In other words, the lighter the blue colour, the greater the extent of complex formation between aroma compounds and amylose. In some cases, there might be an interaction between the aroma compound and iodine, and hence the aroma compound was added to the amylose solution and gently shaken prior to the addition of iodine solution (see Section 5.2.2). However, in all cases the interaction between the aroma compound and iodine produced no colour. A simple kinetic analysis of complex formation was carried out to evaluate the effect of time as well as the concentration of the aroma compounds.

In this chapter, the strength of the interactions between aroma compounds and rice starch is discussed with respect to the relative retention times of the compounds in the rice-flour, amylose, amylopectin and β-cyclodextrin GC columns. Observations of the formation of complexes between amylose and aroma compounds are also discussed. The possible relationships between the rice-flour pore sizes and
the relative retention times of the aroma compounds in a rice-flour GC column are also considered.

5.2. Materials and Methods

5.2.1. Gas Chromatography Columns

The stationary phases that were used for packing the gas chromatography columns were rice flour (Doongara variety, ground and then passed through a 125 μm sieve), pure amylose, amylopectin and β-cyclodextrin (for source and purity see Table 2.1). All of these materials were dried in an oven at 120°C for 2 hours prior to packing in steel tubing with a length of either 86 cm × 4 mm (I.D.) or 40 cm × 4 mm (I.D.). The stationary phases were dried to reduce the effects of the interactions between water and the aroma compounds, and because the column temperatures were greater than 100°C. Each column was installed into a PYE Series 104 gas chromatograph (PYE UNICAM) with oven and detector (thermal conductivity) temperatures of 150°C and 180°C, respectively. The carrier gas (helium) flow rate was adjusted to 30 mL/minute and monitored periodically during the migration of the aromas. All columns were allowed to stay in the gas chromatograph overnight with the carrier gas supply open and oven switched on in order to eliminate any water that remained in the stationary phases. A column temperature of 150°C was found to be optimum for all starch-based columns. At first the column temperature was set to approximately 180°C, as for the SGE BP 5 column used in Section 2.3.1. Unfortunately, the colour of the rice flour in the column, which was initially creamy, changed to brown, indicating that some degradation had occurred. We therefore tested oven temperature settings of 170, 160, 150, 140, 130 and 120°C. Of these
oven temperatures, 150°C was found to be optimum for rice-flour, pure amyllose, amylopectin and β-cyclodextrin columns, with no significant changes in the colour of these stationary phases. An oven temperature lower than 150°C caused longer retention times for the aroma compounds, and we were aware that long exposure of the various columns to heat could damage the starch granules (there were four model aroma compounds to be injected in a column). The properties of the stationary phase should be kept consistent for all of the aroma compounds during the analysis in order to obtain reasonably accurate retention times.

Hexyl acetate, eugenol, cinnamaldehyde and methyl eugenol were used as model aroma compounds. Hexyl acetate was used since it was found to be only weakly bound to the rice (see Section 2.4.1) and we wished to establish a comparison with the five more strongly bound aroma compounds (eugenol, methyl eugenol, isoeugenol, cinnamyl alcohol and cinnamaldehyde) that were used in this project. Unfortunately, it was not possible to use cinnamyl alcohol and isoeugenol in the GC experiments due to their relatively high viscosities, which created difficulties during injection into the columns. We were unable to withdraw 2 μL of pure liquid cinnamyl alcohol and isoeugenol using a 10-μL syringe; a larger syringe (20-μL) was also used, but the syringe plunger was forced back during attempts to inject the compound into the column. Retention times for the aroma compounds were recorded as the length of time from sample injection until the first appearance of the peak front. The choice of retention time of the unretained solute for the columns is discussed in detail in Section 5.3.1.
5.2.2. Complexes between Amylose and Aroma Compounds

Experiments on the interactions between the aromas and amyllose in the solution phase were carried out for comparison with the results of the gas-phase experiments (Section 5.2.1). It was expected that the results could be used to evaluate whether there were differences in the interactions between aroma compounds and amyllose in the gas and solution phases.

Amylose suspensions were prepared by weighing approximately 500 mg of amyllose into a 250-mL glass beaker and adding 100 mL of distilled water. The mixture was stirred until all of the amyllose was dispersed evenly in the water. The suspension was heated on a hot plate until it boiled. Distilled water was then added (approximately 200 mL) while stirring. The mixture was left to cool overnight. This suspension was transferred into a 1-L volumetric flask and distilled water was added to the mark. It was filtered using Whatman 41 filter paper and the filtrate was used as the amyllose solution.

Hexyl acetate, eugenol, methyl eugenol, cinnamaldehyde, isoeugenol, methyl isoeugenol and cinnamyl alcohol were used as model aroma compounds. All of these compounds were dissolved in acetonitrile (HPLC grade). To enable us to observe the changes of the absorbances during the 30-minutes equilibration period, the concentrations of the compounds in acetonitrile were varied (see Section 5.3.2). For some of the aroma compounds, it was difficult to see the changes in absorbance even though the concentrations of the compounds were either increased or decreased, which suggested in the first instance that there was little interaction between the aromas and the amyllose. Acetonitrile was chosen as the solvent since its absorbance
at 680 nm was unchanged after the addition of either amylose or iodine solution. In addition, acetonitrile is a relatively good solvent for both non-polar and polar compounds. Solutions of the aroma compounds and amylose were added directly into a 4-mL cuvette. 2.25 mL of amylose solution and 0.75 mL of aroma compound solution (in acetonitrile) were pipetted into the 4-mL cuvette and shaken for 2 seconds. Then 10 μL of 0.05 M iodine was added and shaken. The 0.05 M iodine solution was prepared by dissolving 20 g of potassium iodide (KI) in 100 mL distilled water and adding 12.69 g of I₂. The mixture was gently swirled until all substances were dissolved, then distilled water was added until the volume reached 1000 mL. The cuvette was placed into a UV-visible spectrophotometer (Cary 3, Varian) for recording the absorbance at 680 nm of the solution immediately after the addition of the iodine. The amylose solution with neither aroma compound nor iodine was used as a blank. The amylose and aroma solutions were mixed prior to the addition of the iodine to allow time for the compounds to interact. The working time for some of the aroma compounds affected the results, and therefore was kept consistent for all samples. In these experiments, observations were made of the effect of time (i.e. 30 minutes equilibrating period) as well as the aroma concentrations on the formation of the complexes between the amylose and the compounds. The results are discussed in Section 5.3.2. If the amylose did not interact with the aroma compound then it would be free to react with the iodine, indicated by the appearance of a blue colour in the solution. This method was used by earlier researchers; for example, in the study of the interaction of monoglycerides in different physical states with amylose in bread (Krog and Jensen, 1970; Guraya et al., 1997; Kaur and Singh, 2000).
The colourless amylose and aroma solutions changed blue after the iodine was added, causing an increased absorbance at 680 nm. The absorbance was measured over a period of 30 minutes. Three replicates were carried out for each treatment. The complexing index was calculated, to determine the degree of complex formation using the absorbances of the amylose-aroma solutions after 2 minutes in the cuvette. This period of time was chosen because some of the aroma compounds reacted very rapidly with the amylose. For the determination of the complexing index, the concentration of each aroma compound was \((0.60 \pm 0.01)\) mg/mL in acetonitrile. The complexing index is defined as follows (Guraya et al., 1997; Krog and Jensen, 1970):

\[
CI = \frac{A - B}{A} \times 100
\]

Where: \(CI\) = complexing index;

\(A\) = absorbance of standard solution (2.25 mL of amylose solution and 0.75 mL of acetonitrile without aroma compound) after the addition of 10 \(\mu\)L of iodine; and

\(B\) = absorbance of the amylose and aroma compound solution (2.25 mL of amylose solution and 0.75 mL of aroma compound solution in acetonitrile) after the addition of 10 \(\mu\)L of iodine.
5.3. Results and Discussion

5.3.1. Rice-Flour, Amylose, Amylopectin and β-Cyclodextrin Gas Chromatography Columns

The retention times of the aroma compounds in amylose, amylopectin and rice-flour chromatography columns in 40 cm × 4 mm (I.D.) and 86 cm × 4 mm (I.D.) tubing are shown in Tables 5.1 and 5.2. Two different lengths of tubing were used in this experiment. Initially, we were interested in the longer tubing (86 cm) which should provide a longer period of time for the injected aroma compounds to interact with the rice flour in the tubing and hence give better separation. Unfortunately, it was not possible to pack the longer section of tubing with amylose powder due to its stickiness, which often created voids in the column. While the sticky nature of the amylose made it difficult to pack what industry standards would consider to be a good GC column, the shorter 40-cm particle beds proved to yield columns of acceptable nature; peak shapes were broad, but nevertheless distinct, as shown in Figures 5.1 to 5.4. Hence, in order to make comparisons between the relative retention times of the aroma compounds in the rice-flour, amylose and amylopectin columns, the short column (40 cm) was used.

In this study, it was not possible to determine with certainty the retention time of an unretained aroma compound, that is, a compound that does not interact with the stationary phase. We first chose to inject butane gas into the starch columns to determine the retention time of an unretained compound. Unfortunately, we were unable to produce a chromatogram of the compound, even though a large amount of butane gas was used, and the detector (thermal conductivity) should have been
sensitive to it. Very little information is available about chromatography on starch columns, hence the establishment of an unretained marker is itself a difficult task, and beyond the scope of this study. For instance, chromatographers themselves still argue about the choice of unretained markers even in well-understood systems. For the sake of comparison, the retention times of all compounds were normalised to the retention time of hexyl acetate (chosen since it had the shortest retention time in this experiment). We assumed, for the sake of argument, that there was no interaction between hexyl acetate and the stationary phase in this experiment. This was only an approximation, since it seemed likely that there would be at least some interactions, possibly through the formation of hydrogen bonds involving the ester linkage of the hexyl acetate. However, these interactions were clearly much weaker than for the other aroma compounds, as the retention times of hexyl acetate were substantially shorter than those of the other compounds (see Tables 5.1 and 5.2). The relative retention times were calculated as the retention time of the aroma compound divided by the retention time of hexyl acetate on the same column. The capacity factor \( k' \) was not calculated, since an unretained void marker was not identified. Hence, relative retention time is a more appropriate indication of retention time; these values are given in parentheses in Tables 5.1 and 5.2. No replicates were carried out for each determination of the retention times due to the difficulty of filling the starch homogeneously and reproducibly into the GC columns and winding the steel columns into spiral shapes prior to installation in the gas chromatograph, and also due to the need for minimising the exposure of the columns to high temperatures.
Figure 5.1. Typical GC band profiles of (a) hexyl acetate, (b) eugenol, (c) methyl eugenol and (d) cinnamaldehyde in an amylose column. Chart speed = 1 cm/minute in all cases.

Figure 5.2. Typical GC band profiles of (a) hexyl acetate, (b) eugenol, (c) methyl eugenol and (d) cinnamaldehyde in an amylopectin column. Chart speed = 10 cm/minute in all cases.
Figure 5.3. Typical GC band profiles of (a) hexyl acetate, (b) eugenol, (c) methyl eugenol and (d) cinnamaldehyde in a rice-flour column. Chart speed = 5 cm/minute (a) and 1 cm/minute (b, c, d).

Figure 5.4. Typical GC band profiles of (a) hexyl acetate, (b) eugenol, (c) methyl eugenol and (d) cinnamaldehyde in a β-cyclodextrin column. Chart speed = 1 cm/minute (b, c, d) and 2 cm/minute (a).
Table 5.1. Retention times and relative retention times (in brackets) of aroma compounds on amylose, amylopectin, β-cyclodextrin and rice-flour columns (40 cm × 4 mm (I.D.)).

<table>
<thead>
<tr>
<th>Aroma Compounds</th>
<th>Retention time (seconds)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amylose</td>
<td>Amylopectin</td>
<td>β-Cyclodextrin</td>
<td>Rice flour</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>16.2 (1.00)</td>
<td>6.0 (1.00)</td>
<td>15.1 (1.00)</td>
<td>13.2 (1.00)</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>45.0 (2.8)</td>
<td>15.6 (2.6)</td>
<td>49.4 (3.27)</td>
<td>91.2 (6.9)</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>47.4 (2.9)</td>
<td>16.8 (2.8)</td>
<td>55.0 (3.64)</td>
<td>102.0 (7.7)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>49.8 (3.07)</td>
<td>18.0 (3.00)</td>
<td>55.9 (3.70)</td>
<td>104.4 (7.9)</td>
</tr>
</tbody>
</table>

Table 5.2. Retention times and relative retention times (in brackets) of aroma compounds on amylopectin and rice-flour columns (86 cm × 4 mm (I.D.)).

<table>
<thead>
<tr>
<th>Aroma Compounds</th>
<th>Retention time (seconds)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amylopectin</td>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>46.8 (1.00)</td>
<td>54.0 (1.00)</td>
<td></td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>108.0 (2.3)</td>
<td>468.0 (8.7)</td>
<td></td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>115.2 (2.5)</td>
<td>528.0 (9.8)</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>128.4 (2.7)</td>
<td>576.0 (10.7)</td>
<td></td>
</tr>
</tbody>
</table>

The results in Table 5.1 show that the relative retention times of the aroma compounds in the short columns varied for each compound. Hexyl acetate had the shortest retention time followed by cinnamaldehyde, methyl eugenol and eugenol. The same pattern was also obtained in the long columns (amylopectin and rice-flour; see Table 5.2). The relative retention times of the aroma compounds in the long and short columns were similar, which indicated that column length had little effect on the interaction between the aroma compounds and stationary phases. The orders of the retention times of the compounds were consistent for all columns. The differences in particle size for the various columns will affect the retention times;
however, due to the constant velocity of the carrier gas for all columns, all components spent the same times in the mobile phase, which resulted in no effect on the relative retention times (see Tables 5.1 and 5.2). Hence, the evaluation of the results in this experiment was based on the relative retention times. The retention times of eugenol, methyl eugenol and cinnamaldehyde were similar in the amylose column, but greater by a factor of approximately three than the retention time of hexyl acetate. Similar results were also obtained for the amyllopectin, β-cyclodextrin and rice-flour columns. This confirms that the model aroma compounds interact significantly with both forms of starch. Clearly the relative retention times were fairly similar for the amylose and amyllopectin columns, with amylose showing slightly greater retention, relative to hexyl acetate. These results were surprising, given the general view in the literature that amylose is significantly more important in forming complexes with aroma compounds (see Section 4.4.2). This does not appear to be the case for pure starches in the gas phase. In marked contrast rice, which consists of approximately 70% amyllopectin and 30% amylose, had relative retention times that were more than double the corresponding values for the amylose and amyllopectin columns. As expected, the relative retention times for the β-cyclodextrin column were higher than for the amylose and amyllopectin, but they were still substantially below those for the rice-flour column. Even though the β-cyclodextrin is known to bind well with most aroma compounds, it was unable in this case to match the ability of the rice flour to bind the aroma compounds. This suggested that either the structural organisation of the amylose and amyllopectin in the rice starch, which is not present in the pure amylose or amyllopectin, or the rice proteins that occupy the spaces between the endosperm starch granules, may be responsible for the enhanced aroma absorption by rice. Unfortunately, we were
unable to develop rice-protein GC columns to test these hypotheses. As mentioned in Section 4.4.2, water is another potential factor in the aroma interactions in milled rice, but this will have been largely excluded in the present GC studies of starch and rice flour. In contrast, the earlier kinetic and diffusivity studies were carried out on rice with a normal moisture content.

As discussed in Section 4.4.2, the molecular characteristics that offer better retention for certain aroma compounds appear to be the presence of hydroxyl and methoxy groups, which are related to the ability of the aroma molecules to form hydrogen bonds with the starch. When designing these experiments we hoped that it would be possible to obtain an independent set of quantitative binding data for the aroma compounds, and it was pleasing to note that for all columns the retention times were in the order eugenol > methyl eugenol > cinnamaldehyde >> hexyl acetate. This is consistent with the effective diffusivities that are tabulated in Table 4.2. However, the retention times were much more evenly spaced than the diffusivities, a point that is considered below. Unfortunately we were unable to analyse the retention times as rigorously as we had hoped, since the absolute retention times for the compounds were only slightly different, and reliable replication was not possible. However the GC results were still novel and useful, and allowed some qualitative analysis and interpretation.

In gas-solid chromatography, as occurs in the rice-flour and starch columns, separations may occur due to differences in vapour pressure and interactions between sample molecules and the stationary phase. According to Jennings et al. (1997), a component that has higher vapour pressure partitions more toward the mobile phase
and is swept towards the detector more rapidly than a component with lower pressure. Jennings and his colleagues further stated that a component which engages in an interaction with the stationary phase will effectively reduce its vapour pressure, resulting in a longer period of time to reach the detector. We expected that the aroma compounds that had lower rates of loss from aromatised rice would have longer retention times in the rice-flour GC column, since the compounds interacted with the rice particles during their migration along the column.

In the first instance we studied the relationship between the vapour pressures and the relative retention times of the aroma compounds. The vapour pressures of hexyl acetate, eugenol, methyl eugenol and cinnamaldehyde at 150°C were calculated and extrapolated as listed in Table 5.3. The extrapolated experimental vapour pressures for eugenol and cinnamaldehyde, and calculated values for the other two compounds, were used in the evaluation. The vapour pressure of hexyl acetate was clearly the highest, followed by methyl eugenol, cinnamaldehyde and eugenol. The short retention time for hexyl acetate is consistent with its high vapour pressure and the observation that it binds only weakly to rice. Similarly the long retention time for eugenol is consistent with its low vapour pressure and hydrogen-bonding capacity. Based on vapour pressures, cinnamaldehyde should have a longer retention time than methyl eugenol. This is not the case, and indicates that aroma-rice interactions are important. As shown by Table 4.2, methyl eugenol binds much more strongly than cinnamaldehyde, and therefore requires a longer period to pass through the column. It is unfortunate that isoeugenol could not be introduced into the column in detectable quantities, as a comparison with eugenol would have been helpful.
Table 5.3. Calculated and extrapolated vapour pressures of hexyl acetate, eugenol, methyl eugenol and cinnamaldehyde at 150°C.

<table>
<thead>
<tr>
<th>Aroma compounds</th>
<th>a Calculated vapour pressures at 150°C (mm Hg)</th>
<th>b Extrapolated experimental vapour pressure at 150°C (mm Hg)</th>
<th>Difference between a and b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexyl acetate</td>
<td>440</td>
<td>390&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eugenol</td>
<td>38</td>
<td>31</td>
<td>22.6</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>83</td>
<td>74&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>41</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>

a The calculated vapour pressures used the Antoine equation (Grain, 1990).

b The extrapolated vapour pressures of the experimental vapour pressures that were obtained from Jordan (1954). The extrapolation used the Clausius-Clapeyron equation (Grain, 1990).

c The difference was assumed to be the mean of the eugenol and cinnamaldehyde differences.

d Calculated value, equal to 88.7/100 of 440 mm Hg (see Section 3.4 for correction factor).

e Calculated value, equal to 88.7/100 of 83 mm Hg (see Section 3.4 for correction factor).

Measurements of the pore dimensions for rice flour and whole rice grains (see Section 3.4) were carried out to assess whether they gave any insights into the better retention of some aroma compounds in the rice-flour GC column and, specifically, to what extent the injected aroma molecules could enter the pores in the rice flour. These measurements were carried out using a Micromeritics poresizer 9310 (Particle and Surface Sciences, Gosford, NSW). Sample preparation and equipment operation were as described in Section 3.4. The smallest and largest measurable pore diameters were 0.006 and 360 μm, respectively. Graphs for the cumulative and percentage pore volumes of rice flour are shown in Figures 5.5a and b.
Figure 5.5a. Cumulative pore volume for rice flour.

Figure 5.5b. Cumulative percentage pore volume for rice flour.
As for the whole rice grains, most of the pore volume for rice flour was derived from pores with a small diameter. The cumulative pore volume plot (Figure 5.5a) shows that the total pore volume for pores with a diameter of more than 60 µm was only 0.064 mL/g, and total pore volume from pore diameters of 0.006 to 0.4 µm was about 0.50 mL/g, indicating that the flour was very porous. Figure 5.5b shows that only 10% of total pore volume was given by pores with diameters from 60 to 360 µm. As shown in Table 3.2, the maximum length of the aroma molecules was found to be 1.3 nm which fitted easily into even the smallest measurable pore diameter (6 nm in this experiment). Unfortunately, these measurements did not allow us to determine the natural shape of the pores in rice flour. They were assumed to be cylindrical, but were probably irregular. It is clear, however, that the opportunity for interaction between the aroma compounds and rice components would increase with a larger pore volume, owing to the greater surface area available for physical contact between the aroma molecules and starch, and the possibility of some degree of temporary entrapment in the pores, as occurs with small solutes in gel-permeation chromatography.

The relative retention times of the injected aroma compounds in the amylose, amylopectin, β-cyclodextrin and rice-flour columns show that amylose, which was thought to be responsible for most of the aroma binding with starch in the solution phase, did not yield a significantly greater interaction than amylopectin for gas–phase aroma compounds. β-cyclodextrin, which was recognised to be a reliable aroma encapsulant, proved to have a higher level of aroma retention than amylose and amylopectin, but not rice flour. The physical properties of the rice flour, such as the relatively large surface area, allow improved opportunities for the aroma compounds
to interact with the rice flour. These findings suggest that this, possibly coupled with
the structural organisation of the amylose and amylopectin in the rice starch, may be
responsible for the better retention of certain aroma compounds. However, a detailed
structural study of rice flour and the starch samples would be required to assess this.
As discussed in Section 4.4.2, volatile compounds can potentially bind to protein.
Solution-phase studies have shown that there are many factors that can affect the
aroma-protein binding process, such as the type of protein, the functional groups of
the aroma compounds, and the presence of water. These studies suggested that
hydrophobic interactions are important, although hydrophilic interactions appear to
dominate in the present gas-phase aroma-binding studies. Nevertheless, we
considered that there was also a possibility that protein in aromatised rice might take
part in the aroma binding, noting that proteins contribute a significant percentage of
the milled rice composition. However, we cannot yet rule out or confirm this
possibility, since we were unable to carry out suitable gas-phase experiments.

5.3.2. Complexes between Amylose and Aroma Compounds in
Solution Phase

As mentioned in Section 5.1, we designed gas-phase experiments that
modelled the aroma-rice interactions in aromatised rice, through the development of
various starch-GC columns. Earlier studies of this type of interaction, especially
those involving starch-based matrices and volatile compounds, were commonly
carried out in solution phase. We therefore carried out some solution-phase
experiments to allow us to evaluate whether there was a correlation between results
from the gas- and solution-phase studies.
Eugenol, methyl eugenol, isoeugenol, cinnamyl alcohol, cinnamaldehyde and hexyl acetate were used as model aroma compounds for studying complex formation and complexing indexes. The absorbances at 680 nm of various mixtures of amylose, iodine and aroma compounds over periods of 30 minutes were measured and plotted, as shown in Figures 5.6 to 5.8. Initially the concentrations of the aroma compounds in the amylose solutions were varied, depending on the rate at which the blue colour of the iodine-amylose complex disappeared. If the colour disappeared almost immediately after the addition of the iodine solution, then the aroma concentration was reduced. Alternatively, if there was no change in the blue colour during a 30-minute period, the aroma concentration was increased. This process of trial and error aimed at finding aroma concentrations that were suitable for assessing whether the aroma compounds had interactions with the amylose during an equilibration period of 30 minutes.

For the following three compounds the absorbances did not change during the 30 minutes equilibration period: hexyl acetate (0.2, 0.4 and 2.0 mg/mL), cinnamaldehyde (0.0022, 0.022, 0.22 and 2.2 mg/mL) and cinnamyl alcohol (0.021, 0.21 and 2.1 mg/mL). These values suggested that either the aroma compounds interacted only slightly with the amylose solution during the period. The complexing indexes (see Table 5.4) show that some reaction occurred when the reagents were mixed, but any subsequent reaction was clearly very slow. In contrast, the rate of the reaction between amylose solution and eugenol, methyl eugenol and isoeugenol produced measurable absorbance changes during the 30-minute reaction time, as shown in Figures 5.6 to 5.8.
**Figure 5.6.** Kinetic plot of the interaction between eugenol and an amylose solution (absorbance at 680 nm).

**Figure 5.7.** Kinetic plot of the interaction between methyl eugenol and an amylose solution (absorbance at 680 nm).
Figure 5.8. Kinetic plot of the interaction between isoeugenol and an amylose solution (absorbance at 680 nm).

Four different eugenol concentrations were used, and despite some scatter in the data, higher eugenol concentrations typically yielded lower absorbances, indicating that most of the iodine had been displaced from the starch by eugenol. Figure 5.6 demonstrates the eugenol interaction with amylose during the 30 minutes reaction period, presumably due to the formation of a complex.

The results for methyl eugenol and amylose solution, as shown in Figure 5.7, are less clear-cut. For low methyl eugenol concentrations (0.04 and 0.35 mg/mL) the absorbances increased slightly, but for 3.5 mg/mL the absorbance decreased, indicating there was an interaction between methyl eugenol and amylose.
Figure 5.8 demonstrates that the amylose-isoeugenol interaction increases markedly for concentrations of 0.010 and 0.006 mg/mL, but the absorbances levelled out during the 30 minute equilibration period. These results showed that the interaction between isoegenol and amylose was faster than that for methyl eugenol at the same concentration (0.04 mg/mL).

The complexing index can be used as a rough measure of the degree of the interaction between the aroma compounds and amylose, where a larger complexing index indicates a stronger interaction. The complexing indexes of the aroma compounds and amylose were calculated from absorbance measurements taken 2 minutes after iodine addition. Earlier workers reported that the reaction mixture was inverted several times before the absorbance was read; for example, in the study of amylose-lipid complex formation during cooking of rice flour (Kaur and Singh, 2000). This would be satisfactory if the aroma-starch reaction occurred very rapidly, but as Figures 5.6 and 5.8 show, this is not always the case, especially for eugenol and methyl eugenol. Thus the complexing indexes would be underestimated for these aroma compounds if the absorbances were recorded too soon after mixing. For the sake of consistency similar conditions were applied for each model aroma compound in this study and, except in the cases of eugenol and isoegenol, the absorbances had reached stable values for 0.6 mg/mL solutions after 2 minutes equilibration. The complexing indexes are plotted on a bar chart for ease of comparison (Figure 5.9), and also tabulated together with relative retention times and rates of aroma loss (Table 5.4).
Figure 5.9. The complexing indexes of eugenol, isoeugenol, methyl eugenol, cinnamaldehyde, cinnamyl alcohol and hexyl acetate after 2 minutes in amylose solution.

Table 5.4. Complexing indexes, relative retention times and rates of aroma loss from compounds that were used in the experiments.

<table>
<thead>
<tr>
<th>Aroma compounds</th>
<th>Complexing Index (%)</th>
<th>RRT on rice-flour column*</th>
<th>Rate constant of aroma loss (mg hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>99 (0.24)</td>
<td>7.9</td>
<td>0.055 ± 0.008</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>97 (0.37)</td>
<td>-</td>
<td>0.0137 ± 0.0009</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>26 (0.23)</td>
<td>7.7</td>
<td>0.033 ± 0.004</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>22 (2.25)</td>
<td>6.9</td>
<td>0.206 ± 0.014</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>23 (0.8)</td>
<td>-</td>
<td>0.0163 ± 0.0014</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>27 (1.5)</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers in parentheses denote standard deviations from three replicates.
RRT = relative retention times.
* Short rice-flour GC column (40 cm × 4 mm(I.D.)).
ThecomplexingindexesinFigure5.9showthateugenol(99%)and
isoeugenol(96%)hadtthehighestcomplexingindexesfollowedbymethyleugenol
(26%),cinnamylalcohol(23%)andcinnamaldehyde(22%).Asmentionedearlier,
thissectionoftheprojectaimedtocomparetheinteractionsbetweenamyloseandtheme-
odelaromacomponentsinthe
gas-andsolution-phases.Unfortunately,wewere
unabletocompareresultsforsallofthearomacomponents,becausedatawere
availableforsomeexperiments,andonlyhexylacetate,eugenol,methyl

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eugenoland
cinnamaldehydewastudiedinthegCexperiments.Onthewholetherewaspoor
correlationbetweenthegas-andsolution-phaseresults.Forexample,eugenolhas
thehighest(99%)complexingindexandthehighestrelativerepresentationtime(7.9)but
itdidnothavethesmallestrateconstantofaromalo ss.Cinnamaldehydehadalow
complexingindex(22%)indicatingweakinteractionwithamylose;italsohada
relativerepresentationtime(6.9)lowerthanthatefeugenolandmethyl

i

eugenol,and

highrateconstant(0.206±0.014)mghr⁻¹.However,hexylacetate,whichhasa
muchlowerGCrepresentationtime,hadalargercomplexingindexthanmethyl

eugenol,cinnamylalcoholandcinnamaldehyde.Thissuggesteddathattheinteractions
betweenamyloseandhexylacetatewererelativelystrongerinthesolutionphase.
Theinconsistenciesbetweenthegas-andsolution-phasebindingstudiessupportour
decisiontostudysolidrice,ricepowderandstarchsamplesintheabsenceof
solvents.We note,however,thatmilledricehasamoisturecontentofabout11-
13%,andaromabindingunderthesecircumstancesmaybeinfluencedbythebound
waterintherice.
5.4. Conclusion

In earlier chapters the results from measurements of the rate of aroma loss from rice (Section 3.4) and the corresponding aroma diffusivities (Section 4.4.1) were discussed in terms of the structural and physicochemical factors that could affect the retention of certain aroma compounds in aromatised rice (Section 4.4.2). In the present chapter further quantitative results were obtained from a series of experiments on amylose, amylopectin, β-cyclodextrin and rice-flour gas-chromatography columns, the measurement of pore volume and entrance diameters for rice flour, the determination of complexing indexes, and simple kinetic analysis of the interactions between aroma compounds and amylose in solution. The relative retention times of the aromas in the gas chromatography column provided, as hoped, an additional set of quantitative results that reflected the strength of rice-aroma binding. The results were not as precise as we had hoped, but they showed that the retention times were consistently in the order eugenol > methyl eugenol > cinnamaldehyde >> hexyl acetate. With allowances for the effects of vapour pressure, this ordering would have been expected on the basis of the hydrogen-bonding ability of the aromas. However, the diffusivity results from Chapter 4 show that the behaviour of aromas in intact rice grains under normal storage conditions is more complex. The different behaviours of the GC columns showed that there were no significant differences in the interactions between solid amylose and amylopectin. Amylose was thought to have stronger interactions with aroma compounds; however, rice flour with a starch composition of approximately 70% amylopectin and 30% amylose was found to have significantly higher aroma retention than pure amylose and amylopectin in the GC experiments. Therefore, we speculated that the overall structural organisation of amylose and amylopectin in the rice flour, which would not
be found in the pure amylose or amyllopectin, or the rice proteins, played a role in aroma retention. Our pore-size measurements were a first step in testing this hypothesis, but further work on rice and solid starch samples will be required, and this lay outside the scope of the present project. The results of the solution-phase kinetic measurements and complexing indexes for the interactions of the aroma compounds and amylose show that, in some concentration ranges, the measured complexing index depends on the equilibration time for the reaction mixtures. Furthermore, the correlation between solution- and gas-phase measurements was rather poor, which supported our decision to try the more realistic but time-consuming gas-phase experiments.

Based on the results from Chapters 2, 3, 4 and 5, we have obtained a substantial amount of information about the absorption, binding and release of aromas by rice. Chapter 6 deals with some practical aspects related to the use of aromatised rice as a foodstuff, namely the stability of the aroma compounds in the aromatised rice during storage, and measurements of the aroma concentration in cooked aromatised rice.
Chapter 6

Aroma Compounds in Stored and Cooked Aromatised Rice

6.1. Introduction

The sensory perception of flavour is an essential factor that can affect the choice and acceptance of a food product by consumers (Bakker et al., 1997). It is, in fact, the interactions between aroma and taste that create what consumers perceive as the flavour of food (Wilson, 1986). Taste relates to the perception of sweetness, sourness, saltiness and bitterness in the mouth; whereas aroma is associated with the smell of the food, as detected by the human olfactory system (deMan, 1990). The perception of aroma is a result of the release of odorous volatile compounds from food. These are usually found in only small amounts in food. In many cases a volatile aroma compound can evaporate continuously, even at room temperature (Wu and Pan, 1997), and the rate of aroma loss can increase with an increase in temperature.

Although nutrient content has been of primary importance when discussing the stability of food during storage, sensory attributes such as aroma also play a significant role, especially for products such as aromatised rice. The concentration of aroma compounds in aromatised rice is the most important factor that should be taken into account in determining the shelf-life of the product, since if the concentration is below its odour threshold, then the aromatised product is effectively the same as ordinary non-fragrant rice. According to Wu and Pan (1997), odour threshold is the minimum concentration of an odorous volatile compound that can be
detected by the human olfactory system. They further stated that the odour threshold of a volatile compound might not be related to its volatility. An aroma compound which has high volatility does not always have a lower odour threshold than another, less-volatile aroma compound. The odour threshold is different for each aroma compound, and thresholds can vary from person to person on the basis of factors like age, mood, and even hunger and satiety (Murphy and Gilmore, 1990; Meilgaard et al., 1991). For this reason, odour thresholds are typically presented as ranges. For example, eugenol has an odour threshold range of $8.0 \times 10^{-6} - 0.23 \text{ mg/m}^3 \text{ air}$ (Appell, 1969; Baldus, 1936; Van Anrooij, 1931; Ohma, 1922 and Tempelaar, 1913 cited in Van Gemert and Nettenbreijer, 1977) and cinnamaldehyde has a range of $1.5 \times 10^5 - 9.8 \times 10^2 \text{ mg/m}^3 \text{ air}$ (Ran debrock, 1971; Appell, 1969; Baldus, 1936; Backman, 1917 and Tempelaar, 1913 cited in Van Gemert and Nettenbreijer, 1977). Clearly, these ranges are very large.

A series of experiments on the rate of aroma losses from aromatised rice in open air at room temperature ($21^\circ\text{C}$) was discussed in Chapter 3. In contrast, the present chapter describes some measurements of the changes that occur in concentrations of injected aroma compounds in aromatised rice during storage in vacuum-sealed plastic packages. The results from these experiments provided valuable information for the determination of the shelf life for the aromatised rice.

The appropriate packaging for a food product, especially aromatised food, is important in order to prevent aroma loss to open air before processing and consumption. Some types of aroma loss can occur during the storage of an aromatised food in a package. According to Debeaufort and Voilley (1994), the
volatile components can interact with the packaging, migrate and be lost to the air. There have been many studies on the interaction between aroma compounds and packaging, for instance the interaction of citrus flavour compounds with polypropylene film (Letinski and Halek, 1992), factors affecting the absorption of aroma compounds into low density polyethylene (Nielsen et al., 1992) and the effect of storage period on instant-fried rice in a polyethylene packaging (Pratama et al., 1997). In this project, we studied aroma concentrations in aromatised rice after being packed for a period of time in aroma-barrier bags (see Section 6.1).

The aromatised rice in this project was designed to be consumed as whole “boiled and steamed” rice. “Boiled and steamed” rice is produced by boiling and steaming the raw rice grains. Firstly, rice is boiled in a volume of water approximately 3 or 4 times the volume of the rice; secondly, the boiled rice is steamed until it is no longer possible to feel a hard centre in a rice grain when held between the fingers. In practice, methods of cooking rice vary between different cultures. For instance, in India, the majority of rice consumers cook rice in excess water which is then discarded (Brooke, 1972), while Japanese consumers prefer rice which is boiled and steamed (Juliano and Sakurai, 1985). No matter what equipment is used to prepare the cooked rice, the basic processes of boiling and steaming are common to these cooking methods. Furthermore, many modern rice consumers also demand a practical method of cooking rice, such as using rice cookers and microwave ovens. For example, a rice cooker is designed to have two functions—cooking and warming. The “cooking” function corresponds to the boiling process and “warming” corresponds to steaming. For the preparation of cooked rice in a microwave oven, rice to which water has been added is first placed in the oven at a
high heat setting in order to boil the rice, then the heat is reduced for the purpose of steaming the boiled rice into a completely cooked rice.

Because substantial proportions of the injected aroma compounds are likely to be lost during cooking, experiments for determining the concentrations of aroma compounds in cooked aromatised rice were also carried out, as discussed in Section 6.3.1. Ideally, enough aroma compound should remain in the cooked rice to be detectable when it is released during serving, in order to provide the consumer with a pleasant odour. Cooked rice is mostly served hot or warm, and its aroma can add to the palatability of the rice. For this reason, naturally fragrant rice is often preferred over non-fragrant rice.

Choosing the right aroma compounds for aromatising rice is important since, in addition to sensory and safety issues, the right balance of aroma retention and release must be achieved. Ideally, sufficient concentrations of aroma compounds should be retained in raw aromatised rice, and then released slowly after cooking to prolong the odour for consumers to enjoy during eating. Two aroma compounds, eugenol and cinnamaldehyde, were chosen as model compounds in the aroma analysis of cooked rice. Both compounds have fairly strong, distinctive odours and occur naturally in other foodstuffs, including cloves and cinnamon, respectively. Of the main model aroma compounds studied in Chapters 3 – 5, eugenol was among the most tightly bound to rice, and cinnamaldehyde was the most weakly bound. These two compounds provided a good range of binding strengths for this study. In practical terms they might potentially be used in commercial aromatised rice products, although cultural and other factors will be important. In particular, we

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wished to determine whether the concentration of cinnamaldehyde in the cooked aromatised rice was still above its odour threshold.

These experiments were carried out in two parts: (1) measurement of the aroma concentration in uncooked aromatised rice after being stored for a period of time in aroma-barrier plastic bags, and (2) the evaluation of the odour of the cooked aromatised rice to determine whether the aroma compounds are detectable by GC analysis and the human olfactory system.

6.2. Materials and Methods

Eugenol was used as the model aroma compound for the shelf-life studies, in which levels of injected aroma compounds in aromatised rice were measured during 6 months storage. The aromatised rice was prepared using the procedures described in Section 2.3.3, with the exception that 10 μL of liquid eugenol was used, rather than 6 μL. Five replicates were used in the experiment, involving a total of 15 samples. After each 2 g of aromatised rice was prepared, it was packed directly into a 100 mm x 80 mm aroma-barrier plastic bag (NY15/ PE30/ LLDPE30 MIC, Globus Co., NSW) using a vacuum packing machine (Kramer Grebe, Western Germany). The concentration of eugenol in aromatised rice was measured at three stages: before packing, and after 3 and 6 months storage in the dark at approximately 21°C. The method used for the measurement of eugenol concentration in aromatised rice is described in Section 2.3.3.

The experiments on the concentration of injected aroma compounds in cooked aromatised rice were carried out using eugenol and cinnamaldehyde as the
model aroma compounds. The aromatised rice samples were prepared using the procedures described in Section 2.3.3, with a volume of 10 µL of the model aroma compound. Five replicates were used. Each sample of freshly prepared aromatised rice was placed in a 100-mL beaker, and 8 mL distilled water was added. The sample was brought to the boil to allow the rice grains to absorb all of the water in the beaker. This beaker was then placed into a 250-mL beaker containing 50 mL of distilled water. To prevent direct contact between the 100-mL and 250-mL beakers during boiling, a filter paper (Whatman 41) was placed between them. The 250-mL beaker was covered with the lid of a petri dish, and the water in it was boiled for 10 minutes; this corresponded to the steaming stage of rice cooking. The cooked rice was left in the beaker until it had cooled to approximately 25°C. This cool cooked rice was analysed for its aroma concentration.

The method that was used to extract the injected aroma compound from the cooked rice was as described in Figure 2.30, but with 6 mL methanol for soaking the rice sample rather than 3 mL. The reason for increasing the amount of methanol was that the volume of the rice increased during cooking, and more methanol was required for soaking and extracting the aroma compound. Gas chromatography was used to measure the concentration of the extracted aroma compound. A Hewlett Packard Model 5890A gas chromatograph, which was equipped with an SGE BP 5 column (2.5 m × 0.22 mm × 1.0 µm) and a flame ionization detector (FID), was used for the quantification of the aroma compounds in cooked rice. The experimental conditions were similar to those described in Section 2.3.1. Due to the larger volume of methanol (6 mL) that was used for soaking the cooked rice sample, the equation for calculating the concentration of the model compound in cooked rice was
modified accordingly. The aroma concentration was expressed as mg of model aroma compound per 100 g of raw rice that was used to prepare the cooked aromatised rice. The concentrations was not expressed as mg per 100 g cooked rice since it was difficult to maintain a uniform mass among the cooked rice samples due to their stickiness.

6.3. Results and Discussion

The discussion of the aroma concentrations in stored and cooked aromatised rice is divided into two sections. Firstly, the measurements of aroma concentration in stored and cooked aromatised rice are evaluated; secondly, the preparation of this product by consumers is discussed, and comparisons are made with existing aromatised foods.

6.3.1. Aromas in Stored and Cooked Aromatised Rice

The effect of storage time (0, 3 and 6 months) on the concentration of eugenol in aromatised rice is shown in Figure 6.1. The average concentrations of eugenol are expressed as mg/100 g rice. Error bars corresponding to standard deviations from 5 replicates are shown for each set of data. Analysis of variance of the data was carried out using the Statistica for Windows, Release 4.5 software, and the changes in eugenol concentrations during storage were not significant (P = 0.7), presumably due to the high level of aroma containment provided by the aroma-barrier plastic bags and the vacuum conditions that were used for packing the rice. It was noted that the rice grains were dry and intact after 6 months storage, indicating that the eugenol was well retained by them.
Figure 6.1. Amount of extractable eugenol in aromatised rice during storage at room temperature.

The results of the measurements on the concentrations of eugenol and cinnamaldehyde in cooked aromatised rice are shown in Figure 6.2. The concentrations of both eugenol and cinnamaldehyde were each reduced to approximately 95% of their initial concentrations, but were still detectable. Substantial amounts of the aromas were lost during cooking and cooling, but the GC analysis confirmed that some aroma remained, and was indeed still detectable by the human nose. We did not undertake a formal sensory evaluation at this early stage of product development, but four colleagues were asked to sniff the rice and identify the odours. They had no difficulty in doing so, which is a very encouraging result. Thus the amounts of eugenol and cinnamaldehyde released by cool samples of cooked aromatised rice were still above the odour thresholds of these compounds; and it
should be noted that cinnamaldehyde was weakly bound in comparison with the other principal aroma compounds that were studied in earlier chapters. As reported above, eugenol has an odour threshold range of $8.0 \times 10^{-6} - 0.23 \text{ mg/m}^3$ air, and cinnamaldehyde has a range of $1.5 \times 10^{-5} - 9.8 \times 10^{-2} \text{ mg/m}^3$. We were unable to quantify the concentrations of the aroma vapours that were released during cooking or serving the cooked rice using the headspace-GC method since, as discussed in Section 2.4.2, there were interferences from other volatile components of the rice, and potentially reaction products from the injected aroma compounds. However, the perceivable odour from the residual injected aroma compound, together with the texture of the cooked rice, would create a pleasant flavour for consumers of cooked aromatised rice to enjoy during eating. Furthermore, the combination of odour and texture would differentiate this product (aromatised rice) from non-fragrant rice.

After conducting a series of experiments on the stability of the aroma compounds in aromatised rice, we conclude that injected aroma compounds can be retained by rice during storage and released during cooking. Although there were substantial losses during cooking (a characteristic that is shared by conventional fragrant rices), the aroma could still be detected before the cooked rice was consumed, especially when it was served hot.
Figure 6.2. The amount of eugenol and cinnamaldehyde in cooked aromatised rice. Aroma concentrations are based on the original mass of uncooked rice.

6.3.2. The Use of Aromatised Rice and Its Comparison to Other Aromatised Foods

As discussed earlier, aromatised rice was designed to be consumed as whole "boiled and steamed" rice. We have now demonstrated that for two well-known aroma compounds the cooked rice contains detectable amounts of the aroma, thus meeting one of our original aims. Clearly the production of aromatised rice is not limited to the five model aroma compounds that were used in this project. Various other aromas could be imparted either singly or in combination, and of course there would have to be further experiments to determine how effectively these injected
compounds are retained in the rice. As well as having potential commercial outcomes, this could also provide further information about aroma-rice binding. Aromatised rice is a unique product which is very different from the majority of commercial aromatised foods, such as flavoured cereals and starch-based snack foods, which are mostly processed by extrusion. The aroma compounds in commercial flavoured snack foods are relatively stable in the products, since they are blended evenly into the matrix during the extrusion process. In contrast, aromatised rice grains remain intact and visually indistinguishable from the unprocessed foodstuff. Another approach to producing an aromatised rice is instant quick-cooking rice, which is pre-cooked or gelatinised rice that can be easily and quickly prepared for serving. It needs a relatively short cooking time in comparison to the conventional methods used for ordinary rice (Syarief et al., 1987; Pratama et al., 1997), but it is a more highly processed, and hence more expensive, product. In this case the aroma compounds may be more stable than in raw aromatised rice, as the gelatinised starch in the rice grains may entrap and hold the volatile compounds more effectively. However, further experiments would be required to test these speculations. Aromatised rice could be used to create new rice products, including wet-pack products that have been processed with the addition of liquid (for example “rice tomato soup”) or dry-pack products, in which no liquid is involved (Burns and Gerdes, 1985).

6.4. Conclusion

The final aim of this project was to produce aromatised rice that can retain its aroma for as long as possible during storage, and release detectable amounts during cooking and eating. A series of experiments measuring the aroma concentrations
during storage in a closed package showed insignificant changes during 6 months storage. It was also found that levels of eugenol and cinnamaldehyde in freshly prepared aromatised rice were each reduced to approximately 95% of their initial concentrations after cooking, but these were still above their odour thresholds.

Although we were unable to carry out experiments that covered all aspects of the aromatisation of rice, the results that are presented in this thesis — including the method of imparting, kinetic measurements, diffusion studies, the interaction of aroma compounds and rice, and the levels of aroma compounds in stored and cooked rice — represent a substantial contribution to further researchers or food manufacturers who seek to further develop aromatised rice as a food product. The use of other model aroma compounds with different odours, and the modification of the aromatised rice to produce other aromatised food products, are two possible directions stemming directly from this research.
Chapter 7

Conclusions

This project has demonstrated the possibility of producing aromatised rice, in which an aroma or a combination of aromas is introduced into raw milled rice. The appearance of the rice is effectively indistinguishable from that of the untreated foodstuff, and the rice can be cooked in the usual way to give a product with a clearly discernible aroma. For two common aroma compounds, eugenol (cloves) and cinnamaldehyde (cinnamon), the aromatised rice retained its aroma when stored in aroma-barrier packaging, and this is expected to be typical of other aromas. Much of the aroma is lost on standing in the open air, and further work on packaging and storage requirements would be required if the product were to be commercialised.

In reaching these conclusions this project has compiled a substantial body of knowledge on the way in which rice absorbs, binds and releases a variety of aroma compounds. This involved a large amount of experimental work, partly due to the experimental uncertainties involved in working with rice samples. Initially the project aimed to find an effective method for aromatising rice. Of the aroma carriers that were evaluated, liquid carbon dioxide was found to be effective for a range of aroma compounds. The optimum method required a pressure of 8 MPa at room temperature, 5 minutes equilibrating period, and no more than 10μL of pure aroma liquid for 2 g of rice. The method, which yields indistinguishable changes in the aromatised rice grains, retained the injected aromas longer than the other carriers that were used in this project, such as carbon dioxide gas and air. Eugenol, isoeugenol,
methyl eugenol, cinnamyl alcohol and cinnamaldehyde were used as model aroma compounds, and from 30-50% of the aroma compounds were absorbed by the rice. Liquid carbon dioxide was able to carry the injected aroma compounds into the rice cores, as shown by Fourier Transform Infrared (FT-IR) spectroscopy and Solid Phase Microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Unfortunately, we were unable to map the distribution of the aroma compounds in the rice grains. Aroma extraction and analysis were important for quantifying the aroma concentrations in rice, and methanol extraction coupled with gas chromatography (GC) was found to be reasonably practical and accurate for the purposes of this study.

Kinetic studies were carried out to determine the rate of aroma loss from aromatised rice to the open air. Aroma loss, as expected, was effectively first order, and rate constants varied among the model compounds, indicating that factors such as vapour pressure and the physicochemical characteristics of the compounds could affect the binding between the aroma compounds and rice. Some of the model compounds showed two patterns of aroma loss: a rapid loss initially, followed by slower aroma release. This suggested that there may be two binding modes for some aromas, but we could not determine what these were. Other compounds demonstrated a gradual loss during the exposure time. Aroma diffusion in the aromatised rice was measured by calculating effective diffusivities from the kinetic measurements, using the typical assumptions that aroma migration can be understood as a process of liquid diffusion, and rice grains are effectively spherical for the purposes of mathematical modelling. The diffusivities of the five aroma compounds spanned an order of magnitude, and attempts were made to use these results to study
the nature of aroma-rice binding. After trying to correlate the vapour pressures and some physicochemical properties of the model aroma compounds with the diffusivities, it was found that there was poor correlation between diffusivity and vapour pressure for these compounds. No single factor seemed to dominate the binding, but these and other experimental results suggested that binding to rice lipids is fairly unimportant, and these compounds probably do not bind significantly to the hydrophobic cores of the amylose in rice starch. Our results suggested that hydrophilic interactions, such as hydrogen bonding, are important.

Starch, which constitutes more than 90% of the white milled rice, and protein (7.3-8.3%), the second abundant component, potentially take part in the aroma binding. Earlier workers have concluded that the linear fraction of starch — amylose — is the most important component of rice starch in the interactions with aroma compounds. To study the interactions between amylose, amylopectin and our model aroma compounds, gas-phase and liquid-phase experiments were undertaken. The gas-phase experiments involved the development of various starch-based GC columns with amylose, amylopectin, β-cyclodextrin and rice flour as stationary phases. Since we could not determine the retention time for an unretained component in these starch columns, the retention times of all compounds were normalised to that of hexyl acetate on the same column, for the sake of comparison. The interaction between hexyl acetate and the starch was clearly small compared to the other compounds. The relative retention times for the rice-flour column were much greater than those for the amylose and amylopectin columns, indicating stronger interactions for the rice flour, but the aroma compounds showed clear evidence of binding to the starch. Surprisingly, amylopectin and amylose gave
similar relative retention times for the aroma compounds, suggesting that amylopectin may play a role in aroma binding by rice. Unfortunately, we were unable to develop a rice-protein GC column to assess the extent to which the protein plays a role in the interaction. However, these chromatographic results suggested some factors that may contribute to the aroma binding by rice: (1) the physical structure of the rice flour, and possibly the organisation of the amylose and amylopectin, (2) the proteins that occupy the spaces outside the starch granules, and (3) a combination of (1) and (2). Fats were not considered to be a major factor affecting the aroma binding, as the degree of rice milling, did not correlate with aroma absorption by rice, and hydrophilic interactions were important for the model aroma compounds. We confirmed that liquid-phase experiments are not an ideal method for evaluating the strength of binding between aroma compounds and solid starch or rice, although earlier studies typically involved liquid-phase experiments. The correlation between gas- and solution-phase experiments was not particularly good for the model aroma compounds in this study, and gas-phase results are more realistic in this case.

The aroma concentrations in aromatised rice were measured as a function of storage time for samples that had been sealed in vacuum aroma-barrier plastic bags. There were no significant losses after 6 months storage. Although there were substantial losses of eugenol and cinnamaldehyde when the aromatised rice was cooked, the compounds were still readily detectable by the human olfactory system.

This project has significantly improved our knowledge of the way in which rice can bind, retain and release a range of aroma compounds. Clearly there is scope
for further experimental and theoretical work to study more closely the distribution and detailed binding mechanisms of aromas in rice. Further work on solid amylose and amylpectin would be valuable, but ultimately it is the properties of rice itself that are of practical importance. This project has shown that the production of aromatised rice is feasible, and these results would be valuable for food manufacturers who might seek to produce aromatised rice as a commercial product. Further work that would be required for commercialisation would include studies of other potential aroma compounds, further studies of the effects of packaging on the aroma retention in rice during storage, and appropriate sensory evaluation of the odour that is released during serving and consumption of the cooked aromatised rice.
Literature Cited


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