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Comparison of aromatic compounds in jasmine rice of different origin

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Declaration

I hereby declare that I have done this thesis entitled **"Comparison of aromatic compounds in jasmine rice of different origin"** independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 15.08.2023

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Aneta Kašíková

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Abstract

Rice is a staple food for the vast majority of people around the world and the different cultures have preferences for various types of rice. Aromatic varieties have special characteristics such as a distinctive and attractive aroma resulting from a complex mixture of volatile substances. Their aroma is therefore the most valuable factor in determining quality and price. The characteristic high intensity odour of aromatic rice comes mainly from a compound 2-acetyl-1-pyrroline (2AP), described as the aroma of popcorn or pandan with an extremely low threshold. The solid phase microextraction was used for the extraction of volatile compounds from Thai jasmine rice. The optimised conditions for SPME were 1 g sample of ground rice in a 4 ml vial, sampling temperature at 80 °C for 30 min and exposure of the polydimethylsiloxane fibre in the headspace for 20 minutes. Identification of volatile compounds of Thai jasmine rice of different origin was performed by gas chromatography mass spectrometry and principal component analysis (PCA) was used to observe similarities in their volatile profile. A total of 59 volatile compounds were identified among the fifteen experimental samples of Thai jasmine rice. From these, 27 were identified in Thai conventional and organic jasmine rice collected in the Czech Republic, 22 in Thai conventional jasmine rice and 18 in Thai organic jasmine rice purchased in Chiang Mai region in Thailand. The volatile compounds in Thai jasmine rice were mainly aldehydes, alcohols, ketones, hydrocarbons, terpenes, and other low-volatile compounds. 2AP was found in two samples purchased in the Czech Republic and one sample collected in Thailand presented a trace amount in the chromatogram. This study found separation into the groups based on origin of the samples using PCA. Although SPME was regarded as a promising method for extraction of plant material, the methodology was not uniform, particularly in terms of extraction time and sample amount, which affected extraction efficacy. For future research, it would be appropriate to optimise SPME conditions for rice by increasing the extraction time and sample amount, which may enhance the final content of volatiles as well as focus on impact of biotic and abiotic stress in detail, in order to minimise aroma loss.

Key words: Thailand, *Oryza sativa*, volatile compounds, aroma, 2-Acetyl-1-pyrroline, SPME, GC-MS

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List of the abbreviations used in the thesis

2AP	2-acetyl-1-pyrroline
AAS	Amino Acid Score
AC	Alternate current
BADH2	Betaine aldehyde dehydrogenase 2
CAR	Carboxene
CZ	Czech Republic
DC	Direct current
DOM	Degree of milling
DVB	Divinylbenzene
EPA	Environmental Protection Agency
ESI	Electrospray ionization
GC	Gas chromatography
GC-FID	GC-flame ionization detector
GLC	Gas-liquid chromatography
GSC	Gas-solid chromatography
HHP	High hydrostatic pressure
HS	Headspace
IRRI	International Rice Research Institute
KDML	Khao Dawk Mali
MALDI	Matrix-assisted laser desorption/ionization
m/z	Mass-to-charge ratio
Mn	Manganese
MS	Mass spectrometry
MSD	Mass selective detector

NIH	National institutes of Health
NaCl	Sodium chloride
NIST	National Institute of Standard and Technology
PCA	Principal component analysis
PDMS	Polydimethylsiloxane
PTFE	Polytetrafluorethylene
QMA	Quadrupole mass analyser
RF	Radio frequency
RI	Retention Index
SCOT	Support-coated open tubular
Si	Silicon
SPME	Solid phase microextraction
TMP	2,4,6-trimethylpyridine
TH	Thailand
THC	Thai conventional rice
THO	Thai organic rice
USA	The United States of America
WCOT	Wall-coated open tubular

1. Introduction

Rice (*Oryza sativa* L.) is a major human dietary component for about half the world's population and contributes to food security in many low-income countries especially in Asia and Africa (Dias et al. 2021). Different cultures have preference for different types of rice. Scented rice or aromatic rice is popular in the South and Southeast parts of Asia and has gained wider acceptance in East Asia, Europe, and the United States (Verma & Srivastav 2022). Because of their aroma, flavour and texture, aromatic varieties command a higher price in the local and international rice markets than do the non-aromatic rice varieties which should help to improve the socio-economic life of farmers in developing countries engaged in rice cultivation (Singh et al. 2000; Sakthivel et al. 2009).

The most popular aromatic rice varieties sold in global trade are Basmati from India and Pakistan and Jasmine from Thailand (Dias et al. 2021). The aroma, flavour, and fragrance of jasmine-type and basmati-type rice varieties have the closest association with 2-acetyl-1-pyrroline (2AP), described with popcorn-like aroma, due to its maximum odour potency in comparison with at least 200 other aroma volatile components found in rice (Rao et al. 1974; Verma & Srivastav 2022).

The growing demand for aromatic rice worldwide has attracted the attention of rice producers and led them to increase the aroma of rice grain as a major target for improving their commercial rice varieties. The grain aroma directly influences the sale value and consumer preference. Thus, aroma management of rice has become an extremely important trait for the cultivation of this grain due to its commercial importance for the world market (Aryadeep Roychoudhury 2020).

2. Literature Review

2.1. Rice

The domestication of wild rice species probably began 9,000 years ago. Domestication in Asia may have occurred independently and simultaneously in several places within or bordering a large area that stretches from the lowlands below the eastern foothills of the Himalayas in India through upper Myanmar, northern Thailand, Laos, and Vietnam to southwestern or southern China or its borders (Singh et al. 2000).

Genus *Oryza* L. which originated in Southeast Asia and Philippines, has two cultivated species *Oryza sativa* L. and *Oryza glaberrima* L. Asia is where the *Oryza sativa* species originated before being exported to other countries while *Oryza glaberrima* species are native to Africa and are only grown in that continent (Chang 1976; Ito & Lacerda 2019).

More than half of the world's population relies on rice, a significant cereal crop, as their primary source of nutrition. This cereal has a broad genetic diversity, with thousands of varieties grown around the world. All these rice varieties are part of the grass family known as Gramineae or Poaceae as well as other cereals such as wheat, corn, rye, oats, and barley (Liu et al. 2022).

2.2. Aromatic rice

The aroma of fragrant rice was first analytically examined and classified popcorn-like in the early 1980s. Rankings of perceived popcorn odour intensity in various fragrant rice cultivars revealed that 2-acetyl-1-pyrroline was the main source of this fragrance. This volatile component can contribute to a popcorn-like smell with a low detection threshold (0.02 ng/l in air) (Wei et al. 2021). For the first time, it was found in cooked fragrant rice (Kongchum et al. 2022). This substance can be found in a wide variety of unprocessed food items, such as hazelnuts, pandan leaves, and Manuka honey. It is also present in some processed food items, such as popcorn and wheat bread crusts, as well as on the surface of Mediterranean dried sausages, Parma ham, and Italian-style salami, where it contributes to the salami's distinctive odour (Wei et al. 2017).

India, Pakistan, and Thailand are the main producers of aromatic rice. Basmati rice makes up most of the fragrant rice exported from Pakistan and India, whereas jasmine rice is Thailand's major export (Singh et al. 2000). According to World Bank (2015), in 2010, Thailand was the biggest exporter of fragrant rice: 2.65 million tonnes of jasmine rice were exported, followed by India (1.80 million tonnes basmati) and Pakistan (1.05 million tonnes basmati).

Compared to non-fragrant rice, fragrant rice is substantially more expensive. Premium fragrant basmati rice, for instance, costs three times as much as non-fragrant rice of the high quality. Because fragrant rice varieties are generally low yielding, their economic worth is higher than that of non-fragrant rice. Aromatic rice is more susceptible to disease and insect pests, and is prone to high shedding, resulting in yield losses (Berner & Hoff 1986; Faruq et al. 2011). It has been demonstrated that crops cultivated in drought and saline conditions produce grains of higher quality and with stronger aroma. But these unfavourable conditions are not conducive to high yields (Yoshihashi et al. 2004). Also, drying, milling and storage technologies affect both texture and preserved aroma of the grains (Singh et al. 2000).

2.2.1. Jasmine rice

Jasmine rice has a distinct aroma reminiscent of pandan-leaves and popcorn, due to the occurrence of natural aromatic compounds in the rice plant. The key aromatic compound responsible for these flavour notes is 2-acetyl-1-pyrroline (2AP), which is recognised as the most important fragrance component in aromatic rice out of more than 200 other volatile components that have been found in aromatic rice (Rao et al. 1974; Jinakot & Jirapakkul 2019).

KDML 105

Jasmine rice or Khao Dawk Mali rice is referred to as KDML and is the most significant variety of aromatic long grain rice in Thailand (Jinakot & Jirapakkul 2019). It is a traditional cultivar that was discovered by a farmer in the Chon Buri area of eastern Thailand in 1945. Khao means white and Dawk Mali means jasmine flower. The line was later released as Khao Dawk Mali 105 in 1995 (Singh et al. 2000).

KDML 105 can produce the aroma when growing in the fields and also during cooking (Jinakot & Jirapakkul 2019). The yielding potential of KDML 105 is considered low with the typical yield around 1.7 t/ha. However, the outcome of several rice experiment stations of the International Rice Research Institute (IRRI) with the appropriate technology, resulted in 4.5–5 t/ha yield. This demonstrates the potential of achieving higher yields with good management (Singh et al. 2000; Kukusamude & Kongsri 2018).

Figure 1. Thai jasmine rice sold on local market in Chiang Mai region in Thailand

2.3. Nutritional properties and chemical composition

The hull, which is the outer layer of rice, makes up around 20% of the total weight of paddy rice weight and contains minerals (silica) and cellulose. Beneath the hull, there is the caryopsis. Brown rice is created when the hull is removed from the grain during the hulling process. The caryopsis, which is made up of the germ, endosperm, and bran, is retrieved after the hull has been removed (Rahim et al. 2022). The bran layer and germ, which are rich in protein, fibre, oil, minerals, vitamins, and other phytochemicals, are removed during the milling process, which transforms brown rice into white rice (Burlando & Cornara 2014). The majority of rice cultivars have around 20% rice hull or husk, 11% bran layers, and 69% starchy endosperm (Dong et al. 2008a).

All rice varieties contain starch, which is mostly composed of two different forms of α-glucans called linear amylose and branched amylopectin. Linear amylose has a low molecular weight (105-106), and branched amylopectin has a high molecular weight (107-108). α-glucan chains are spontaneously organized into semi-crystalline granules that contain both crystalline and amorphous regions. The parallel association of hydrogen bonds between the linear and branching macromolecules, which are organized in the radial orientation, gives the starch its semi-crystallinity. Due to the parallel arrangement of its chains creating a double helix, amylopectin has a stronger impact on crystallinity than amylose chains, which intertwine in this structure and make up the amorphous phase of the starch (Liu et al. 2011). According to Amagliani et al. (2016), 98–99% of the dry weight of starch granules is made up of amylose and amylopectin.

The starch in non-glutinous rice is made up of 10–30% amylose and 70–90% amylopectin. In contrast to regular rice, which has an amylose level that ranges from extremely low (5-12%), low (12-20%), moderate (20-25%), and high (25-33%), glutinous (waxy) rice has an amylose content that is less than 2% (Juliano 2003). Various rice types have different amylose contents. The amount of amylose in rice has a significant influence on the rice quality (Ito & Lacerda 2019).

As major ingredient in rice, starch is the main source for providing energy. Rice proteins can be categorized based on how soluble they are. The primary type is glutelins, which make up 60% of total protein and are soluble in alkaline solutions, followed by prolamin (alcohol-soluble; 25%), globulin (salt-water-soluble; 10%), and albumin (water-soluble; 5%) (Dong et al. 2008a; Ito & Lacerda 2019).

According to Nunes et al. (2016), the nutritional value of a protein depends on its concentration of essential amino acids, its capacity to meet human metabolic needs, and the bioavailability of these amino acids.

Due to its higher concentrations of lysine and sulphur-containing amino acids, rice [Amino Acid Score (AAS) of 68] has a more complete and balanced amino acid composition than wheat (AAS of 43) and corn (AAS of 35). In addition, compared to other cereals, rice has superior biological values, protein efficiency, and digestibility (Carvalho et al. 2013).

The lipid content of rice grains is very low. From a chemical point, a distinction is made between saponifiable lipids (triacylglycerols, diacylglycerols,

monoacylglycerols, free fatty acids, and waxes) and unsaponifiable lipids (phytosterols, triterpene alcohols, γ-oryzanol and vitamin E homologues, tocopherols, and tocotrienols) (Frei & Becker 2005).

2.3.1. Volatile compounds

Rice aroma quality is determined by the combination of many different volatile compounds, such as oxygen-containing group, nitrogen group, sulphur group, and aromatic group. Alkanes possess higher odour thresholds, contributing to limited aroma. Aroma intensity is also related to alkane chain length and unsaturated bonds, with unsaturated compounds being stronger than saturated ones (Hu et al. 2020).

2-Acetyl-1-pyrroline

The most crucial aroma compound 2-acetyl-1-pyrroline (2AP), that gives rice (particularly fragrant rice) its popcorn-like scent, was first identified by Buttery, Ling and Juliano (Buttery et al. 1982). 2AP has an odour threshold of 0.1 ng/l in water and 0.02-0.04 ng/l in air (Gemert 2003). Maximum 2AP content is reached during grain development four to five weeks after heading, and it drops while grain is stored. Due to variations in harvest timing and postharvest processing, 2AP content in rice of the same variety cultivated under the same conditions may change significantly. Compared to milled rice, brown rice had a higher concentration of 2AP, indicating that the distribution of 2AP was initially heterogeneous. 2AP content reduced and became more homogeneous during storage. It has been discovered that 2AP is the main distinguishing factor between fragrant and non-fragrant rice. The concentration of 2AP in various rice cultivars has been the subject of numerous studies, and the findings revealed that the concentration of 2AP in fragrant cultivars varies substantially (Wei et al. 2017). The 2AP level of aromatic rice was 10 times higher than that of non-aromatic rice, indicating that volatiles play a role in rice scent (Hu et al. 2020). It was also found that 2AP is not created during cooking (Itani et al. 2004).

Aldehydes

Due to their low smell threshold, aldehydes, which are mostly formed by lipid oxidation and decomposition, contribute most to overall flavour (Suzuki et al. 1999). One of the most substantial volatiles in rice, hexanal, which is generated from linoleic acid,

adds a fruity, grassy, and green flavour (Bergman et al. 2000). The low odour threshold for nonanal is 3.1 ng/l, and the low odour threshold for octanal is 0.88 ng/l (Monsoor & Proctor 2004). Hexanal and pentanal concentrations are higher for linoleic acid oxidation products than for oleic acid oxidation products because linoleic acid oxidizes more quickly than oleic acid (Hu et al. 2020).

Heterocyclic compounds

The main aromatic volatile compounds formed by the Maillard reaction were heterocyclic compounds such as furans, pyrazines, thiophenes, thiazoles, pyrroles, imidazoles, and pyridines. The most abundant compounds, furans, contributed to the caramel-like odour of the burned carbohydrates. The major alkylfuran found in rice grains, 2-pentylfuran, has a distinctive nutty smell in low concentrations and a less pleasant aroma typical of soybeans in higher concentrations (Zeng et al. 2007). It was also discovered to be an odour-active compound in aromatic, non-aromatic and black rice having floral, fruity, nutty, green, almond, buttery, and beany aromas (Dong et al. 2008a; Hinge et al. 2016). Aromatic rice cultivars contained greater amounts of 2-pentylfuran than non-aromatic ones (Dong et al. 2008b; Grimm et al. 2011). Pyrazines, which have an offensive, strong, and pervasive odour, were also significant food flavouring substances (Hu et al. 2020).

Alcohols and phenols

Alcohols with low odour thresholds, such as 1-octen-3-ol, linalool, 1-nonanol, and 1-hexanol, as well as most of phenols, contribute to the flavour of rice. The most prevalent volatiles in cooked white rice were hexanol, 1-octen-3-ol, and benzyl alcohol; they made up 20.3% of the total, which was less than aldehydes (60.9%) (Dong et al. 2008a).

White rice contained a higher relative proportion of alcohols than black rice. The researchers found that black glutinous rice has higher aliphatic alcohol content than white glutinous rice (Ajarayasiri & Chaiseri 2008). This means that the proportion of various volatiles differed within varieties (Champagne et al. 2004). Among the most abundant volatiles in rice were also lipid-derived alcohols such as hexanol (herbaceous) and 1-octen-3-ol (mushroom, straw). One of the main alcohols in rice was hexanol, which contributed to the green, herbal, and sweet flavour. In comparison to non-aromatic rice varieties, basmati cultivars have significantly greater concentrations of hexanol and 1-

octen-3-ol (Mathure et al. 2014). Aromatic rice contained more benzyl alcohol, which contributed a slightly sweet flavour, than non-aromatic rice (Sansenya et al. 2018). Alcohols thus played an essential role in distinguishing aromatic from non-aromatic rice varieties.

According to reports, guaiacol, which adds a smoky or black rice-like scent, is the main odorant in black rice (Sukhonthara et al. 2009). Black glutinous rice contains phenol and 4-vinyl-2-methoxyphenol, but white glutinous rice does not contain these substances (Ajarayasiri & Chaiseri 2008; Hu et al. 2020).

2.4. Factors affecting aroma formation

Although all the aromatic rice traits are genetically governed and inherited, their expression under natural condition is very much dependent on environmental and soil and management practices. Basmati rice, for instance, loses its aroma when grown outside of the Punjab region of Pakistan and India. The Punjab climate and/or soil are regarded to be important in developing a strong aroma. The most valuable aromatic rice cultivar in Thailand, Khao Dawk Mali 105, is thought to have the strongest aroma and highest quality when cultivated in the Tung Kula Rong Hai region in northwest Thailand (Tran & Ho 2017).

2.4.1. Genetic factors

According to Wakte et al. (2017), rice aroma is a highly heritable characteristic. It had been discovered that the genes governing the aroma attribute were relatively complex and genetically regulated (Routray & Rayaguru 2018). Aroma was researched as a quantitative trait, and numerous genes were included in the expression. Genetic investigation revealed that the key component responsible for the aroma is 2-acetyl-1-pyrroline (2AP), which arises via a recessive allele (fgr) at a locus on chromosome 8 that corresponds to the gene encoding betaine aldehyde dehydrogenase (BADH2) (Hashemi et al. 2013; Dutta et al. 2022). Furthermore, Ahmad Sarhadi et al. (2009) found that the number of genes regulating the inheritance of aromatic traits differed. In summary, rice aroma genes were complex, and more research was required to find both the major and minor genes influencing rice aroma.

2.4.2. Cultivation conditions

Temperature influence

Temperature is known to have a significant impact on aromatic rice quality attributes, notably during flowering, grain filling, and maturity. Aroma is due to certain chemicals present in the endosperm. It is known that aroma is best developed and retained when aromatic rice is grown in areas where temperature is lower during maturity. It was also found that rice cultivated at a mean daily temperature of 18°C was of the highest quality (Singh et al. 2000).

Salinity

Salinity was believed to improve the quality of rice aroma and certain popular aromatic rice varieties were traditionally grown in the areas with salty soils or saltwater intrusion from the sea. Three enhanced aromatic rice varieties showed an increase in 2AP concentration in grains with salinity (Gay et al. 2010). However, it was primarily attributed to the change of some yield components and grain physical characteristics instead of the direct impact of salt on 2AP biosynthesis. Contrary to this, some suggested minimal effect of salt (NaCl) on the 2AP based aroma (Fitzgerald et al. 2008).

Plant nutrition and fertilizer application

Because aromatic rice was more susceptible to pests and diseases, diverse agricultural chemicals, such as fertilisers, growth regulators, etc. were utilised in its cultivation. Manganese (Mn) application significantly increased the amount of 2AP in rice grains (Li et al. 2016), which was most likely due to an improved enzyme activities that contribute to the synthesis of 2AP.

More 1-proline, one of the precursors to 2AP, was produced when the overall nitrogen concentration of the soil was higher. Thus, total soil nitrogen was one of the most substantial contributors in the formation of rice aroma. Additionally, it was discovered that silicon (Si) application increased 2AP contents in grain to some extent by positively correlating Si contents in leaves with 2AP contents in grain at the flowering stage (Mo et al. 2017).

The impact on aromatic properties could be achieved by influencing yield, proline accumulation and proline dehydrogenase activity. Treatments with growth regulators,

such as gibberellic acid, paclobutrazol, 3-indoleacetic acid, and a mixture of regulators consisting of paclobutrazol, proline, and zinc chloride, suppressed the metabolic processes related with the formation of volatiles (Goufo et al. 2011). Despite increased grain yield and quality, aromatic rice treated with a regulator at panicle emergence had decreased aroma content and lower sensory ratings. The levels of 2AP, 3-indoleacetic acid, and paclobutrazol diminished. Therefore, adequate growth regulators need to be controlled to achieve aromatic rice of excellent quality.

In some rice varieties, shading treatments during grain filling raised 2AP levels and had a selective impact on other volatiles metabolism (Mo et al. 2015). While it lowered the relative content of octane and benzyl alcohol and raised the relative content of (E)-2-hexenal in both varieties, it had little to no effect on the relative content of 1-hexanol, 1-heptanol, and benzene acetaldehyde.

2.4.3. Processing

Cooking

Cooking is a key process for rice before consumption. Water cleansing, pre-soaking, and appropriate cooking techniques are all part of the cooking process. The aroma of the rice would change with each step. The content of volatiles of stored milled head and broken rice was substantially lowered after 5 minutes of washing with water (Monsoor & Proctor 2004). Pentanal, pentanol, and hexanal concentrations were decreased. This was mostly due to the effect of water washing on the total surface lipid content, which resulted in the removal of 60-70 % of the total surface lipid content. Traditional preparation before cooking involves pre-soaking. Cooking would be more uniform and take less time. An increase in sulphur-containing free amino acids and their breakdown products was the main cause of the major increase in sewer/animal flavour and summed unfavourable flavour characteristics that resulted from pre-soaking for 30 minutes prior to cooking (Champagne et al. 2008).

Rice has a strong flavour when cooked because flavour compounds and compounds created by thermal degradation and the Maillard reaction can be released. The cooking process was separated into four stages. Aldehydes such n-nonanal, n-decanal, and (E)-4-nonenal were the main rice compounds found during cooking stage I (25 minutes from the start of heating to the start of steam flowing out of the rice cooker).

Hexadecanoic acid and tetradecanoic acid were the two most prevalent substances found during cooking stage II (13 minutes from the beginning of steam coming out of the rice cooker to the end of steam coming out of the rice cooker). Aldehydes and fatty acids were the main components discovered at cooking stage III (10 minutes from the end of steam flowing out of the rice cooker to automated stop of heating) and stage IV (keeping the rice warm for a further 30 minutes). They hypothesised that the initial heating at stage I resulted in the evaporation of aldehydes present in the rice prior to cooking. During cooking stage II, a huge amount of steam was released from the cooker, which caused fatty acids to be distilled out of the rice and significantly rise (Zeng et al. 2007). Whereas some studies reported that 2AP was produced during cooking (Hofmann & Schieberle 1998), others claimed that it was not (Yoshihashi 2002).

High hydrostatic pressure and superheated steam

High hydrostatic pressure (HHP) was regarded to be an efficient processing to enhance product flavour since it had stabilised effects on low-molecular-weight volatiles (Xia & Li 2018). Before cooking, pre-soaked samples underwent a 10-minute HHP processing treatment (Deng et al. 2013), which increased the amount of alcohols, ketones, esters, and olefins in the jasmine rice while lowering the amount of heterocycles, alkanes, and arenes. Pressure level and rice cultivars affected how much the volatile profile changed. HHP was considered to be a good pretreatment choice to improve the cooked rice's aroma quality. The HHP procedure increased the synthesis of aldehydes, alcohols, and ketones in germinated brown rice, which improved its flavour (Xia et al. 2017). And, (E,E)-2,4-decadienal, (E)-2-hexenal, (E,E)-2,4-heptadienal and benzyl alcohol were considered as volatile biomarkers of high pressure.

According to Takemitsu et al. (2016), the volatiles of rice cooked using a superheated steam (SS) rice cooking system were compared to those of conventionally cooked rice. The amount of hexanal and (E,E)-2,4-decadienal after extraction with methyl tert-butyl ether was discovered to be nearly identical. Headspace analysis, on the other hand, revealed that superheated steamed rice had less volatiles than 10% of standard cooked rice. The oneba layer of ordinary cooked rice was amorphous and consisted of a number of rice solids that were water soluble. Additionally, the volatiles in this layer were easily released, contributing to the rice odour. The internal cell wall structure of

superheated steamed rice was retained, and almost any soluble substance leached out, leading to less oneba on the surface. Thus, fewer aroma components were emitted.

Roasting

Rice aroma can be considerably enhanced by roasting, a traditional method of pre-gelatinization of maturing and aromatizing materials, while also altering the morphological, structural, and functional characteristics of grain starch and protein (Fischer et al. 2017). Roasting, for instance, reduced carbohydrates and proteins while enhancing the content of heterocyclic compounds (AL Juhaimi et al. 2018; Shi et al. 2018). According to some research, roasting caused the grains to undergo a Maillard reaction, and the amount of Maillard reaction by-products (e.g. pyrazine, pyrrole, furan, and their derivatives) increased in the total content of volatiles. This in turn caused significant changes in colour and flavour, which were more noticeable as a result, and it usually increases consumer popularity (Shi et al. 2018; Adelina et al. 2021).

For instance, in brown rice, roasting created bumps and fractures on the surface as well as expanding the space between the granules and altered the microstructure in a way that facilitated the release of aromatic compounds (Hu et al. 2020).

Dehulling

Although not properly defined method of dehulling might influence the aroma content of the processed grains. Farmers at Tapovan claim that manual dehulling results in more aromatic and better-quality grains as compared to mechanical dehulling (Singh et al. 2000).

Milling

Milling is a common method of improving white rice quality by removing the fibre-rich bran which is the cause of brown rice's low palatability. Brown rice's external bran and embryo are removed during the physical process of milling, causing physical damage which can result in structural alterations that affect the physiochemical characteristics and perceived quality of white rice (Bhattacharya 2011; Hasjim et al. 2012).

The most vital volatile compound defining aromatic characteristics in fragrant rice is considered to be 2-acetyl-1-pyrroline, which gives the rice its popcorn flavour. Therefore, any 2AP losses caused by milling will result in severe market value losses.

Mahmud et al. (2017) found that there were no substantial differences observed in 2AP levels as a function of degree of milling (DOM). These results imply that rice's aromatic characteristics are unlikely to be reduced by higher milling levels intended to improve eating quality. These findings correspond with those of Grim et al. (2001), who observed constant 2AP levels in 21 rice varieties throughout various milling fractions. According to contradictory reports, the declining 2AP levels at high degrees of milling might be partially related to differences in rice varieties (Liu & Yang 2011).

2.4.4. Storage

Because of a decrease in favourable volatiles and an increase in undesired volatiles, storage will negatively affect the flavour of rice. The entire degradation process was rather complex. Proteins, lipids, and carbohydrates were observed to decompose into volatiles that contributed to rice odour during storage (Guan et al. 2017). Amine macromolecules were created by the decomposition of protein. The lipids were mostly first hydrolysed by lipases, releasing free fatty acids that were then further decomposed into aldehydes and acids. Alcohols, aldehydes, ketones, and carboxylic acid gases were formed after the decomposition of carbohydrates. Overall, with extended storage time, the concentration of aldehydes, acids, and nitrogen heterocyclics increased, producing a displeasing scent (Lin et al. 2018).

Rice's biochemical responses during storage were strongly influenced by the length of storage and storage conditions such vacuum levels, temperature, packing, and moisture. During storage, there were significant correlations among volatiles and vacuum levels (Liu et al. 2018) The main cause of the unfavourable odour was the development of undesirable volatiles specifically production of lipid oxidation during storage. Throughout the ageing of rice, aldehydes were the major volatiles. Hexanal was identified as a lipid oxidation marker in rice and was directly associated to oxidative off-flavours (Wang & Ha 2013). The rate of fragrance deterioration would be impacted by storage temperature. Longer storage times at higher temperatures led to a quicker rise in fat acidity and rancid scent as well as a decrease in sensory quality (Sung et al. 2014). For

aromatic rice, it is generally recommended to store it at a lower temperature and use superior packaging materials to preserve the rice's desirable aroma.

2.5. Extraction methods

Instrumentation and sampling methods for isolating and determining 2AP concentration levels in aromatic and nonaromatic rice samples have been improved during the last two and a half decades, and new techniques are provided (Verma & Srivastav 2022). Numerous extraction techniques are available, and they vary from each other in a wide range of aspects. This includes pricing, effectiveness, simplicity, total time spent, duration of the extraction process, need for solvent, selectivity, compatibility with various instruments and techniques, and others. Table 1 compares the SPME method with the performances of various other extraction methods and shows the advantages provided by SPME. The fact that sampling, extraction, concentration, and sample introduction are all merged into one step is another benefit of SPME (Mottaleb et al. 2019).

Table 1. Comparison of performances of SPME and other conventional techniques *(Source: Mottaleb et al. 2019)*

Detection limit (MS)	Precision (RSD)	Expense	Time	Solvent used	Simplicity
Purge and Trap (ppb)	1 – 30	High	30 min	None	No
Stripping (ppt)	3 – 20	High	2 h	None	No
Headspace (ppm)		Low	30 min	None	Yes
Liquid-liquid extraction (ppt)	5 – 50	High	1 h	1000 ml	Yes
Solid-phase extraction (ppt)	7 – 15	Medium	30 min	To 100 ml	Yes
SPME (ppt)	<1 – 12	Low	5 min	None	Yes

2.5.1. Solid-phase microextraction

Solid-phase microextraction (SPME) is a relatively new extraction sampling technique. It is simple, solvent-free, quick, easy to automate, precise, and extremely sensitive for the identification of volatile and non-volatile compounds of solid, liquid, and gaseous analytes (Balasubramanian & Panigrahi 2011; Shirey 2012). The SPME approach is gaining popularity and is being used in an expanding number of disciplines because of its numerous advantages over other extraction techniques. The method's basis is the absorption of analytes onto a fused-silica optical fibre covered with an absorbent (Sgorbini et al. 2014; Mottaleb et al. 2019). 1 cm is the most common fibre length. Fibres

longer than 2 cm are never utilized for efficiency reasons. The maximum number of extractions with a single fibre depends on the coating durability of the fibre (Kyle 2017).

If the fibre is overused, it will lead to its "bleeding" and siloxane contamination which is one of the most common types of "ghost peaks" in gas chromatography (GC). An inner needle or tube that is concealed inside the outward piercing needle has a coated fibre linked to it. The sealing septum that covers the outer needle is a crucial component of this simple device. It prevents leaks from happening when a needle is inserted into a pressurized injection port on a gas chromatograph (GC). The type of coating put to the fibre is indicated by the colour of the hub at the top of the tubing (plunger) (English 2022). See Figure 2 for a comprehensive list of commercially available fibres with technical characteristics.

Analyte Type	Molecular Weight Range (g/mol)	Recommended Fiber
Gases and Low Molecular Weight Compounds	30-225	75 µm/85 µm Carboxen®/polydimethylsiloxane
Volatiles	60-275	100 µm polydimethylsiloxane
Volatiles, Amines, and Nitro-aromatic Compounds	50-300	65 µm polydimethylsiloxane/divinylbenzene
Polar Semi-volatiles	80-300	85 µm polyacrylate
Non-polar High Molecular Weight Compounds	125-600	7 µm polydimethylsiloxane
Non-polar Semi-Volatiles	80-500	30 µm polydimethylsiloxane
Alcohols and Polar Compounds	40-275	60 µm CARBOWAX® (PEG)
Flavor Compounds: Volatiles and Semi-volatiles, C3 - C20	40-275	50 µm/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane on a StableFlex™ fiber
Trace Compound Analysis (ppb)	40-275	50 µm/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane on a 2cm StableFlex™ fiber
Amines and Polar Compounds (HPLC use only)		60 µm polydimethylsiloxane/divinylbenzene

Figure 2. Fibre coating selection based on analyte properties (*Source: Sigma-Aldrich 2018)*

Retracting the fibre after the extraction and desorption is done by a spring, which is a component of the manual assembly. The spring is missing in the assembly used with autosamplers (Shirey 2012).

The assembly is placed into the manual holder for easier manipulation (Figure 3). The holder is equipped with a needle guide depth gauge that measures how deep the needle travels either into the vial or in the injection port by being screwed down or up. This is done since the needle is quite brittle and may be quite easily destroyed. A z-slot attached to the manual holder can be used to secure fibre in the exposed position (Shirey 2012; Sigma-Aldrich 2018).

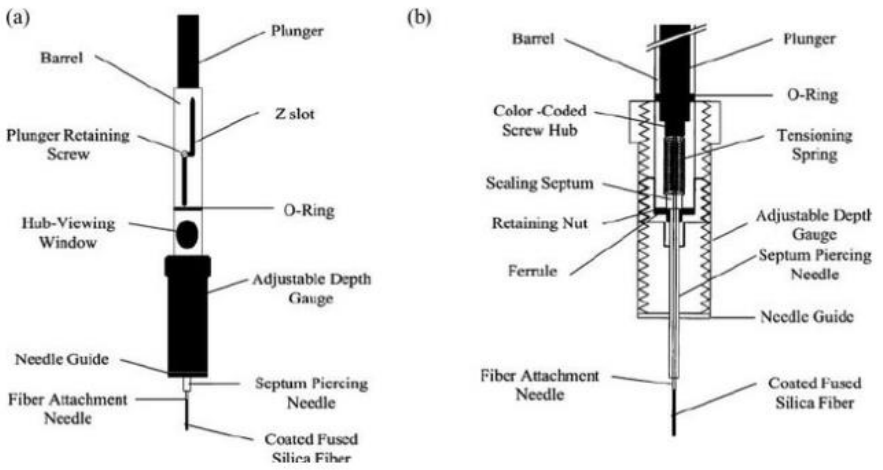

Figure 3. Commercial SPME device: (a) SPME fibre holder and (b) cross section of SPME fibre assembly *(Source: Abdulra'uf et al. 2012)*

When the device is being transferred, the plunger is in the topmost position in the z-slot, indicating that the fibre is safely concealed inside the hollow needle. During extraction and subsequent injection into the chromatograph (GC), the plunger's downward motion along the z-slot forces the fused-silica fibre out of a hollow needle (Kusch 2017). Figure 4 provides a thorough illustration of the entire process.

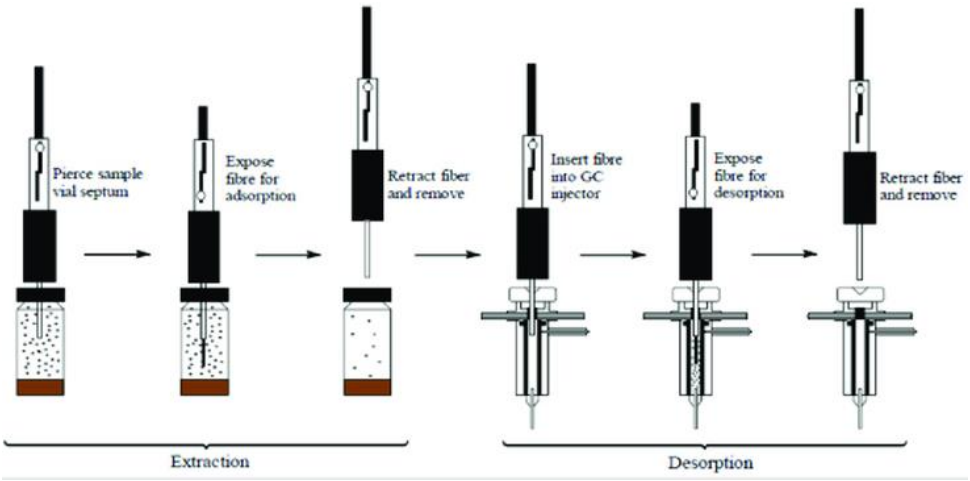

Figure 4. A schematic diagram showing steps of typical SPME extraction and subsequent thermal desorption in GC injector *(Source: Ahmed 2019)*

There are three basic SPME operation modes. The first one is a direct extraction, in which the needle is inserted right into the sample. When the fibre is introduced solely into the headspace just above the sample without contact to the sample at all, this is

referred to as headspace-SPME (HS-SPME) (Lancioni et al. 2022). This technique is frequently used in analysis of samples with high-molecular-weight interferences. Heating the vial speeds up the release of volatile compounds and shortens the extraction process. By reducing headspace volume, extraction sensitivity can be improved. The last technique, membrane-protected SPME, is ideal for accurate identification of samples that simultaneously include non-volatile target analytes and high-molecular weight interfering chemicals. The extraction time is influenced by the amount of time needed for the analyte concentration to balance between the sample matrix and the fibre coating (Pawliszyn 2000). The coating's thickness, for instance, has a big impact on this time among other factors. Thin coatings are needed to absorb or desorb semi-volatile compounds with the greatest amount of efficiency, whereas thick coatings are utilized to monitor volatile substances (Kusch 2017; Mottaleb et al. 2019)

2.6. Gas chromatography and mass spectrometry

By combining two methods gas chromatography-mass spectrometry (GC-MS) compensates the weakness of each method and enhances the quantification and identification of volatile and semi-volatile organic chemical substances in complex mixtures (Settle 1997). Gas chromatograph can physically separate the sample compounds. However, it is unable to properly detect separated particles once they have been physically separated. Soon after its development in the mid-1950s, the gas chromatograph was linked with the mass spectrometer, which has the opposite issue - it gives extensive information about the structure of the compounds, enabling for their precise identification but the device is unable to easily separate the mixture (Settle 1997; Sneddon et al. 2007).

The high-pressure cylinder with a carrier gas supply, pressure regulator, flow controller, sample injector port, column, detector, electrometer, and data processing unit make up a standard gas chromatograph (Aniszewski 2007; Evers 2014). The sample is delivered by an injection port into the inlet (referred to as the injector), either in the form of a liquid solution or an accumulation of molecules absorbed on the outer layer of the fibre (SPME method). Vaporization and on-column injectors are the two main categories of injectors (Forgács & Cserháti 2003). Injectors for vaporization expose the sample to very high temperatures (between 200 and 300 °C). The extract instantly volatilizes and

combines with a steady stream of a carrier gas. The carrier gas must be inert or non-reactive because it merely serves as a background gas to aid in detection.

The most used carrier gases for gas chromatography are often nitrogen or helium as the result of a compromise between inertness, efficiency, and operating cost. Other frequent gas carriers include hydrogen as well as argon (Stauffer et al. 2008; Evers 2014; Stashenko & Ren 2014). On-column injectors, which belong to the second category, do not vaporize. Sample is deposited directly into the column without applying any heat (Forgács & Cserháti 2003).

The mobile phase (gas) and the stationary phase [solid in the case of gas-solid chromatography (GSC) or liquid in the case of gas-liquid chromatography (GLC)], are the two phases that separate the substances in a specific way. The long capillary tube, known as the column, is where the gaseous mobile phase enters the separating section (Forgács & Cserháti 2003; Stashenko & Ren 2014). According to Stauffer et al. (2008), the column is housed inside a temperature-controlled oven. In overall, the chromatographic columns can be divided into two different categories: packed and capillary columns, also referred to as open tubular columns (Harvey 1999). As their name implies, packed columns constructed from glass, stainless steel, copper, are tightly packed with a stationary phase, which is represented by a thin layer of high molecular weight polymer, and a solid support, such as fluorocarbons, diatomaceous earth, graphitized carbon black, or glass beads (Harvey 1999; Forgács & Cserháti 2003). Capillary columns are further split into two main categories. The first of which is wall-coated open tubular column (WCOT) containing a thin layer of stationary phase and coated on the capillary's inner wall. The second type is support-coated open tubular column (SCOT), in which a thin layer of a solid support, such as diatomaceous earth, coated with a liquid stationary phase is attached to the inner wall of the capillary (Harvey 1999; Poole 2003).

The gaseous substance reacts with the stationary phase as the sample and carrier gas stream are passed through the column. The retention times are the intervals at which the molecules continuously elute from the column after being kept by it. According to their physical features, such as boiling point, polarity variations, or molecule size (molecular sieve columns), the column separates individual components of the sample. The volatile compounds are the first to get out of the column. Detectors measure the concentration of each component leaving the column (Harvey 1999; Emerson Automatic

Solutions 2019). The major result of the method is a chromatogram which is a plot of the detector signal as a function of time produced by the GC system. Each chromatogram peak represents a separate combination compound. Peak regions are determined through incorporating the chromatogram, allowing for the quantification of each mole fraction. Figure 5 displays an illustration of a chromatogram that was created from a sample of rice.

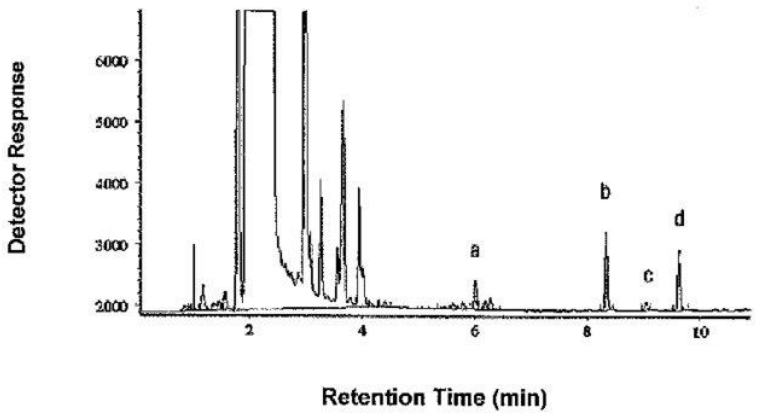

Figure 5. Chromatogram of typical aromatic rice extract obtained by using GC-MS. Peak a, Hexanal; b, 2-Acetyl-1-pyrroline; c, aromatic rice associated peak; and d, 2,4,6-trimethylpyridine (TMP*) (Source: Bergman et al. 2000)*

The typical background signal known as a column bleed may be attributed to degradation products that are eluted from the stationary phase. No matter the quality or origin, it happens to a certain degree in all cases, and does not always indicate a column damage. Bleeding from the column increases with increasing column length, column diameter, and film thickness. Polar phases show slightly higher bleeding rates compared to non-polar phases, whose column life, temperature limits and efficiency tend to be however higher than polar stationary phases (Harvey 1999; Teutenberg et al. 2006; Agilent Technologies 2012).

To obtain peaks, a single point introduction of compound or compounds into the column is needed. Throughout the blank runs, the presence of discrete peaks invariably indicates the contamination of the inlet or front portion of the column. Solvent raising can be used to clear contaminated columns. Discrete peaks in a blank run cannot be caused by a degrading stationary phase since stationary phase degradation is a continuous process while peak formation is the outcome of a single event (momentary sample input into the column) (MSP Kofel 2005).

Mass spectrometry is a very sensitive and precise analytical method for determining molecular structure. A modern mass spectrometer consists of at least an ion source, a mass analyser, a detector, and a data processing system (de Hoffmann 2005).

Ionization is the initial step after inserting the sample into the mass spectrometer. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the two most prevalent ionization techniques. The ionized sample proceeds to a mass analyser, where the ions are sorted based on their mass-to-charge ratio (m/z). Quadrupole, ion trap, time-of-flight, and orbitrap are among the different types of mass analysers which are available (Harvey 1999; Pan et al. 2014; Vandell & Limbach 2017). Finally, selected ions are fragmented and evaluated in the second mass analyser. The ions are detected, their quantity is determined, and they are then transformed into electrical signals after passing through the last analyser. After that, the electrical impulses are analysed and transferred to a computer, where they are presented as a mass spectrum of the molecules (de Hoffmann & Stroobant 2007). The mass spectrum is most often displayed as a bar graph. Every bar represents an ion with a certain mass-to-charge ratio, noted m/z. The length of the bars represents the relative abundance of each ion. The most intense ion (the highest bar) is given an abundance of 100%, when the abundance is stated in absolute form, and the remaining ions are normalised to this value (de Hoffmann 2005; de Hoffmann & Stroobant 2007; Stauffer et al. 2008). In figure 6, the mass spectrum of 2-acetyl-1-pyrroline is shown as an example.

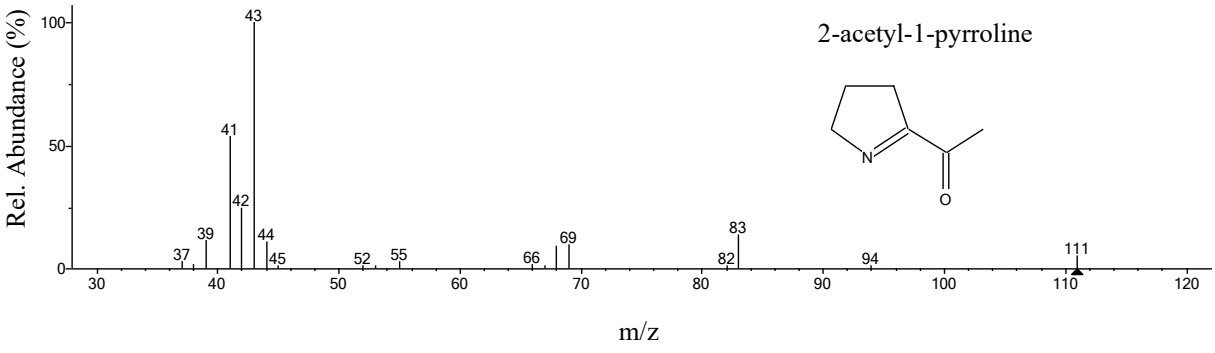

Figure 6. Mass spectrum of 2-acetyl-1-pyrroline library standard

The mass spectrum along with the retention time of the molecule are the most essential properties for the final identification of sample compounds as these two

characteristics are compared and aligned to the standard reference compounds analysed under the same conditions (Kusch 2017).

A quadrupole mass analyser (QMA) is one of the most frequently used mass analysers in contemporary GC-MS. The QMA consists of four metallic rods set parallel to each other with a space in the middle, which act as electrodes. Each opposing pair of rods is electrically connected. The principle that opposite electrodes have identical voltage is used to apply both direct current (DR) and alternate current (AC) voltages (Settle 1997; de Hoffmann 2005; Clarke 2017). Oscillating electrical fields formed around the rods can selectively stabilize or destabilize ions traveling through a radio frequency (RF) quadrupole field formed among the rods, allowing the ions to be filtered based on their mass-to-charge ratio values (m/z) (Thomas 2019). The main drawbacks of this relatively quick and simple operation, which does not require a very high vacuum (> 107 Torr), are particularly poor (usually unit) resolution, a relatively low m/z cut-off, as well as low transmittance (Somogyi 2008). Figure 7 depicts a schematic diagram of a GC-MS system with a quadrupole mass analyser.

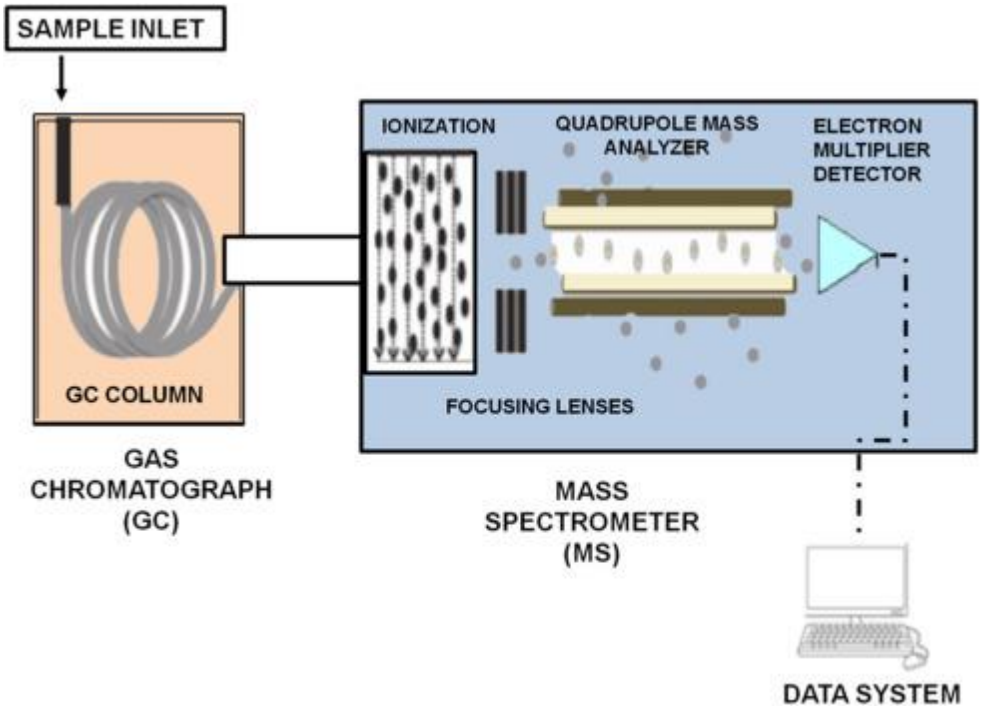

Figure 7. A schematic diagram of a GC–MS with quadrupole mass analyser *(Source: Rudrapal et al. 2022)*

2.7. Principal component analysis

Principal components analysis (PCA) is a multivariate ordination technique that is widely used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible with a minimal loss, as well as to investigate correlations between dependent variables. It is a hypothesis-generating technique used to describe patterns in a data table rather than testing formal statistical hypotheses. PCA assumes linear responses of variables, and performs well over short ecological gradients, with few zeroes in the data. Other than data display, it offers a variety of applications such as multiple regression and variable reduction (Syms 2008; Pongpiachan 2022).

Many applied analytical scientists, spectroscopists, and other users of PCA lack a sufficient knowledge of the linear algebra that underpins it. Also, the meaning of features identified through PCA is frequently ambiguous (Beattie & Esmonde-White 2021).

3. Aims of the Thesis

The main aim of the diploma thesis was to analyse volatile chemical compounds of fifteen samples of grains of Thai jasmine rice. The detection was performed by gas chromatography-mass spectrometry with particular emphasis on the search of the key aroma component, 2-acetyl-1-pyrroline (2AP), which is the distinctive aroma compound in jasmine rice causing popcorn-like flavour.

The specific objectives of this thesis were to determine and compare content of aromatic compounds present in ten samples of the grain of Thai Jasmine rice collected in Chiang Mai region in Thailand with five samples collected in the Czech Republic. The changes in contents of tested components were assessed and evaluated.

Hypothesis

Various studies suggested that the amount of 2AP could be influenced by the type of rice, growing systems, storage conditions and processing; nevertheless, there was no final conclusion on crucial factors influencing 2AP content in rice. That is why this study worked with the null hypothesis that there was no difference in the content of 2AP.

4. Materials and Methods

The experimental part was carried out under the supervision of a competent person in the laboratories of the Faculty of Tropical AgriSciences at the Czech University of Life Sciences Prague.

4.1. Experimental material

Ten samples of grains of Thai Jasmine rice were collected in agricultural company, local market, organic health food store and supermarkets in Chiang Mai region in Thailand and five samples were collected in organic health food stores and supermarkets in the Czech Republic.

Table 2. Explanation of sample coding used in the study

Sample	Code*	Country of purchase	Farming	Type of rice	Point of purchase
S1	CZ_ORG_WHI_21_HLS	Czech Republic	organic	white	health food store
S2	CZ_ORG_WHI_22_HLS	Czech Republic	organic	white	health food store
S3	CZ_ORG_BRO_23_HLS	Czech Republic	organic	brown	health food store
S4	CZ_CON_WHI_24_SUP	Czech Republic	conventional	white	supermarket
S5	CZ_CON_WHI_25_SUP	Czech Republic	conventional	white	supermarket
S6	TH_CON_WHI_1_MAR	Thailand	conventional	white	local market
S7	TH_CON_WHI_2_MAR	Thailand	conventional	white	local market
S8	TH_CON_WHI_3_MAR	Thailand	conventional	white	local market
S9	TH_CON_WHI_2_ACO	Thailand	conventional	white	agricultural company
S10	TH_CON_BRO_11_SUP	Thailand	conventional	brown	supermarket
S11	TH_ORG_RED_7_SUP	Thailand	organic	red	supermarket
S12	TH_ORG_BRO_5_HLS	Thailand	organic	brown	health food store
S13	TH_ORG_WHI_18_SUP	Thailand	organic	white	supermarket
S14	TH_ORG_WHI_26_SUP	Thailand	organic	white	supermarket
S15	TH_ORG_WHI_27_SUP	Thailand	organic	white	supermarket

* codes were used in GC-MS analysis

Abbreviations (Table 2):

CZ = Czech Republic
TH = Thailand
CON = conventional
ORG = organic
WHI = white
BRO = brown
RED = red
HLS = health food store
SUP = supermarket
MAR = market
ACO = agricultural company

4.2. Sample preparation

Three samples from Thai local market were packed in transparent polyethylene plastic bags with reusable zipper closure and twelve samples were maintained sealed in their original packing up to the testing period.

Prior to the analysis, the rice was ground in RETSCH Knife Mill Grindomix GM 100 at an operating speed of 10,000 rpm for approximately half a minute. A 4 ml clear vial was filled with 1 g of milled rice and sealed with a hole cap and PTFE-faced silicone septa.

Figure 8. Preparation of rice samples in the laboratory

4.3. Optimisation of extraction method

Headspace solid phase micro-extraction (HS-SPME), together with gas chromatography and mass spectrometry (GC-MS), were used to investigate volatile compounds in Thai jasmine rice. Experimental conditions including extraction time, temperature, the amount of sample and equilibrium time, were optimised. Optimal conditions were that a 1 g sample was heated at 80 °C for 30 minutes prior to headspace absorption and extracted for 20 min with HS-SPME followed by GC-MS analysis for 32 minutes.

Measurements of volatile substances were performed on fifteen samples. Each measurement was repeated three times and the mean value was calculated. A total of forty-five samples were analysed.

4.4. Extraction

Each sample was equilibrated for 30 minutes in a thermostatic bath at sampling temperature of 80 °C prior to extraction. After being removed from the bath, the head of

the SPME silica fibre with 100 μm thick polydimethylsiloxane (PDMS) film in the manual SPME device was inserted into the vial through a hole in the top and exposed to the headspace for 20 minutes.

The microextraction procedure was optimised according to (Lin et al. 2010; Dias et al. 2019).

4.5. GS-MS analysis

Each day, the fibre was reconditioned at 250 °C before being used for analysis, and the blank measurement took 30 minutes in total. The GC-MS analysis was performed on an Agilent 7890B GC & 5977A Series GC/MSD (Agilent Technologies, USA) equipped with a HP-5MS column 5% Phenyl Methyl Silox (30 m length x 250 μm internal diameter x 0.25 μm film thickness).

After the extraction period was complete, the fibre was removed and inserted quickly into the injection hole of a gas chromatogram. Thermal desorption was carried out directly into the GC injection port at 250 °C and maintained throughout the entire chromatography run, which was set at 32 minutes. With a 0.75 mm i.d. liner, the GC injector port was operated in splitless mode. The optimised GC oven temperature program was 45 °C (5 min) to 250 °C at 10 °C/min (final temperature kept for 5 minutes). Helium was used as the carrier gas with a flow rate of 1 ml/min. Retention Index (RI) values for the volatile substances were calculated by running *n*-Alkanes (Sigma Aldrich) under the identical conditions.

The temperature of the MSD transfer line was maintained at 250 °C with the electron energy of 70 eV. Using a scan time of 1 s, mass spectra were obtained in the mass scan range of m/z 30 to 550.

MassHunter Workstation Software Qualitative Analysis Version B.07.00 was used for data processing. The software also enabled acquiring the peak areas by integration. The identification of the volatile compounds was performed by comparing their mass spectra with the mass spectra contained in the NIST/EPA/NIH Mass Spectral library. The accuracy of identification was confirmed by comparing RI. Due to the lack of some retention indices, not all substances could be verified by RI comparison.

4.6. Data analysis

All data were organised and saved in Microsoft Excel and then further analysed by principal component analysis (PCA). The processed data were uploaded into freely available MetaboAnalyst software for statistical analysis (www.metaboanalyst.ca) (Xia et al. 2009; Xia & Wishart 2011). The table of absolute intensity peaks from GC-MS was transformed into a table of relative intensities in each particular sample and imported into MetaboAnalyst for the statistical analysis (matrix of 5 samples in 3 groups). Samples were normalised by median; the data were normalised using the log transformation and data were auto scaled. Data were separated in three groups using unsupervised PCA.

5. Results

A total of 59 compounds were identified among 15 samples of Thai jasmine rice of different origin from which the highest amount of detected volatile substances were found in the samples collected in the Czech Republic (27) followed by samples of Thai conventional jasmine rice (22) and samples of Thai organic jasmine rice (18) collected in Thailand as shown in Table 3. Where possible, the characteristic odour of the volatile compounds was determined using available literature (see Appendix IV.)

The aroma components of Thai jasmine rice were mainly aldehydes, alcohols, ketones, hydrocarbons, and terpenes, as well as a large number of other volatile compounds with a low abundance, which make up the specific aroma flavour of Thai jasmine rice. Of these, aldehydes were the most abundant.

Although the profile of compounds of each sample group (Thai conventional and organic rice purchased in the Czech Republic S1-S5; Thai conventional rice S6-S10; Thai organic rice S11-S15) was different, the five most abundant volatile substances (listed in the descendent order, as followed) hexanal; acetone; pentanal; 2-pentylfuran and heptanal were found in all the samples, indicating the existence of characteristic compounds in Thai jasmine rice samples. The complete versions of the tables were attached in Appendices (see Appendix V, VI and VII). These compounds belong to the chemical classes of aldehydes (3), ketones (1) and furans (1).

1-Hexanol and (2-aziridinylethyl)amine were found in 14 samples, but were absent in S12. Also, 14 samples contained 1-pentanol except for sample S5; 5-methyl-2-hexanone (except for S6). Hydroxyurea; 2-methylbutanal; 3-methylbutanal; dimethyl disulfide; octane; 2,2,4,4,6-pentamethylheptane; octanal; nonanal and 2,6-dimethyldecane were present in some samples of each group (Thai conventional and organic rice purchased in the Czech Republic; Thai conventional rice; Thai organic rice).

Only the rice samples purchased in the Czech Republic contained 2-methyl-1-propanol; α-pinene; 1-(2-methoxypropoxy)-2-propanol; 2-furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-; O-decylhydroxylamine; 2-nonen-1-ol; 3-carene; camphor; m-xylene. 3-Methylpentanal; o-cymene; 3,5-octadien-2-ol; 5-undecene; 2-hexyl-1-decanol; heptane; 2-octene; dodecane, 2,6,11-trimethyl-; and 4-methylundecane were only present in samples of rice purchased in Thailand. As shown in Appendix V and

Appendix VII), a trace amount of 2AP was found manually in rice samples (S1, S2 and S15).

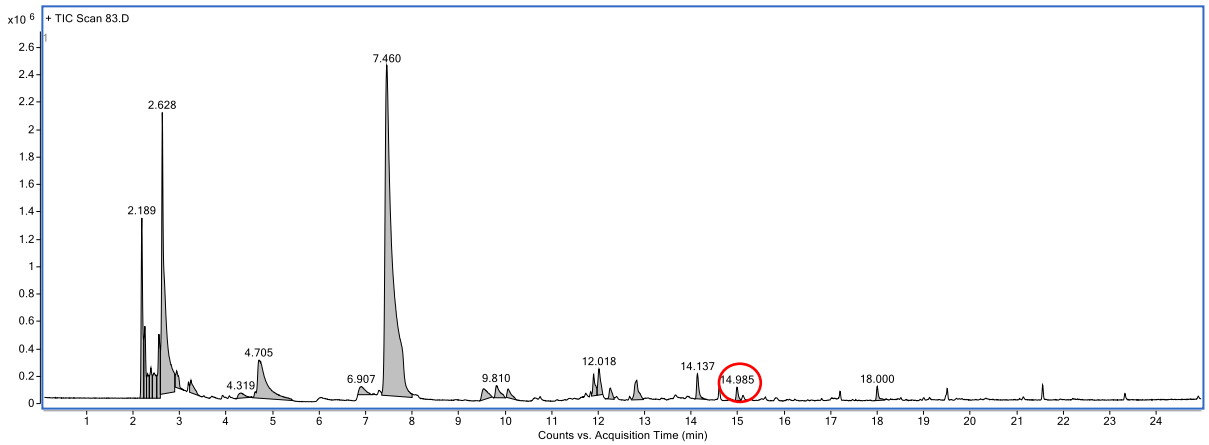

Figure 9. Example chromatogram of the single sample CZ_ORG_WHI_22_HLS (S2) containing camphor (highlighted in red)

Table 3. Overview of the volatile compounds identified in Thai jasmine rice

Volatile compound	RI (C)	RI (L)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Acetone	*	**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2-Butanone	*	**		x	x	x	x					x					
1-Butanol	*	**		x	x	x			x	x							
2-Methyl-1-propanol	*	**			x												
Hydroxyurea	*	**	x	x	x	x	x	x		x	x				x	x	x
(2-Aziridinylethyl)amine	*	**	x	x	x	x	x	x	x	x	x	x	x		x	x	x
2-Methylbutanal	*	**	x		x		x				x		x				
3-Methylbutanal	*	**	x		x		x				x		x			x	
Pentanal	*	**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2-Hydroxypropanamide	*	**	x	x	x	x	x	x	x	x	x						
1,3-Butanediol	*	**	x	x	x	x	x										
(3S)-1,3-Butanediol	*	**						x	x	x			x	x	x	x	x
3-Methylpentanal	*	**											x				
Heptane	*	**										x					
5-Methylhexanal	*	**			x					x							
3-Methylbutan-1-ol	748	747			x								x				
Dimethyl disulfide	749	751	x	x		x	x		x	x	x		x		x	x	x
Toluene	778	778			x	x	x										
1-Pentanol	790	779	x	x	x	x		x	x	x	x	x	x	x	x	x	x
2-Octene	790	810										x					
Octane	810	**			x						x	x		x		x	x
Hexanal	816	812	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
5-Methyl-2-hexanone	871	857	x	x	x	x	x		x	x	x	x	x	x	x	x	x
m-Xylene	890	888					x										
1-Hexanol	896	880	x	x	x	x	x	x	x	x	x	x	x		x	x	x

Table 3. (Continued)

Volatile compound	RI (C)	RI (L)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
o-Xylene	900	908			x		x										
Heptanal	931	914	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2-Acetyl-1-pyrroline	939	935	x	x													x
(2Z)-2-Heptenal	960	964										x		x			
α-Pinene	966	954			x												
1-Heptanol	987	978										x		x			
2,2,4,4,6-Pentamethylheptane	1018	997		x							x		x		x	x	
Octanal	1020	1009	x	x		x			x				x	x	x		
2-Pentylfuran	1020	1010	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
o-Cymene	1033	1027												x			
Decane	1034	**					x				x	x					
3-Carene	1052	1031			x												
Hexanoic acid	1054	1036										x		x			
3,5-Octadien-2-ol	1060	1039												x			
1-(2-Methoxypropoxy)-2-propanol	1062	**			x												
5-Ethylcyclopentene-1-carbaldehyde	1067	1053										x		x			
Eucalyptol	1070	1053						x	x								
D-Limonene	1072	**	x	x		x											
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-	1075	1074			x												
O-Decylhydroxylamine	1093	1100				x											
2-Nonen-1-ol	1096	1105			x												
(E)-2-Nonenal	1111	1144	x					x	x	x							
Isopulegol	1112	1138				x	x										
5-Undecene	1116	1092												x			

Table 3. (Continued)

Volatile compound	RI (C)	RI (L)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Nonanal	1124	1113	x	x		x			x	x				x	x		
4-Methylundecane	1132	1158									x						
2-Hexyl-1-decanol	1140	**														x	
2-Hexyl-1-octanol	1159	**									x					x	
2,6-Dimethyldecane	1164	1112					x				x						x
Dodecane	1197	1200					x				x						x
Camphor	1204	1180		x													
Isomenthol	1225	1182	x					x	x	x							
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-	1234	1232						x	x								
2,6,11-Trimethyldodecane	1239	1275									x						

* data out of calibration

** not available in the literature

Abbreviations (Table 3):

RI (C) = Retention index (calculated)
RI (L) =Retention index (library)

S1 = CZ_ORG_WHI_21_HLS
S2 = CZ_ORG_WHI_22_HLS
S3 = CZ_ORG_BRO_23_HLS
S4 = CZ_CON_WHI_24_SUP
S5 = CZ_CON_WHI_25_SUP
S6 = TH_CON_WHI_1_MAR
S7 = TH_CON_WHI_2_MAR
S8 = TH_CON_WHI_3_MAR
S9 = TH_CON_WHI_2_ACO
S10 = TH_CON_BRO_11_SUP
S11 = TH_ORG_RED_7_SUP
S12 = TH_ORG_BRO_5_HLS
S13 = TH_ORG_WHI_18_SUP
S14 = TH_ORG_WHI_26_SUP
S15 = TH_ORG_WHI_27_SUP

Identified volatile compounds were used for the principal component analysis (PCA), since this analysis took into account both the particular characteristics of the samples and the relationships between the studied variables, thus showing similarities and differences between the samples.

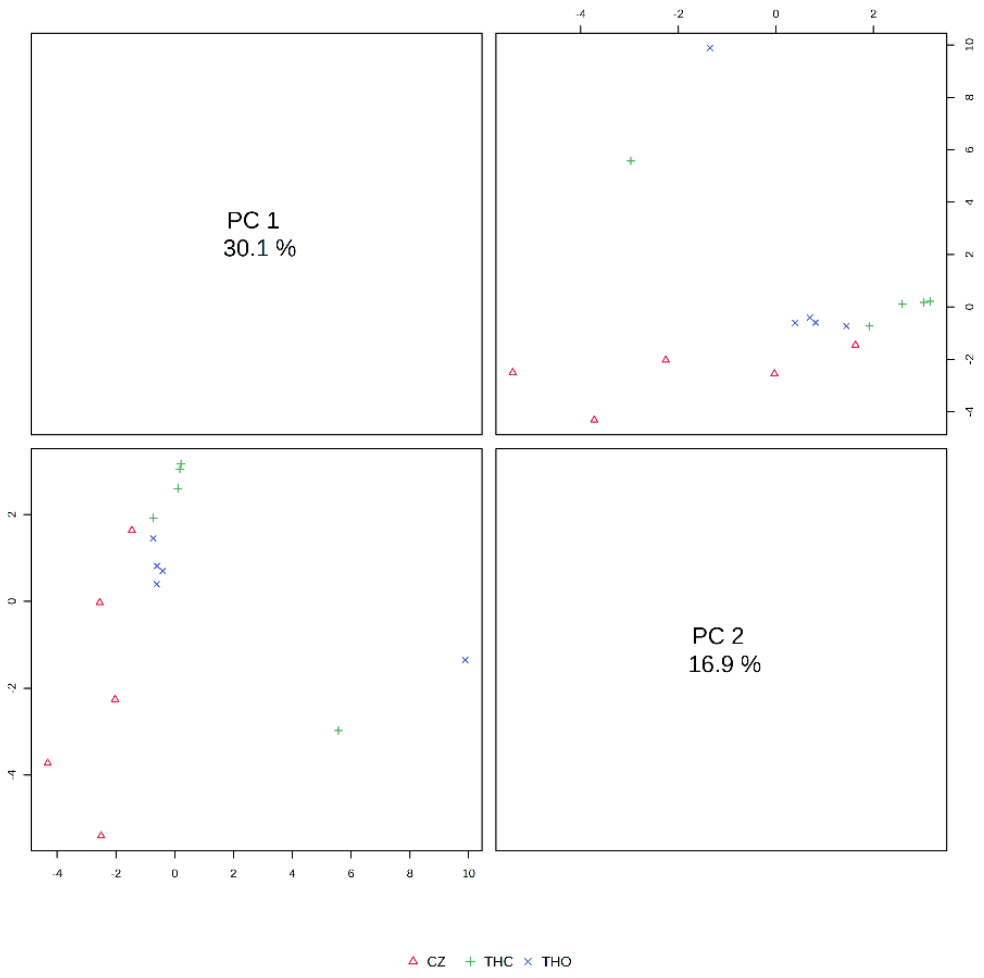

Figure 10. Principal component analysis (PCA) score plot showing sample clustering of volatile components of Thai jasmine rice of different origin I

Abbreviations (Figure 10; Figure 11):
CZ = samples of conventional and organic rice (S1-S5) purchased in the Czech Republic
THC = samples of conventional rice (S6-S10) purchased in Thailand
THO = samples of organic rice (S11-S15) purchased in Thailand

For the results of PCA of aromatic compounds in Thai jasmine rice samples of different origin, see Figure 10. As shown in Figure 11, the contribution of PC 1 is 30.1%

and PC 2 is 16.9%, and the cumulative contribution of both is up to 47%, which indicated clear separation in the different groups (CZ, THC and THO).

The differences in characteristics between the samples can be visualised in Figure 11, where all three sample groups are mostly close to each other, as well as the parallel results of each sample are close to one another and some of them almost overlap with the exception of the CZ sample group where the distances between the samples are nearly equal. This is due to the relatively obvious differences in the characteristics of the sample groups of Thai jasmine rice of different origin and the good reproducibility of the individual samples.

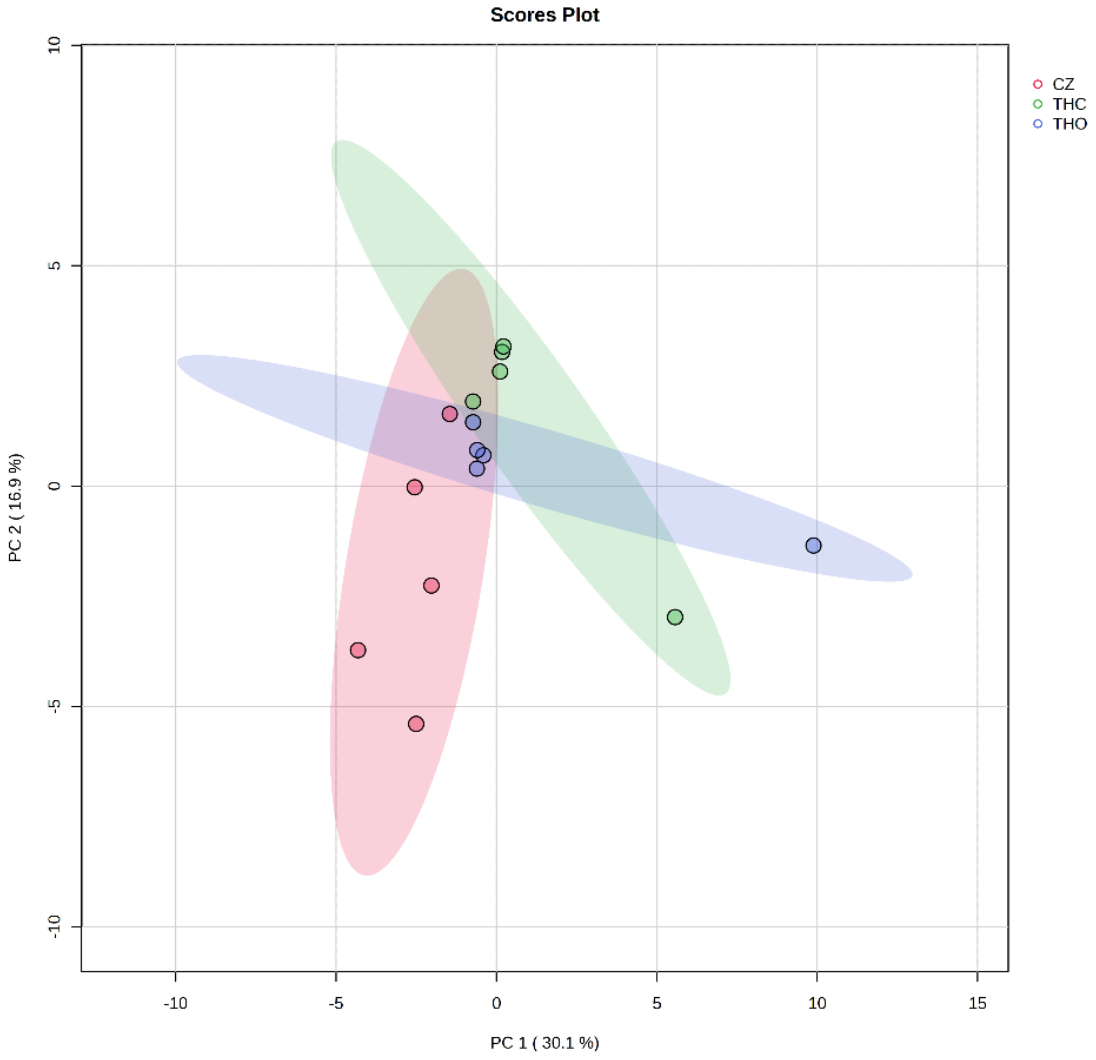

Figure 11. Principal component analysis (PCA) score plot showing sample clustering of volatile components of Thai jasmine rice of different origin II

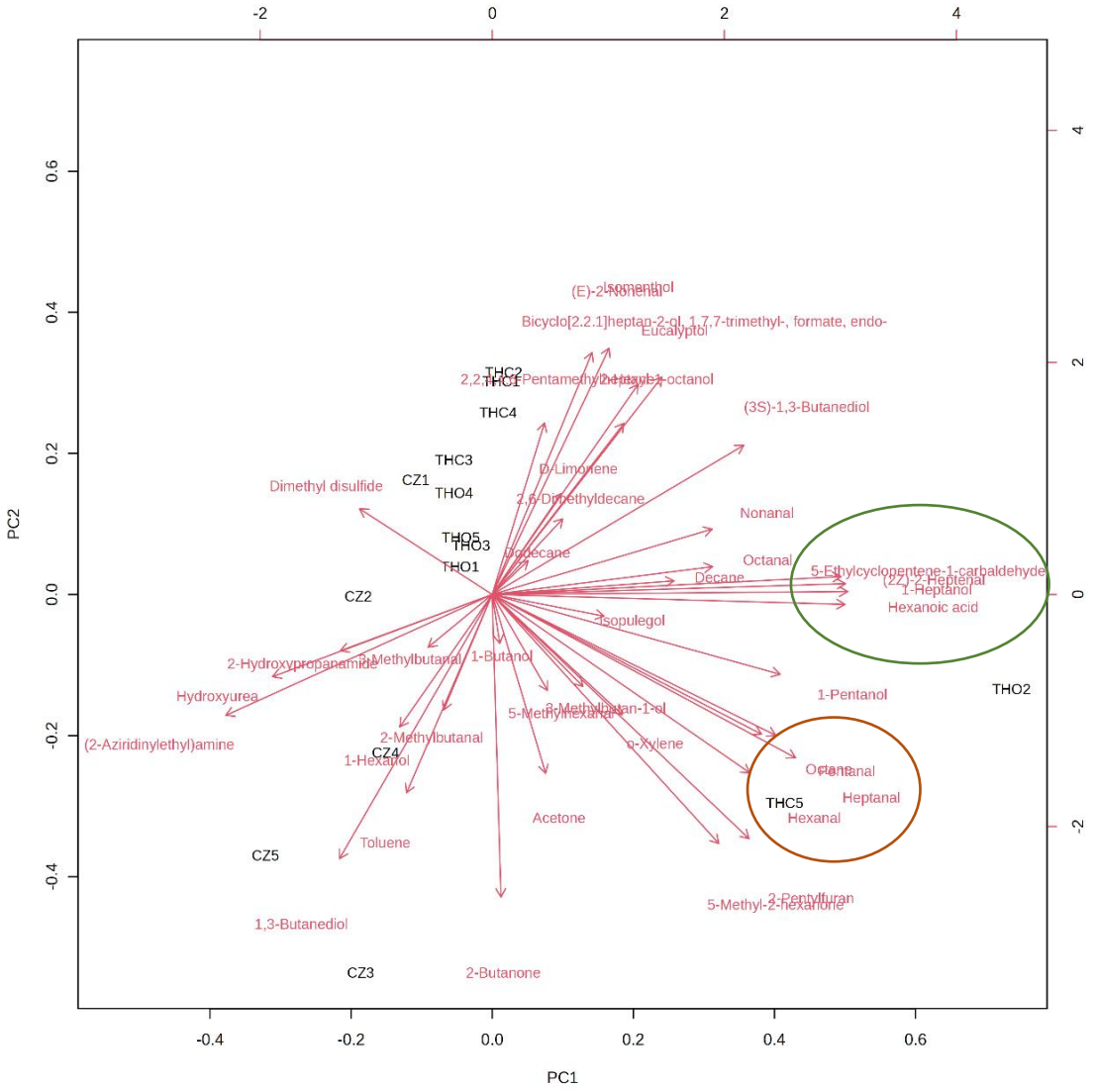

Figure 12. Principal component analysis (PCA) plot of volatile organic compounds contained in all Thai jasmine rice samples collected at different locations

Abbreviations (Figure 12):

CZ1-CZ5 = samples of conventional and organic rice (S1-S5) purchased in the Czech Republic

THC1-THC5 = samples of conventional rice (S6-S10) purchased in Thailand

THO1-THO5 = samples of organic rice (S11-S15) purchased in Thailand

Figure 12 shows the volatile compounds heptanal, hexanal, pentanal, and octane (highlighted in brown) that are responsible for the different volatile profile of brown rice sample THC5 (S10). The volatile profile of another brown rice sample THO2 (S12) is influenced by hexanoic acid, 1-heptanol, (2Z)-2-heptenal, and 5-ethylcyclopentene-1-carbaldehyde (highlighted in green). These four aromatic compounds were found only in samples THC5 and THO2 (both highlighted in Figure 12), and the difference among them

is that THO2 contained them in higher concentrations (for the exact amount, see Appendix VI and VII). The rice samples collected in the Czech Republic contain more toluene, 2-butanone, 1,3-butanediol, acetone, (2-aziridinylethyl)amine and hydroxyurea than samples collected in Thailand. Groups THC and THO containing samples S6-S15 show similarities in content of volatile compounds and differ from samples S1-S5 from group CZO.

Samples CZ1 (S1), CZ2 (S2), CZ3 (S3) and THO2 (S12) purchased from health food stores showed almost no similarities except that they were organic. Samples S4 (CZ4), S5 (CZ5), S10 (THC5), S11 (THO1), S14 (THO4) and S15 (THO5) also differed from each other. Similarities were observed for the conventional white rice, of which S6 (THC1), S7 (THC2) and S8 (THC3) were purchased in the local market and S9 (THC4) was purchased from the agricultural company.

6. Discussion

The aromatic rice has a distinctive aroma for the whole time - "from field to plate". The strength of the aroma is considered to be very important due to the key compound 2AP, which is responsible for the pleasant popcorn-like aroma (Itani et al. 2004). The results of the search for 2AP in our study corresponded with the previously published data on volatile flavour components in rice showing that their research did not detect 2-acetyl-1-pyrroline in the cooked rice (Singh et al. 2000). We found 2AP manually only in two samples purchased in the Czech Republic and one sample collected in Thailand, not giving us the information about the concentration amount of this key volatile compound of scented rice. It was assumed that their detection method was not sensitive enough. In comparison to our study, in which 27 volatile compounds were identified in Thai conventional and organic jasmine rice collected in the Czech Republic, 22 volatile compounds were identified in Thai conventional jasmine rice and 18 in Thai organic jasmine rice purchased in Chiang Mai region in Thailand, their study analysed 29 aromatic compounds in total. If this was a consequence of the low sensitivity of the chosen method, it is also possible to use a different method for the determination of aromatic compounds in rice. Mathure et al. (2011) used HS-SPME together with GC-Flame Ionization Detector (FID) to quantify 2AP and other rice aroma volatiles. The extraction was performed under the same optimised conditions as in our study with the only difference that they added 300 µl of odour-free water to the samples. The standard addition approach was used to generate aroma calibration curves. In 33 scented and 2 non-scented rice samples, the optimised conditions were employed for quantitative analysis. 2AP was present in all samples examined. The GC-FID method was rapid and efficient but required the use of standards, which were not used in our study due to their high cost.

Lin et al. (2010) used headspace solid phase micro-extraction, together with gas chromatography and mass spectrometry, to identify volatile compounds in rice. 100 ml glass bottles with 20 g milled samples of brown rice were used for their testing. In our research, there was an absence of water in glass vials with prepared plant material (white, brown, and red rice), compare to their rice samples prepared with added water in ratio 1:2 which were heated for 30 minutes at 80 °C prior to headspace absorption, and then extracted for 30 minutes with HS-SPME. The volatile compounds found in indica rice

were alcohols, aldehydes, ketones, esters, hydrocarbons, organic acids, as well as heterocyclic compounds. According to their research, aldehydes were the most abundant as it was found in our study. As in our study, the presence of the aromatic compound 2AP was not detected. It could be caused due to the addition of water to the samples but more likely due to the shorter extraction time (30 minutes). According to their findings, the selection of the length of extraction duration is the most efficient at 60 minutes. This would also support previous findings that extraction duration is one of the key attributes for obtaining higher volatile content.

Dias et al. (2019) studied aroma profile of rice varieties with an effort to maximize 2AP and minimize hexanal extraction. The volatile compounds were obtained by using HS-SPME with 60 minutes incubation of 2.5 g grounded sample at 80 °C and exposure of the divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibre in the headspace for 10 minutes. In this study, specifically jasmine rice was analysed for volatile compounds using this technique coupled to a gas chromatograph with mass spectrometer detector (GC-MS). 39 volatile compounds were found including 2AP, the essential compound in aromatic rice, in comparison to our 59 analysed aromatic compounds which however excluded searched 2AP indicating that not all the compounds present in a sample contribute to its overall aroma. In our study, we used the same methodology, but a smaller amount of sample (1 g) and a shorter extraction time (20 minutes) were used. Therefore, the extraction time and the size of the sample may have a significant impact on extraction efficiency. This optimised method could help in obtaining higher content of volatile compounds and releasing the desired aroma of rice.

In our study, hexanal was identified in all 15 samples and it was the compound with highest concentration in 11 of them. That could be one of the reasons for the low abundance of 2AP in our samples. The 2AP may have slowly volatilized of rice samples by the time. There are numerous other factors that affect the quality of the rice itself and its aroma, as the aroma strength of aromatic rice varies with the genetic and environmental conditions (Itani et al. 2004). In the case of our samples, we have no knowledge of how and where they were grown, what chemicals were used, how the rice was stored, in what packaging materials, or at what temperature. In addition to the development of unfavourable volatiles as above mentioned hexanal, the loss of 2AP during storage was a significant factor in the degradation of rice aroma according to Tulyathan &

Leeharatanaluk (2007). In their research, they found that after two months of storage, the 2AP content in KDML 105 rice decreased by around 75%, and by eight months, it had dropped to 7%. Another study also proved that the concentration of 2AP in Khao Dawk Mali 105 reduced as storage time increased. Also, higher 2AP and lower concentrations of off-flavouring compounds were produced at temperatures that were lower (Dutta et al. 2022). Yoshihashi (2002) assumed that lower storage temperatures would reduce the volatilization of 2AP from rice, but Widjaja et al. (1996) reported that no method had been discovered to help preserve the desired 2AP. After three months, the 2AP concentration in the rice decreased to 40–50% regardless of how it was stored - whether it was white rice, brown rice, or paddy rice, and whether it was vacuum- or air-stored. Zhao et al. (2020) found that storage duration and temperature had an impact on the volatile contents in samples of jasmine rice. As a result, this study revealed how the flavour of jasmine rice changed under various storage conditions and recommended that it was essential to avoid long-term storage, particularly at high temperatures, to minimise flavour loss during storage.

According to the results of PCA, the sample groups divided, and we could observe that the samples of rice purchased in the Czech Republic contained the highest amount of volatiles (27), from which 9 were only observe within their group. They were not very similar in their content of aromatic compounds, and also contained higher amounts of 6 common aromatic compounds than the samples collected in Thailand. Unlike the Czech rice samples, samples of rice collected in Thailand showed similarities in their content of aromatic compounds and differed only within 2 samples as they contained some volatiles in higher concentrations. Regarding the occurrence of 2AP, it was found only in one sample of organic rice purchased in Thailand and in two organic samples purchased in the Czech Republic. This could be explained by the allegation that the jasmine rice of premium quality is mainly for export and the fact that these samples were in certified organic quality. Even though 2AP has been established as a universal aroma principle, no economically feasible method has been scaled up to quantify 2AP for the purpose of ensuring the quality of marketed scented rice. As a result, consumers are subjected to fragrant rice admixing and are deceived by paying higher costs for less aromatic rice (Wakte et al. 2011).

7. Conclusions

SPME was a simple, sensitive, and rapid method for the screening of volatile compounds in the headspace of Thai jasmine rice samples. The combination of SPME with GC-MS provided detailed and accurate information on the chemical composition of fifteen samples of *Oryza sativa* L. ssp. *indica*. Single compounds and compounds with higher occurrence were found, however only 5 compounds common to all rice samples were identified namely acetone, pentanal, hexanal, heptanal, and 2-pentylfuran, which could be classified as characteristic volatile compounds from the rice samples analysed. The rice samples collected in Thailand, in both organic and conventional quality, showed very similar composition of aromatic compounds in comparison with rice samples collected in Czechia which differed in the composition of its volatiles. Although this study found separation of the samples into the groups, the origin was not confirmed to have a major effect on the amount of substances in the volatile profile within our sample groups, and there were no major differences in the place of purchase of the examined samples of rice. Since many previous studies suggested that the amount of 2AP could be affected by great deal of various factors, we found not sufficient evidence to support the claim that there would be no difference in the content of 2AP, so we failed to reject the null hypothesis. Based on prior research, it was evident that the methodology was not uniform, particularly in terms of extraction time and sample amount, which had a substantial impact on the extraction efficacy. Therefore, it is crucial to optimise SPME conditions for aromatic rice, fix the optimum length of extraction, increase the amount of the samples as well as prevent long-term storage and avoid high temperatures in order to minimise aroma loss during storage. Such an optimised method could be further employed to obtain a higher content of volatile compounds and thus help to release the desired aroma of rice.

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Appendix III. Photos of Thai organic rice samples (S11-S15)

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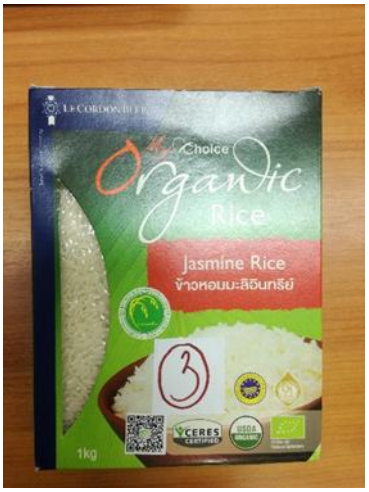

Appendix IV. Odour description of the volatile compounds found in Thai jasmine rice (S1-S15)

Volatile compounds	Odour description*
Acetone	Pungent, fruity
2-Butanone	Sharp, sweet
1-Butanol	Rancid, sweet
2-Methyl-1-propanol	Ethereal
Hydroxyurea	–
(2-Aziridinylethyl)amine	–
2-Methylbutanal	Malty
3-Methylbutanal	Malty
Pentanal	Woody, fruity
2-Hydroxypropanamide	–
1,3-Butanediol	Fruity
(3S)-1,3-Butanediol	Fruity
3-Methylpentanal	–
Heptane	Alkane
5-Methylhexanal	–
3-Methylbutan-1-ol	Malty, burnt
Dimethyl disulfide	Sulphury
Toluene	Sweet, pungent
1-Pentanol	Moderately strong
2-Octene	–
Octane	Alkane
Hexanal	Green tomato, green, grass-like
5-Methyl-2-hexanone	Fruity, pleasant
m-Xylene	Plastic
1-Hexanol	Woody, sweet, herbaceous
o-Xylene	Geranium
Heptanal	Fatty, rancid, fruity
2-Acetyl-1-pyrroline	Popcorn, sweet, and pleasant
(2Z)-2-Heptenal	Fruity
α-Pinene	Pine, turpentine
1-Heptanol	Herbal, sweet, woody
2,2,4,4,6-Pentamethylheptane	Irrigating odour
Octanal	Citrus, fruity, floral, fatty
2-Pentylfuran	Floral, fruit, nutty, beany
o-Cymene	Gasoline, citrus
Decane	Alkane
3-Carene	Sweet, pungent
Hexanoic acid	Low heavy waxy with a creamy, candle waxy nuance
3,5-Octadien-2-ol	Fruity, fatty, mushroom
1-(2-Methoxypropoxy)-2-propanol	Mild odour

Appendix IV. (Continued)

Volatile compounds	**Odour description***
Eucalyptol	Eucalyptus, herbal, camphor
D-Limonene	Fresh, sweet
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis	–
O-Decylhydroxylamine	–
2-Nonen-1-ol	Green type
(E)-2-Nonenal	Beany, cucumber, fatty, woody and tallowy
Isopulegol	Minty
5-Undecene	–
Nonanal	Grassy, citrus, floral
4-Methylundecane	–
2-Hexyl-1-decanol	Mild, sweet
2-Hexyl-1-octanol	Waxy
2,6-Dimethyldecane	–
Dodecane	Gasoline like
Camphor	Slightly minty
Isomenthol	Minty, musty, woody
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-	Green, earthy
Dodecane, 2,6,11-trimethyl-	Fried-oil-like

** (Source: Singh et al. 2000; Bryant & McClung 2011; Hinge et al. 2016; Setyaningsih et al. 2019; Zheng et al. 2022)*

Appendix V. Area (× 10^3) of the volatile compounds identified in samples of Thai conventional and organic jasmine rice collected in the Czech Republic

Volatile compound	**Area ø**				
	S1	**S2**	**S3**	**S4**	**S5**
Acetone	4330	9038	33215	36360	32070
2-Butanone		543	3832	964	3213
1-Butanol		321	1081	225	
2-Methyl-1-propanol			401		
Hydroxyurea	472	2146	4554	804	2874
(2-Aziridinylethyl)amine	2375	3378	13702	7466	11643
2-Methylbutanal	90		649		221
3-Methylbutanal	346		392		532
Pentanal	900	3747	1703	7045	8151
3-Methylbutan-1-ol			2585		
1-Pentanol	547	551	10142	2773	
2-Hydroxypropanamide	1298	1172	4800	3637	7513
1,3-Butanediol	102	509	2351	871	2130
(3S)-1,3-Butanediol					
Toluene			344	911	32353
Dimethyl disulfide	224	331		2765	1964
Heptane					
Hexanal	6980	24736	16295	63764	67870
3-Methylpentanal					
1-Hexanol	2147	8787	3392	17573	27766
m-Xylene					1232
o-Xylene			469		524
(2Z)-2-Heptenal					
2-Octene					
5-Methyl-2-hexanone	194	739	7467	2512	1769
Heptanal	147	508	561	905	768
2-Acetyl-1-pyrroline	trace*	trace*			
5-Methylhexanal			6368		
Octane			4904		
1-Heptanol					
Hexanoic acid					
5-Ethylcyclopentene-1-carbaldehyde					
3,5-Octadien-2-ol					
Octanal	196	357		336	
o-Cymene					
α-Pinene			4782		
3-Carene			6736		
D-Limonene	304	775		321	
2-Pentylfuran	443	855	14440	2907	2059
Decane					1054
Nonanal	550	604		1516	

Appendix V. (Continued)

Volatile compound	Area ø				
	S1	**S2**	**S3**	**S4**	**S5**
1-(2-Methoxypropoxy)-2-propanol			4368		
Camphor		298			
5-Undecene					
Eucalyptol					
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-					
Isopulegol				2103	1348
Isomenthol	142				
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-			477		
Dodecane, 2,6,11-trimethyl-					
2,2,4,4,6-Pentamethylheptane		604			
4-Methylundecane					
O-Decylhydroxylamine				922	
2-Nonen-1-ol			1767		
Dodecane					1383
2,6-Dimethyldecane					2284
2-Hexyl-1-octanol					
2-Hexyl-1-decanol					
(E)-2-Nonenal	127				
TOTAL COMPOUNDS IDENTIFIED	20	20	27	21	22

* concentration below the limit of detection

Abbreviations (Appendix V):
S1 = CZ_ORG_WHI_21_HLS
S2 = CZ_ORG_WHI_22_HLS
S3 = CZ_ORG_BRO_23_HLS
S4 = CZ_CON_WHI_24_SUP
S5 = CZ_CON_WHI_25_SUP

Appendix VI. Area (× 10^3) of the volatile compounds identified in samples of Thai conventional jasmine rice collected in Thailand

Volatile compound	**Area ø**				
	S6	**S7**	**S8**	**S9**	**S10**
Acetone	9566	10078	8370	1663	6483
2-Butanone					1286
1-Butanol		181	202		
2-Methyl-1-propanol					
Hydroxyurea	447		208	82	
(2-Aziridinylethyl)amine	1199	1563	1161	413	1267
2-Methylbutanal				55	
3-Methylbutanal				79	
Pentanal	499	1360	1185	275	13258
3-Methylbutan-1-ol					
1-Pentanol	274	526	1396	201	7099
2-Hydroxypropanamide	1289	756	1620	416	
1,3-Butanediol					
(3S)-1,3-Butanediol	303	600	295		
Toluene					
Dimethyl disulfide		294	195	213	
Heptane					2560
Hexanal	2806	6384	6836	1634	65176
2-Acetyl-1-pyrroline					
3-Methylpentanal					
1-Hexanol	669	995	1781	556	33627
m-Xylene					
o-Xylene					
(2Z)-2-Heptenal					1909
2-Octene					4045
5-Methyl-2-hexanone		90	269	358	9261
Heptanal	94	81	172	90	2410
5-Methylhexanal			468		
Octane				136	9784
1-Heptanol					867
Hexanoic acid					1877
5-Ethylcyclopentene-1-carbaldehyde					2426
3,5-Octadien-2-ol					
Octanal		69			
o-Cymene					
α-Pinene					
3-Carene					
D-Limonene					
2-Pentylfuran	120	205	256	223	14720
Decane				976	1447
Nonanal		363	453		

Appendix VI. (Continued)

Volatile compound	Area ø				
	S6	**S7**	**S8**	**S9**	**S10**
1-(2-Methoxypropoxy)-2-propanol					
Camphor					
5-Undecene					
Eucalyptol	330	135			
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-	146	152			
Isopulegol					
Isomenthol	140	182	172		
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-					
Dodecane, 2,6,11-trimethyl-				1035	
2,2,4,4,6-Pentamethylheptane				3201	
4-Methylundecane				737	
O-Decylhydroxylamine					
2-Nonen-1-ol					
Dodecane				287	
2,6-Dimethyldecane				1228	
2-Hexyl-1-octanol				101	
2-Hexyl-1-decanol					
(E)-2-Nonenal	87	134	191		
TOTAL COMPOUNDS IDENTIFIED	15	19	18	22	18

Abbreviations (Appendix VI):
S6 = TH_CON_WHI_1_MAR
S7 = TH_CON_WHI_2_MAR
S8 = TH_CON_WHI_3_MAR
S9 = TH_CON_WHI_2_ACO
S10 = TH_CON_BRO_11_SUP

Appendix VII. Area (× 10^3) of the volatile compounds identified in samples of Thai organic jasmine rice collected in Thailand

Volatile compound	Area ø				
	S11	S12	S13	S14	S15
Acetone	10071	6205	9248	6213	5460
2-Butanone					
1-Butanol					
2-Methyl-1-propanol					
Hydroxyurea			970	369	915
(2-Aziridinylethyl)amine	3897		2091	3213	2730
2-Methylbutanal	287				
3-Methylbutanal	443			158	
Pentanal	5015	67008	3600	1610	1977
3-Methylbutan-1-ol	468				
1-Pentanol	2351	32988	1307	520	752
2-Hydroxypropanamide					
1,3-Butanediol					
(3S)-1,3-Butanediol	1122	7116	678	845	558
Toluene					
Dimethyl disulfide	302		631	347	330
Heptane					
Hexanal	21314	294746	25086	13105	14271
2-Acetyl-1-pyrroline					trace*
3-Methylpentanal	336				
1-Hexanol	5116		9518	4603	5344
m-Xylene					
o-Xylene					
(2Z)-2-Heptenal		4672			
2-Octene					
5-Methyl-2-hexanone	480	18173	1071	502	509
Heptanal	423	13403	538	232	247
5-Methylhexanal					
Octane		4928		194	257
1-Heptanol		4008			
Hexanoic acid		36090			
5-Ethylcyclopentene-1-carbaldehyde		3670			
3,5-Octadien-2-ol		5610			
Octanal	628	11829	316		
o-Cymene		5973			
α-Pinene					
3-Carene					
D-Limonene					
2-Pentylfuran	1998	38572	850	569	727
Decane					
Nonanal		5802	1017		

Appendix VII. (Continued)

Volatile compound	**Area ø**				
	S11	**S12**	**S13**	**S14**	**S15**
1-(2-Methoxypropoxy)-2-propanol					
Camphor					
5-Undecene		4535			
Eucalyptol					
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-					
Isopulegol					
Isomenthol					
2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-					
Dodecane, 2,6,11-trimethyl-					
2,2,4,4,6-Pentamethylheptane	1254		338	800	
4-Methylundecane					
O-Decylhydroxylamine					
2-Nonen-1-ol					
Dodecane					463
2,6-Dimethyldecane					2408
2-Hexyl-1-octanol				292	
2-Hexyl-1-decanol				427	
(E)-2-Nonenal					
TOTAL COMPOUNDS IDENTIFIED	17	18	15	17	15

* concentration below the limit of detection

Abbreviations (Appendix VII):
S11 = TH_ORG_RED_7_SUP
S12 = TH_ORG_BRO_5_HLS
S13 = TH_ORG_WHI_18_SUP
S14 = TH_ORG_WHI_26_SUP
S15 = TH_ORG_WHI_27_SUP