IDENTIFICATION OF AROMA COMPOUNDS IN THAI COLORED RICE VARIETIES

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การพิสูจน์เอกลักษณ์ของสารให้ความหอมในพันธุ์ข้าวสีของไทย

นายศุภวัสส์ จินดาวัฒน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ผู้บริโภคมีความสนใจข้าวสีเนื่องจากมีประโยชน์ต่อสุขภาพ อีกทั้งยังมีปริมาณไฟเบอร์และโปรตีนสูง โดยปกติกลิ่นของข้าวสีโดยทั่วไปจะไม่หอมเท่าข้าวหอมมะลิ นำมาสู่วัตถุประสงค์แรกของงานวิจัย เพื่อศึกษาเวลาและอุณหภูมิที่เหมาะสมในการสกัดสารระเหยในข้าวสีพันธุ์พื้นเมืองของไทยด้วยวิธีเฮดสเปซแก๊สโครมาโตกราฟฟี-แมสสเปคโตรเมทรี และวัตถุประสงค์ประการที่สอง เพื่อระบุสารระเหยที่สำคัญของข้าวสีพันธุ์พื้นเมืองของไทย สำหรับการศึกษาแรกนำข้าวหอมแดง (KDML105R-PSL-E-14) มาทำการศึกษาพบว่า สภาวะที่เหมาะสมคือ ระยะเวลาการอุ่นในเตาอบที่ 80 องศาเซลเซียส เป็นเวลา 5 ชั่วโมง ตามด้วยการสกัดบริเวณหัวฉีดเฮดสเปซที่อุณหภูมิ 120 องศาเซลเซียส จากนั้นนำสภาวะดังกล่าวมาใช้ในการศึกษาที่สอง ซึ่งทำการศึกษาพันธุ์ข้าวจำนวน 23 พันธุ์ แบ่งเป็น 4 ประเภท คือ ข้าวขาวหอม ข้าวสีดำมีกลิ่นหอม ข้าวสีดำไม่มีกลิ่นหอม และข้าวสีแดงไม่มีกลิ่นหอม (n=10 ต่อสายพันธุ์) จากการศึกษานี้พบว่า ข้าวสีดำและสีแดงที่ไม่มีกลิ่นหอมมีปริมาณสารหอมระเหยต่ำกว่าข้าวขาวและข้าวสีดำที่มีกลิ่นหอม เมื่อมุ่งความสนใจไปที่ข้าวสีดำที่มีกลิ่นหอม พบว่ามีสารหอมระเหยที่น่าจะเป็นสารหลัก 2 กลุ่มคือ อัลดีไฮด์ (3-เมทิลบิวทานัล 2-เมทิลบิวทานัล 2-เมทิลโพรพานัล เพนทานัล และ เฮกซานัล) และแอลกอฮอล์ (บิวเทน-2,3-ไดออล เพนทาน-1-ออล และ เฮกซัน-1-ออล)

สาขาวิชา	อาหารเคมีและโภชนศาสตร์ทางการแพทย์	ลายมือชื่อนิสิต ....
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Supawat Jindawatt : IDENTIFICATION OF AROMA COMPOUNDS IN THAI COLORED RICE VARIETIES. Advisor: Asst. Prof. ROSSARIN TANSAWAT, Ph.D. Co-advisor: Prof. WANCHAI DE-EKNAMKUL, Ph.D.

Consumers are interested in colored rice because its health benefits and its high fiber and protein content. Normally, the colored rice smell is generally not as strong as the jasmine rice. The first objective of this research was to investigate the proper preheated time and headspace incubation temperature to extract as many volatile compounds as possible from Thai-colored rice using the static headspace GC-MS approach. Secondly, this study aimed to identify the key volatile aroma compounds in Thai native-colored rice varieties. For optimum conditions, Red Hawm Rice (KDML105R-PSL-E-14) was used in this study. The optimal condition was preheated in a hot air oven for 5 hours at 80 °C, followed by a headspace extraction temperature of 120 °C. Afterward, Such conditions were utilized in the second study. Twenty-three rice varieties in four categories: aromatic white, aromatic black, non-aromatic black, and non-aromatic red, were investigated (n = 10 per variety). This study found that the non-aromatic black and red rice containing much lower content of most volatile constituents than the aromatic black and white rice. Focusing on the aromatic black rice, the samples appeared to contain high level of both compound groups of aldehydes (3-methylbutanal, 2-methylbutanal, 2-methylpropanal, pentanal, and hexanal) and alcohols (butane-2,3-diol, pentan-1-ol, and hexan-1-ol).

Field of Study:	Food Chemistry and Medical Nutrition	Student's Signature ....
Academic Year:	2022	Advisor's Signature ....
		Co-advisor's Signature ....

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RESEARCH ORIENTATION

This compilation thesis was composed of two published articles and constructed in accordance with Chulalongkorn University's guidelines. The thesis outline includes the following five chapters:

Chapter I: BACKGROUND AND RATIONALE, which clarifies the main research question of thesis and explains connections with these two articles

Chapter II: LITERATURE REVIEW, a comprehensive overview of relevant information from other research documents

Chapter III: PUBLISHED ARTICLE I, which is written according to the Thai Journal of Pharmaceutical Sciences's guideline, Red Hawm Rice (KDML105R-PSL-E-14), a popular Thai colored rice, was developed from aromatic Jasmine Rice 105 (KDML105). Thus, aromatic compound research should begin with it.

Jindawatt, S., Ekkaphan, P., De-Eknamkul, W. and Tansawat, R. (2021). Static headspace GC-MS analysis for determination of colored rice volatile profile. *The 36th International Meeting in Pharmaceutical Sciences & Herbal Tradition Medicines 2021*, 17-20.

Chapter IV: ARTICLE II (under review), part of a research paper written in accordance with Frontiers in Plant Science guidelines

Tansawat, R., **Jindawatt, S.**, Ekkaphan, P., Ruengphayak, S., Vanavichit, A., Suttipanta, N., Vimolmangkang, S., De-Eknamkul, W. (2022). Metabolomics Approach to Identify Key Volatile Aromas in Thai Colored Rice Cultivars. *Frontiers in Plant Science*.

Chapter V: SUMMARY, a conclusion that integrates all pieces of work in this thesis and coherently presents key findings of both publications.

This chapter also includes a discussion of the study's limitations and suggestions for future avenues of inquiry building on this research.

Chapter I

BACKGROUND AND RATIONALE

Alterations are being made all the time in consumer behavior (Ali & Ali, 2020). As an integral component of modern health care, the consumption of nutritious foods is a major focus of modern society. Consumers are more willing to pay greater prices than usual to get the foods that are cultivated rich in antioxidants and essential nutrients (De Magistris & Gracia, 2008; Krystallis & Chryssohoidis, 2005). One of the keys to a healthy diet and sustainable food production is to eat more plant-based meals and fewer that are animal-based (Langyan et al., 2021; Päivärinta et al., 2020). The global market for plant-based foods is anticipated to increase from 29.4 billion USD in 2020 to 161.9 billion USD in 2030 (Statistica, 2022). Consumers are interested in colored rice because to its health benefits, especially its antioxidant effects, which are stronger than in white rice (Sansenya & Nanok, 2020; Walter et al., 2013), and its high fiber and protein content, which makes it perfect as a plant-based food.

Rice, a member of the Poaceae (Gramineae) family, has a long breeding history. It is one of the major crop grains, particularly in Asian countries, because it is a primary energy source for human body. More than 400,000 rice germplasm accessions are stored in the gene bank around the world (Toriyama et al., 2004), suggesting that the chemical diversity across rice varieties is large. Thailand is the outstanding source of many fragrant rice varieties including Thai Jasmine rice (Khao Dok Mali 105 and RD 15) which are the most famous fragrant rice in the world. For the medical perspective, aromas of food can help increase appetite (Zoon et al., 2016). Thus, fragrant rice may be useful in the elderly or patients with chronic illness who can eat less than usual.

In addition to Thai Jasmine rice, which is white rice, Thailand also has many colored varieties of rice, such as Jao Hom Nin, Riceberry, Black Sticky Rice, Red Rice, Purple Rice, Sang Yod Rice, and Sang Rak Arun Siam Rice, which are of interest to many consumers. These rice varieties are primitive species with specific identities. It has dark in color ranging from red, brown to black, due to the accumulation of the antioxidant compounds such as proanthocyanidins, anthocyanins, flavonoids, and phenolic acids, which bring even more attention from several health-conscious consumers. Additionally, the characteristic of fragrant rice is that it has special breeding to produce and store the aroma compounds in each part of it, especially in the kernel. Nowadays, 2-acetyl-1-pyrroline (2AP) is the identified compound known to the key contribute to rice aroma.

Aroma compounds are classified as secondary metabolites. More than 300 aroma components have been identified in rice. In addition to the 2AP, fragrant rice also contains many aroma compounds such as hexanal, nonanal, octanal, *trans*-2-nonenal, (E, E)-nona-2,4-dienal, heptanal, 1-octen-3-ol, 4-vinyl phenol, 4-vinyl guaicol, decanal, pentanal, *trans*-2-octenal, guaiacol, and vanillin (Daygon et al., 2016). However, most of the previous studies just reported the aroma profiles of white fragrant rice. There is lack of needed information about the chemical characteristics of aroma profiles of these colored rice varieties, and the specific identities of their aromas that differ from the typical white fragrant rice.

Conceptual framework and Objectives

The conceptual framework are summarized as follows in this thesis: Supplemental Figure S1. Red Hawm rice was used to modify two parameters, preheated time and incubation headspace temperature, beginning with the optimization procedure described in Chapter III. The resulting volatile profile was then utilized to determine the optimal condition to be implemented in Chapter IV. As a result, volatile profiles that can be used to determine the distinctiveness of Thai native-colored rice are developed.

The contents are as follows: the first part of this thesis requires the development of a method for identifying its volatile components (Jindawatt et al., 2021). The objectives of the first study were to investigate the proper preheated time and headspace incubation temperature to extract as many volatile compounds as possible from Thai colored rice using the static headspace GC-MS approach. After finding a suitable condition, this method was used in the second part of this research. The second objective of this study was to analyze the types of aromatic compounds in Thai colored rice varieties including aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice.

Benefit of the study

The findings of volatile profiles may one day be put to use to persuade individuals to consume colored rice varieties rather than white rice types for the purpose of improving their overall health and increasing the market worth of the nation.

Chapter II
LITERATURE REVIEW

Rice

Botanical information

Rice, a member of the Poaceae (Gramineae) family, is classified into 24 species (two cultivated and 22 wild). The two cultivated species, including Asian cultivated rice (*O. sativa*), and African cultivated rice (*O. glaberrima*) (Wei & Huang, 2019). More than 400,000 rice germplasm accessions are stored in the gene bank around the world (Toriyama et al., 2004), suggesting that the chemical diversity across rice varieties is large. The rice plants consist of root, culm, leaf, panicle and spikelet. It usually takes 3–6 months from germination to maturity, depending on the variety and the environment under which it is grown (Yoshida, 1981b). The growth of rice plants is divided into 3 stages which are vegetative, reproductive, and ripening (Figure 1) (Moldenhauer et al., 2013). First, the vegetative stage refers to a period from germination to the initiation of panicle, which means active tillering, gradual increase in plant height, and leaf emergence at regular intervals. Second, the reproductive stage refers to a period from panicle initiation to heading, which means culm elongation, heading (panicle exsertion), and flowering (spikelet anthesis). Third, the ripening stage refers to a period from heading to maturity, which means leaf senescence and grain growth. The length of ripening, most of which is affected by the temperature, ranges from about 30 days in the tropics to 65 days in cool and temperate regions such as Hokkaido, Japan and New South Wales, Australia (Yoshida, 1981b). The rice grain is shown in Figure 2, which enveloped by the husk (hull). When rice is brought into the shelling process, and brown rice which composed of the seed coat, nucleus, endosperm, is produced (Khir & Pan, 2019). The endosperm consists of the aleurone layer which enclose the embryo, and the starchy (Juliano, 2003). The white rice is produced by removing of outer layer of brown rice or milling process (Khir & Pan, 2019).

Geography and climate for cultivation

Presently, every continent (except Antarctica) can be used in the cultivation of rice (Figure 3), from northeastern China at latitude 53°N to New South Wales, Australia, at 40°S and from sea level in Kerala, India to an altitude of 3 km in Kashmir, India, and Nepal (Juliano, 2003; Yoshida, 1981a), with a concentration in tropical and temperate regions of Asia (Wei & Huang, 2019). In addition, It can be cultivated in both rainfed and irrigated conditions (Laborte et al., 2017), in both flooded (anaerobic soil) and upland (aerobic) conditions because it has air channels that connect to its roots (Juliano, 2016). Consequently, rice is cultivated two or three times per year in irrigated areas (Laborte et al., 2017).

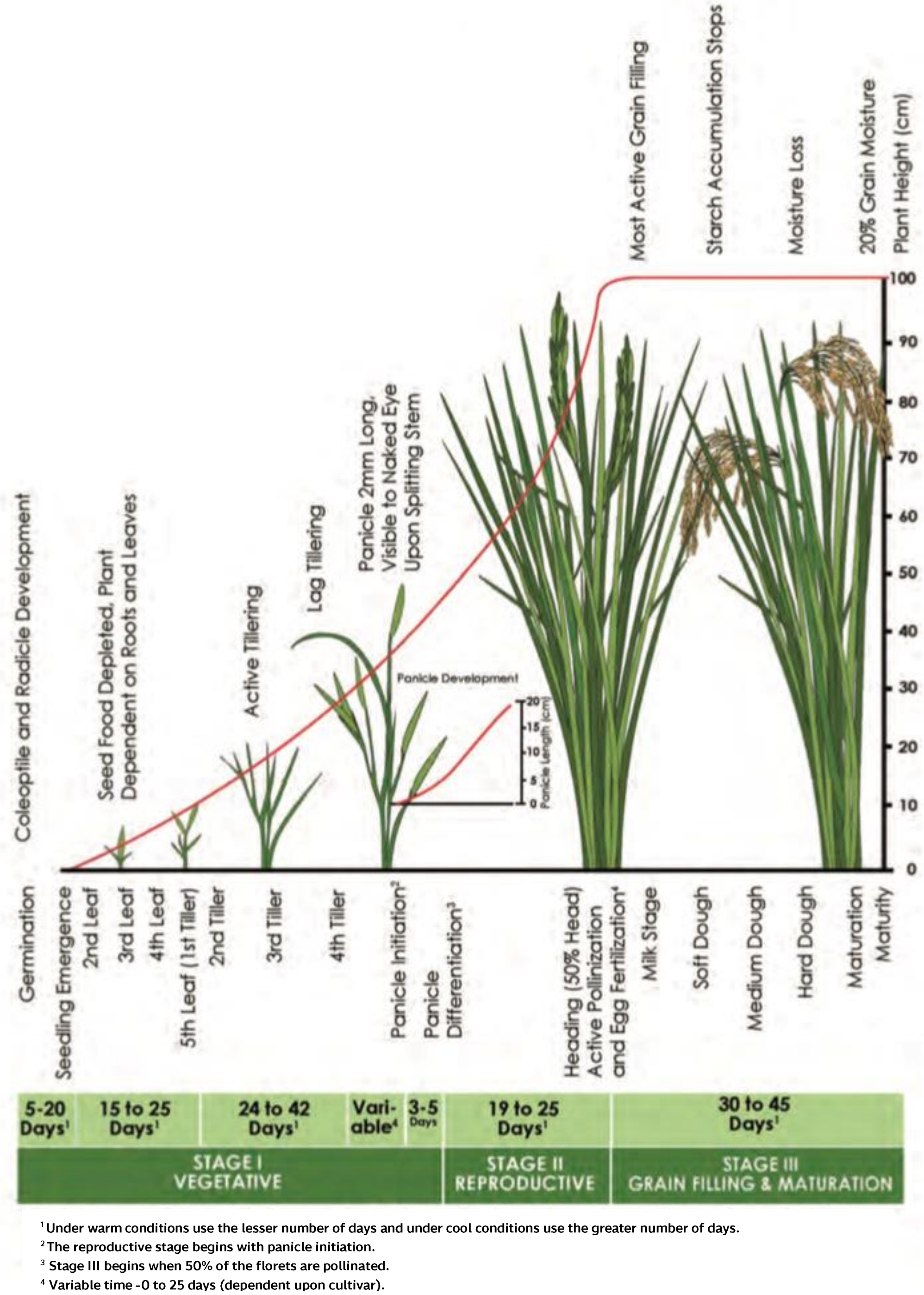


Figure 1 Developmental stages of the rice plant (adapted from Moldenhauer et al. (2013))

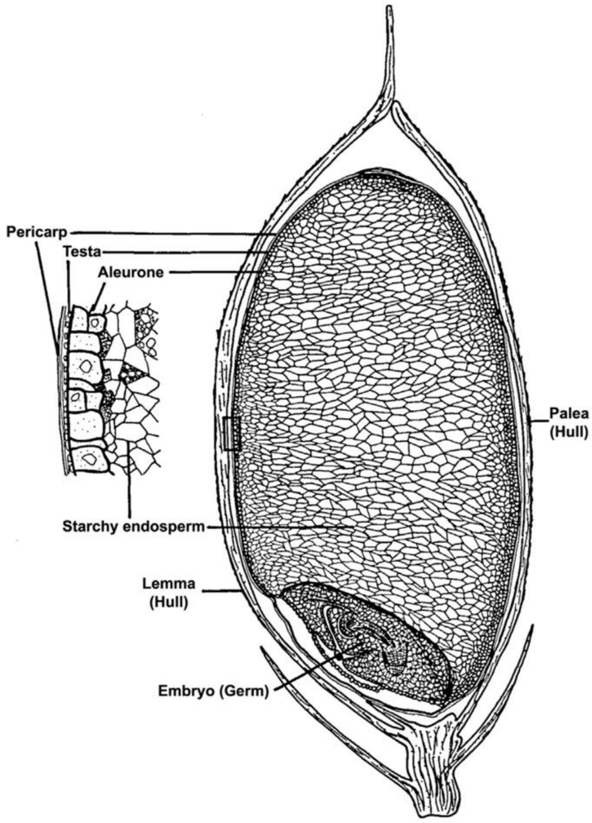


Figure 2 Structure of rice grain (Khir & Pan, 2019).

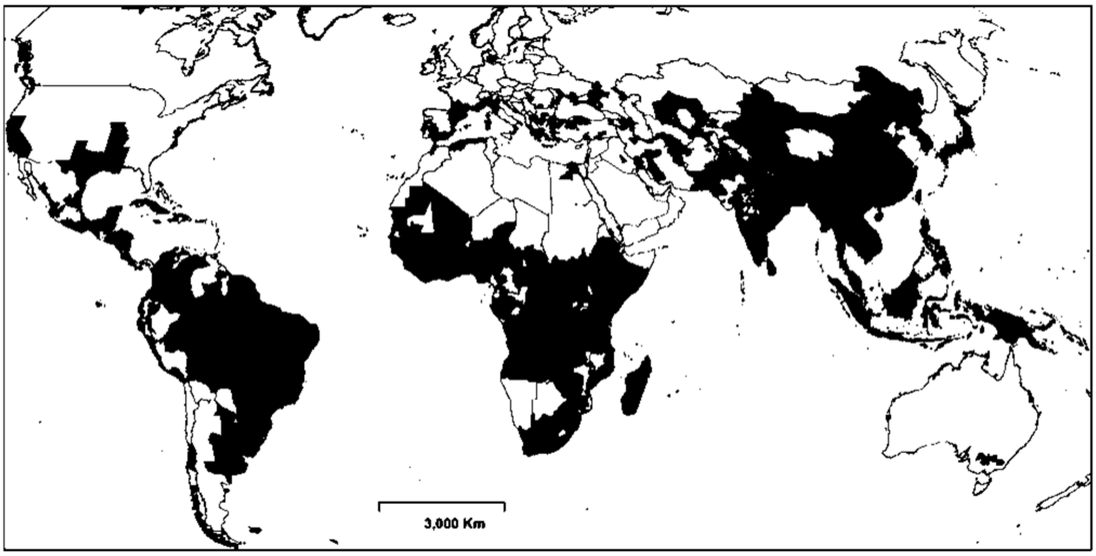


Figure 3 Spatial photo shows rice cultivating regions. (adapted from Laborte et al. (2017))

Since, the climate is influenced by geography, which temperature, solar radiation, and rainfall influence rice growing by directly affecting the physiological processes involved in grain production, and indirectly through diseases and insects. In the temperate regions, irrigated rice cultivation starts when spring temperatures are between 13°C and 20°C; the crop is harvested before temperatures drop below 13°C in the autumn. For, the tropics regions where temperature is suitable for rice growth throughout the year and irrigation is not available in most places, cultivation starts with the rainy season. In both the tropics and the temperate regions, rice yields are primarily determined by the level of incident solar radiation. In the tropics, the dry season rice usually produces higher yields than the wet season because it receives more sunlight (Yoshida, 1981a). Consequently, China and India are the major rice production countries, which can be cultivated about 50% of the world's rice. It is mainly grown in the Yangtze River basin, southeast coastal area, and northeast area of China, while India use the Gangetic Plain and coastal areas are the major rice cultivation (Wei & Huang, 2019).

Rice processing and storage

Rice processing can be defined as a set of operations, which consists of harvesting, drying, and milling. Initial. When the rice is ripening, it is inputted to harvesting process, which consisted of cutting, collecting, threshing the rice crop, separating, and cleaning the grains. This process can be used technology to increase yields, is resulting loss of only 1-2% yields. Harvesting process brings about rough rice (paddy rice) as a major product and straw as a by-product (Khir & Pan, 2019). Then, rough rice needs to be dried as soon as possible for prolonging the storage life of rice by slowing down respiration and preventing deterioration due to molds and insect attack (Khir et al., 2011). Nowadays, infrared (IR) heating is widely used in drying process because it offers many advantages compared to conventional drying (sun) methods (Sharma et al., 2005), which provides a high heating rate, rapid moisture removal, effective disinfestation and disinfection, good rice milling quality, and storage stability (Pan et al., 2008). Afterward, rough rice is entered to milling process, consisted of dehusking, whitening, polishing, and separation, which remove the outer layer from the endosperm simply accessible for human consumption. Approximately 65-72% of rough rice in milling process is edible rice (Singh et al., 2014).

Storage condition after rice processing have a big impact on yields and quality of the final product (Champagne, 2008). Many of the key factors effect in rice quality degrade during storage, such as rodents, birds, insects, microorganisms like fungi, moisture, and temperature. Especially, storage temperature and humidity are the most important environmental factors affecting rice quality (Chen et al., 2015). During storage, the rice aroma can change, mainly because of oxidation and losses over time (Griglione et al., 2015). Therefore, the deterioration of rice quality was accelerated by undesirable storage conditions (Pomeranz & Zeleny, 1971).

Park et al. (2012) reported that milled rice was stored at high temperatures (30 °C and 40 °C) showed higher fat acidity than rice stored at low temperatures (4 °C and 20 °C), which fat acidity is commonly used as an index of quality deterioration during rice storage because lipid dissolution progresses more rapidly than that of protein and starch (Genkawa et al., 2008). Likewise, the study by Choi et al. (2019) reported that high temperature (35 °C) was not appropriate for storage of unmilled or milled black rice because it promoted lipid oxidation, producing volatile compounds. Additionally, previous studies showed that the levels of straight chain aldehydes, such as octanal, hexanal, (*E*)-2-octenal, and 2-nonenal in rice were significantly increased during storage of non-colored rice and various factors affect lipid-oxidation-related volatiles (Griglione et al., 2015; Tananuwong & Lertsiri, 2010). Similarly, Biao et al. (2019) reported that volatile compounds such as aldehydes, ketones, and furans increased when rice was stored under high temperature-high humidity conditions, leading to a pronounced deterioration in rice quality. The relatively high content of aldehydes in the rice produced a detectable rancid odor.

Furthermore, higher storage temperatures (30 °C and 40 °C) significantly decreased all sensory values ($P < 0.05$) even after 1 month of storage (Park et al., 2012). Undesirable temperature affects rapidly the deterioration of rice quality. So, the grain should be stored in bags/vessels, bulk or hematic containers. The study by Norkaew et al. (2017) reported that Nylon/LLDPE pouches containing N_2 was the most suitable packaging for preserving the key aroma compound 2-acetyl-1- pyrroline (2AP) (Figure 4), total phenolic, and anthocyanin contents of unpolished aromatic black rice.

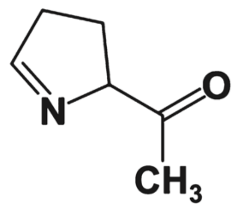


Figure 4 Structure of 2-acetyl-1-pyrroline (2AP) (Wakte et al., 2017).

Fragrant rice varieties and volatile compounds

"Farmers are the backbone of the nation." It has been said for generations in Thailand. If this saying is true; consequently, rice which cultivated by farmers, is one of the important economic plants of Thailand. In 2010, Thailand was the biggest exporter of fragrant rice; 2.65 million tons of jasmine rice were exported (Wei & Huang, 2019), but in 2017 China's rice production was approximately 172 million tons, ranking first in the world (Nie & Peng, 2017), overtaking India, Pakistan and Thailand.

However, quality is still more important than quantity, in the opinion of consumers, the aroma of the rice has a big impact on consumers (Griglione et al., 2015). As a recent sensory study of Champagne et al. (2010), using popular varieties grown throughout South and Southeast Asia, revealed a characteristic flavor profile for those varieties commonly grown and consumed in Southeast Asia consisting of sweet, floral, grassy and dairy notes. In addition, globalization of cuisines and migration of rice consumers from asia to western countries have both led to an increase in demand for both Basmati rice (Ferrero, 2004) and Jasmine rice in western countries (Suwansri et al., 2002). Consequently, Thai Jasmine rice (e.g. Khao Dawk Mali 105) and Basmati rice (Indian fragrance rice) have remained world-renowned and have been in demand among consumers until now.

Moreover, the fragrant rice varieties are a high value agricultural product in the economy of the producing country, which are sold at a premium price in local and export markets because of their superior grain qualities and pleasant and distinctive aroma (Calingacion et al., 2014). In terms of aroma, study has shown that fragrant rice from different parts of the world contain the popcorn-like fragrant compound, 2-acetyl-1-pyrroline (2AP) (Buttery et al., 1983), and carry the same mutation in the fragrance (Bradbury et al., 2005; Fitzgerald et al., 2008). Hence, aroma is one of the quality markers for rice (Kovach et al., 2009) and 2AP is the key discriminator between fragrant and non-fragrant rice and many studies have focused on the concentration of 2AP in different rice varieties.

2AP was first identified in rice by Buttery et al. (1982), and is regarded as the most important aroma compound in rice, especially fragrant rice (Buttery et al., 1983). The IUPAC name of 2-acetyl-1-pyrroline (2AP) is 1-(3,4-dihydro-2H-pyrrol-5-

yl)ethanone, its CAS number is 85213-22-5 and its FEMA (Flavour and Extract Manufacturers Association) number is 4249 (Wei et al., 2017). It was described as popcorn-like aroma, together with its low odor threshold (Schieberle, 1991), but it an important contributor to a food's aroma when present (Bradbury et al., 2005; Fitzgerald et al., 2009).

Later, Buttery et al. (1988) found that 2AP in fragrant rice as 15 times more than in non-fragrant rice. Also, it can only be formed in the aerial parts of plants during growing in paddy fields (Yoshihashi, 2002). 2AP synthesis starts in the early vegetative stage, and accumulates to increase concentration in mature grains of fragrant rice is three times higher than in leaves, and finally gets accumulated in rice seeds (Routray & Rayaguru, 2018). The formation, accumulation, and forms of 2AP present in rice are determined by external factors, such as chemical parameters, agricultural practices, geographical locations, prevailing climatic conditions, cultivar types and genetic makeup (Bhattacharjee et al., 2002; Champagne, 2008; Routray & Rayaguru, 2018).

Various studies have shown that environmental factors affect to 2AP synthesis in rice. Initially, Yoshihashi et al. (2004) showed that more 2AP would be synthesized when rice is grown in a dry climate, due to increased accumulation of its precursor proline. For example, Khao Dawk Mali 105, a famous Thai fragrant rice variety. Samples of this variety were collected from an area under drought conditions, and

2AP accumulation was higher in these samples than that in rice samples collected from an area under non-drought conditions. Likewise, The effects of varying draining and harvesting time on rice sensory properties was studied and stable flavor was observed with timing of field draining (14-day span) and harvesting (32–48 days after flowering) (Champagne et al., 2005).

In addition to, some of studies noted that aroma formation and retention in grain was enhanced at low temperature during the grain filling stage. Basmati requires relatively cooler temperature (25 °C/21 °C day/night during crop maturity) for best retention of aroma (Mann, 1987). Also, Singh et al. (2000) reported that a Basmati variety would be more aromatic if cultivated in an area having relatively cool temperatures in the afternoon (25–32 °C) and night (20–25 °C) with humidity of about 70 – 80% during primordial and grain filling stages. Later, several studies confirmed that drought (Mannan et al., 2012), salinity (Gay et al., 2010; Wijerathna et al., 2014), and crop management practices such as plant populating, harvesting time (Goufo, Wongpornchai, et al., 2010), and storage conditions (Tananuwong & Lertsiri, 2010; Widjaja et al., 1996a) all affected 2AP accumulation or concentration in fragrant rice grains, due to higher proline accumulation in plants from environmental stresses (Szabados & Savouré, 2010).

Additionally, Yang et al. (2010) have found that application of nitrogenous fertilizer strengthens the aroma, by increasing free proline content in rice whereas low fertilizer tends to result in lower 2AP content. In consequence, nitrogen is an important factor which increased 2AP formation in aromatic rice during the development (Itani et al., 2004; Yoshihashi et al., 2002). Moreover, other organisms can influence the increment of 2AP synthesis in rice. Li et al. (2019) found that rice-duck co-culture not only improved the yield and grain quality of fragrant rice, but also promoted both the precursors of 2AP biosynthesis and 2AP accumulation itself. Because, ducks increase the availability of nutrients, and stimulate the physiology of rice plants as they move around the rice fields.

Biosynthesis of 2AP in rice was discovered to be inhibited by a "dominant *BADH2* allele (located on chromosome 8) encoding betaine aldehyde dehydrogenase (*BADH2*)" which are shown in Figure 5 (Jodon, 1944; Kadam & Patankar, 1938; Lorieux et al., 1996). The mechanism was described as follows, normally, gene *BADH2* encodes an enzyme, betaine aldehyde hydrogenase (*BADH2*) which catalyzes the oxidation of 4-aminobutanal to 4-aminobutanoic acid (GABA). Non-fragrant rice varieties contain the *BADH2* gene and a functional *BADH2* enzyme. Whereas fragrant varieties have the mutated *BADH2* gene and so produce a non-functional enzyme. The mutated *BADH2* gene incurs a deletion of eight base pairs in exon 7, leading to early gene termination and production of a truncated non-functional *BADH2* enzyme (Bradbury et al., 2008; Kovach et al., 2009; Vanavichit et al., 2008). Consequently, this non-functional enzyme will not be able to oxidize 4-aminobutyraldehyde, leading to a build-up of 1-pyrroline and hence increased 2AP synthesis (Bradbury et al., 2005; Bradbury et al., 2008).

Afterwards, Huang et al. (2008) proposed additional biosynthetic pathway of 2AP that did not involve *BADH2*. Glutamate was converted to 1-pyrroline-5-carboxylate then, it undergoes a reaction with methylglyoxal, giving rise to 2AP. Similarly, Fitzgerald et al. (2008) found that additional mutations in the pathway leading to 2AP synthesis came from a rigorous study involving a diverse panel of fragrant germplasm that identified several accessions, mostly from Southeast Asia, that had elevated levels of 2AP but did not carry the *BADH2* allele.

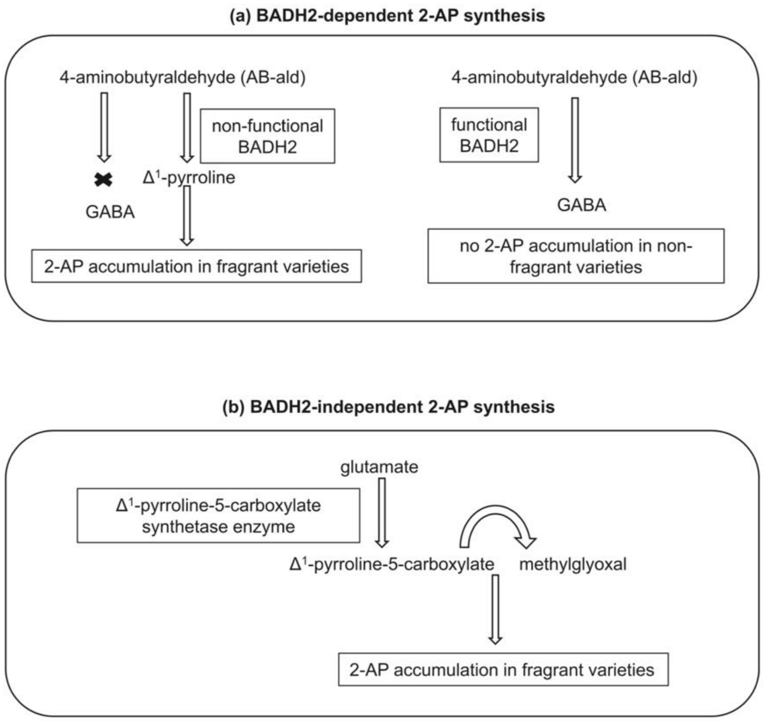


Figure 5 A comparison of the (a) BADH2-dependent 2AP biosynthetic pathway (Bradbury et al., 2008) and the (b) BADH2-independent 2AP biosynthetic pathway (Huang et al., 2008; Sakthivel et al., 2009).

From the aforementioned proposed mechanism showed that 1-pyrroline was a limiting substrate of the biosynthesis of 2AP, a recent study by Poonlaphdecha et al. (2016) found that increment of 2AP production in both fragrant and non-fragrant rice calli which were incubated with 1-pyrroline. Finally, even though the rice is of good quality, but rice aroma can be lost over time if rice was kept for a long time at high temperature. Thus, 2AP has been reported as a possible marker of ageing, because it decreases over time (Widjaja et al., 1996b; Wongpornchai et al., 2004). The study of Goufo, Duan, et al. (2010) found that higher 2AP was reported during storage of 3 months at $-4\ ^{\circ}C$.

Aroma compounds are classified as secondary metabolites. More than 300 aroma components have been identified in rice by comparing their mass spectra and those of the authentic standard and/or the mass spectra found in reference libraries (Jezussek et al., 2002; Widjaja et al., 1996a). Not only fragrant rice has 2AP but also contains many aroma compounds which are shown in Figure 6; such as hexanal, nonanal, octanal, *trans*-2-nonenal, (E,E)-nona-2,4-dienal, heptanal, 1-octen-3-ol, 4-vinyl phenol, 4-vinyl guaiacol, decanal, pentanal, *trans*-2-octenal, guaiacol, and vanillin (Daygon et al., 2016). Several studies confirmed that rice aroma included saturated and unsaturated aldehydes, alcohols, and cyclic compounds; in particular, hexanal, 1-octen-3-ol and 2-pentylfuran were the markers of both quality and ageing

(Buttery et al., 1988; Champagne, 2008; Grimm et al., 2001; Laguerre et al., 2007; Mahatheeranont et al., 2001; Widjaja et al., 1996a).

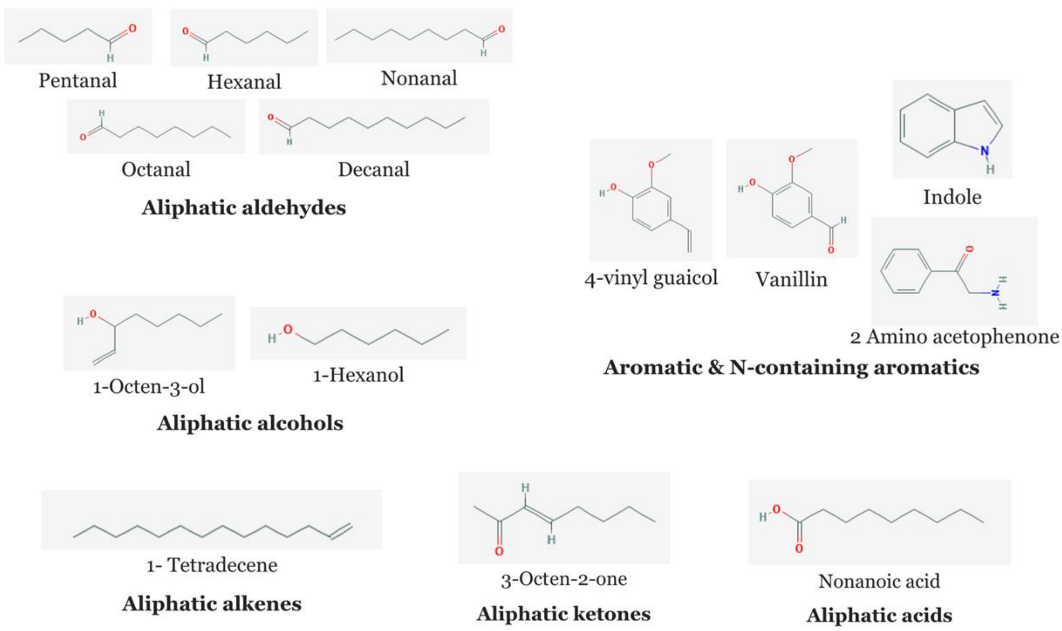


Figure 6 Other volatiles in rice (adapted from Daygon et al. (2016); source: https://pubchem.ncbi.nlm.nih.gov).

Besides, further studies showed that the candidate volatiles included 2-acetyl-1-pyrroline, (E,E)-2,4-decadienal, nonanal, hexanal, (E)-2-nonenal, octanal, decanal, 4-vinyl-guaiacol, 4-vinylphenol, 2-amino acetophenone, and 4,5-epoxy-(E)-2-decenal (Buttery et al., 1988; Jezussek et al., 2002). The study from Yang et al. (2008) found 25 aroma-active compounds in Korean black rice. Compounds that had a high aroma intensity in Korean black rice were 2AP, (E)-2-nonanal, nonanal, hexanal and 3-octen3-one. Some of the key markers were found to be related to off-flavor depending on storage; most of these are products of lipid degradation, such as hexanal, octanal, 2-(E)-nonenal, dec-(2E)-enal, 2-pentylfuran, and (E,E)-2,4-decadienal (Champagne, 2008). Thus, the effects of ageing can be monitored through several compounds: 2-(E)-octenal was identified as an universal marker of ageing for all rice varieties, while 2-pentyl furan, 1-octen-3-ol and dec-(2E)-enal were varieties-specific markers (Griglione et al., 2015).

Eating quality is a major determinant of customer approval of a specific rice variety, and most consumers would not consume the cooked rice they dislike despite its possible health benefits (Wang et al., 2022). Texture is only one component of the more nuanced concept of eating quality. Flavor is often evaluated by taking into account scent, taste, and other sensory properties in order to arrive at a conclusion on which rice type will be preferred by consumers (Biao et al., 2019; Verma & Srivastav, 2022). There have been identified about five hundred volatile organic compounds that contribute to the flavor and odor of cooked rice (Hu et al., 2020). For examples, hexanal, 2-acetyl-1-pyrroline, and E-2-nonenal have been identified as essential aromatic components of cooked rice (Bergman et al., 2000). Juliano et al. (1981) noted that if the cooking method is a variable, then the eating quality may be affected (Juliano et al., 1981). This could be due to the alteration of aromatic compounds in the rice.

Chemical group of rice grain volatiles

More than 300 types of volatile organic chemicals have been discovered in rice (Jezussek et al., 2002), which can be classified into seven groups: aldehydes, alcohols, ketones, acids, hydrocarbons, esters, and heterocyclic compounds(Hashemi et al., 2013; Wakte et al., 2017). 2-AP belongs to the pyrrolines class of chemical compounds, which is considered the most representative fragrance for identifying the overall aroma of cooked rice (Wei et al., 2017). Rice aroma can be traced back to the early 198s, when it was initially analyzed and evaluated under the term "popcorn aroma" (Buttery et al., 1988).

The most numerous chemicals were hydrocarbons, followed by aldehydes. Aldehydes and hydrocarbons account for the greatest proportion by weight of volatile chemicals in rice. alcohols, ketones, organic acids, esters, and heterocyclic compounds (Lin et al., 2010). Hydrocarbons found in rice, for example, 2,6,10-trimethyl-12alkane, pentadecane, 2,6,10-trimethyl-15alkane, hexadecane, and heptadecane.

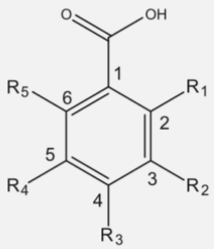
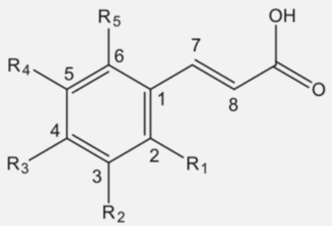
Pentanal, hexanal, heptanal, 2-heptene aldehyde, octanal, nonanal, decyl aldehyde, 2-methypropanal, 2-methylbutanal, 3-methylbutanal and benzene formaldehyde are examples of aldehydes. Hexanal and nonanal are present in higher proportions than the other chemicals in this category (Lin et al., 2010; Zheng et al., 2022). Among alcohols, there are substances that can be found such as 1-octen-3-ol, hexanol, 1-octanol which obtained a higher odor threshold, and they are considered to be more abundant compounds than aldehydes (Yang et al., 2008).

Even so, there was considerable variation in the types of ketones detected in rice. The ketone content was much lower than the aldehyde content. There are other volatiles in ketones that can be found, such as acetone, 2-heptanone, 2-octanone, 2,3-octandione, and 6,10,14-trimethyl-2-pentadecanone. Heterocyclic compounds and alcohols Heterocyclic compounds, which are comprised of pyridines, thiazoles, pyrazines, furans, oxazoles, pyrroles, and their derivatives (Demyttenaere et al. 2002), The compounds discovered include dihydrobenzofuran, 2-pentylfuran, and 2-alkyl furans (Zheng et al., 2022).

Phenolic compounds and health benefits

Phenolics are secondary metabolites present ubiquitously throughout the plant kingdom (Khalid et al., 2019), which comprise of simple phenolic acids (Table 1), flavonoids (Table 2), anthocyanidins (Table 3), proanthocyanidins (Figure 7), lignins, and lignans (Soto-Vaca et al., 2012). Phenolics are synthesized in plants partly as the response to ecological and physiological stresses such as pathogens and insects, salt, drought, ultraviolet radiation, and wounding (Kong et al., 2004; Park et al., 2013). Major roles of flavonoids in plants include modulation of reactive oxygen species (ROS) by reducing the singlet oxygen’s, hindering of enzymes involved in ROS generation (lipoxygenase, cyclooxygenase, xanthine oxidase, monooxygenase), by chelating transition metal ions which trigger the ROS production, and quenching lipid peroxidation by number of free radical reactions, and help in the recycling of other antioxidants (Arora et al., 2000; Cotelle et al., 1996; Mierziak et al., 2014; Rice-Evans et al., 1996). In addition, resisting to harsh environment was observed in further studies (Armero et al., 2001; Schmidt et al., 1994) and found that the exudation of flavonoids to the rhizosphere was shown as signal molecules in response to symbiont or pathogenic microorganism, and abiotic stresses such as drought stress, nitrogen, temperature (Coronado et al., 1995; Dixon & Paiva, 1995; Juszczuk et al., 2004). Likewise, further studies showed that flavonoids transported to infection site gave prompt hypersensitivity reaction, which was the basic defense mechanism adopted by the infected plants. It was observed that flavonoids was assimilated into the walls of necrotic and adjacent cells (Beckman, 2000; Blount et al., 1992).

Table 1 Structures of simple phenolic acids (hydroxybenzoic and hydroxycinnamic) in rice grains (Shao & Bao, 2019).

Groups	Name	R_1	R_2	R_3	R_4	R_5
	GA	H	OH	OH	OH	H
	p-HA	H	H	OH	H	H
	2,5-DHA	OH	H	H	OH	H
	PA	H	OH	OH	H	H
	VA	H	OCH_3	OH	H	H
	SYA	H	OCH_3	OH	OCH_3	H
Hydroxybenzoic acid	SAA	H	H	H	H	OH
	CA	H	OH	OH	H	H
	p-CA	H	H	OH	H	H
	SIA	H	OCH_3	OH	OCH_3	H
	FA	H	OCH_3	OH	H	H
Hydroxycinnamic acid	IFA	H	OH	OCH_3	H	H

GA, gallic acid; p-HA, p-hydroxybenzoic acid; 2,5-DHA, 2,5-dihydroxybenzoic acid; PA, protocatechuic acid; VA, vanillic acid; SYA, syringic acid; SAA, salicylic acid; CA, caffeic acid; p-CA, p-coumaric acid; SIA, sinapic acid; FA, ferulic acid; IFA, isoferulic acid.

Table 2 Structures and groups of flavonoids in rice grains (Shao & Bao, 2019).

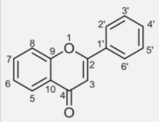
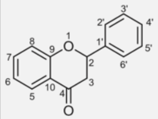
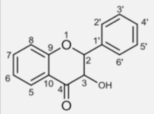
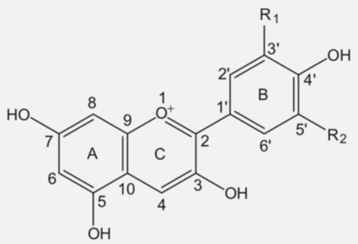
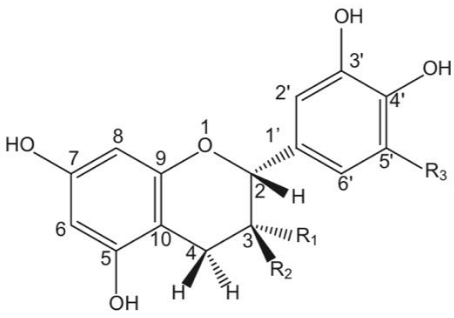
Structure formula	Group	Description	Examples
	Flavone	2-Phenylchromen-4-one	Luteolin, Apigenin, Tangeritin
	Flavonol or 3-hydroxyflavone	3-Hydroxy-2-phenylchromen-4-one	Quercetin, Kaempferol, Myricetin, Fisetin,Galangin, Isorhamnetin, Pachypodol, Rhamnazin, Pyranoflavonols, Furanoflavonols
	Flavanone	2,3-Dihydro-2-phenylchromen-4-one	Hesperetin, Naringenin, Eriodictyol, Homoeriodictyol
	Flavanonol or 3-Hydroxyflavanone or 2,3-Dihydroflavonol	3-Hydroxy-2,3-dihydro-2-phenylchromen-4-one	Taxifolin, Dihydrokaempferol

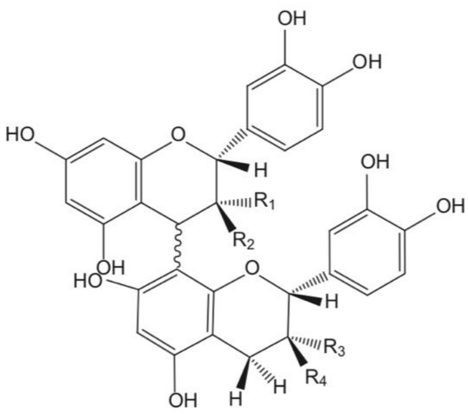
Table 3 Structures of anthocyanidins in rice grains (Shao & Bao, 2019).

Groups	Name	R_1	R_2
	Cyanidin	OH	H
	Peonidin	OCH3	H
	Malvidin	OCH3	OCH3
	Pelargonidin	H	H
	Delphinidin	OH	OH

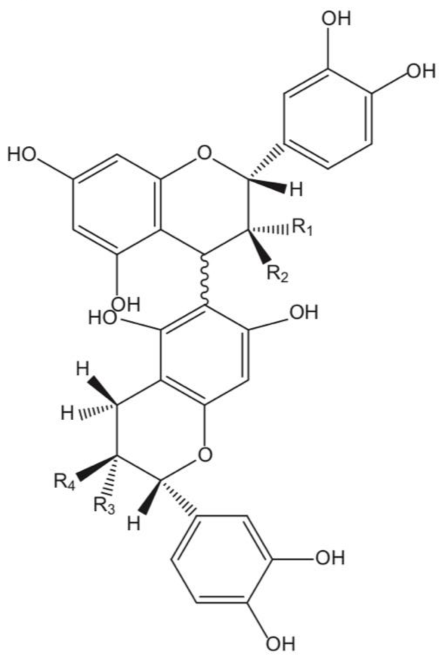
Anthocyanidins are the sugar-free counterparts of anthocyanins.



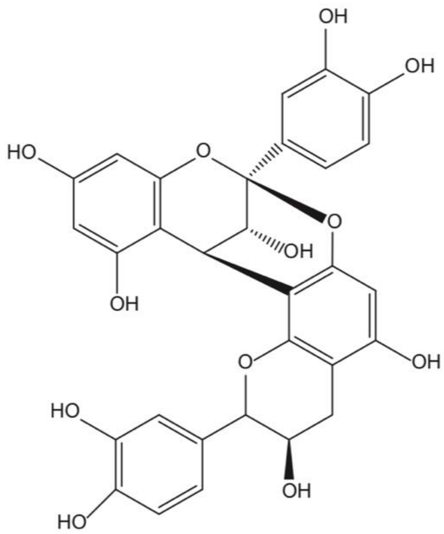
R_1=H, R_2=OH, R_2=H: (+)-catechin
R_1=OH, R_2=H, R_3=H: (–)-epicatechin
R_1=H, R_2=OH, R_3=OH: (+)-gallocatechin
R_1=OH, R_2=H, R_3=OH: (–)-galloepicatechin



Proanthocyanidins dimmers B (C4-C8)
R_1=OH, R_2=H, R_3=OH, R_4=H
R_1=H, R_2=OH, R_3=H, R_4=OH
R_1=OH, R_2=H, R_3=H, R_4=OH
R_1=H, R_2=OH, R_3=OH, R_4=H



Proanthocyanidins dimmers B (C4-C6)
R_1=OH, R_2=H, R_3=OH, R_4=H
R_1=H, R_2=OH, R_3=H, R_4=OH
R_1=OH, R_2=H, R_3=H, R_4=OH
R_1=H, R_2=OH, R_3=OH, R_4=H



Proanthocyanidins dimmers A (C4-C8, C2-O7)

Figure 7 Structures of some proanthocyanidins in red rice grains (Shao & Bao, 2019).

Plants containing a high amount of flavonoids have been used to cure many ailments in humans due to their antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory and anticancer properties (Khalid et al., 2019). Antimicrobial activity of flavonoids has been shown in several studies; for instance, inhibitory activity of flavonoids against the human immunodeficiency virus (HIV) is the most important and dynamic area of research in plant sciences.

Most of the research work has focused against pandemic HIV-1 strain and its enzyme profiles. Flavonoid (Baicalein) has shown pronounced inhibitory activity to HIV-1 infection and replication of many other viruses (González-Molina et al., 2010; Tripoli et al., 2007).

Also, antibacterial activity was found in many phytochemical extracts with high flavonoids content (Ghandchi & Jamzad, 2015; Tereschuk et al., 1997). Similarly, some commercially available flavonoids like ponciretin (Kim et al., 1999), apigenin (Herrera et al., 2010; Sato et al., 2000), pinocembrin (Fukui et al., 1988; Ye et al., 2017), genkwanin (Palacious et al., 1983), naringin and naringenin (Rauha et al., 2000), luteolin and luteolin 7-glucoside (Sato et al., 2000) were found to be potent antibacterial agents. Additionally, propolis also showed activity against *Candida* spp. and dermatophytes because of its high flavonoid contents (Agüero et al., 2014), while another flavonol (galangin) most commonly found in propolis had inhibitory activities against *A. flavus*, *A. tamari*, *Penicillium digitatum*, *P. italicum* and *Cladosporium sphaerospermum* (Afolayan & Meyer, 1997; Famewo et al., 2017).

There are many chemical reactions in human metabolism which generate free radicals as by products all the time. It has been estimated that the average person has around 10,000 – 20,000 free radicals attacking each body cell each day. Uncontrolled free radical activity might combine with other factors to cause chronic diseases such as neurodegenerative diseases, diabetes, heart disease, cancers etc. (Pala & Gürkan, 2008). Fruits and vegetables, which usually rich in phenolic compounds (Chun et al., 2005; Kaur & Kapoor, 2001; Szajdek & Borowska, 2008; Vasco et al., 2008), are associated with a lower risk of chronic diseases (Block et al., 1992; Steinmetz & Potter, 1996). Thus, World Health Organization (WHO) recommends a daily intake of at least 400 g fruits and vegetables (potatoes not included) (WHO, 2002), and US health authorities recommend a minimum of 5 servings of fruits and vegetables a day (USDHSS, 1991).

Generally, in human metabolism, reactive oxygen species (ROS) formation is enhanced by metal ions, the mechanism involved in this reaction is that hydrogen peroxide is reduced by these metal ions resulting in the generation of hydroxyl radical, which is highly reactive (Mishra, Kumar, et al., 2013). Subsequently, cell membrane is attacked by ROS when an unbalance between the ROS and antioxidant, leading to loss of membrane integrity and normal function (Schneider & Oliveira, 2004).

Cardiovascular disease is one of noncommunicable disease (NCDs) which is a leading cause of disability and death (Smith et al., 2012). The common phenomenon involved in the development of CVD is the atherosclerotic plaque formation which is initiated by endothelium damage. Atherosclerosis is characterized by the plaque formation in large arteries, and it is one of the major factors contributing to incidence of stroke and myocardial infarction. Also, inflammation and oxidative stress are the key factors contributing to the damage of endothelium (Kaleem & Ahmad, 2018). When the inflammation takes place for a long period of time, it can mediate the development of several chronic diseases such as CVD, cancer, arthritis, neurodegenerative diseases, and pulmonary diseases (Rubio-Perez & Morillas-Ruiz, 2012).

Several previous studies have shown that high intake of fruits and vegetables rich in flavonoids reduce several risk factors for development of atherosclerosis including high tolerance to glucose, maintaining good body mass index, lowering blood pressure (Mulvihill & Huff, 2010). As flavonoids are natural compounds, they can target multiple steps in the inflammation pathway as compared to monotargeted synthetic anti-inflammatory drugs (Sung et al., 2012). The capability of flavonoids to control oxidative stress and act as anti-inflammatory agents is responsible for their cardioprotective properties. The anthocyanins present in black rice grain and the proanthocyanidins found in red rice and grape seeds scavenge hydroxyl radicals and superoxide ion (Kruger et al., 2014; Walter & Marchesan, 2011)

Furthermore, production of nitric oxide (NO) is stimulated by proanthocyanidins which are present in red grapes. The availability of NO in acute oxidative stress like reperfusion/ischemia is protective to cardiomyocytes, because it inhibits the cardiomyocytes apoptosis (Jones & Bolli, 2006). Likewise, recently study by Bondonno et al. (2019) found the associations of flavonoid and flavonoid-rich wholefood intakes with all-cause mortality and the moderating effects of early mortality risk factors. The study included 2,349 participants of The Blue Mountains Eye Study, with a mean ± SD age at baseline of 64.7 ± 9.2 years. After 14 years of follow-up, moderate to high intakes of flavonoids and certain flavonoid subclasses may provide health benefits, particularly for individuals with at least one early mortality risk factor.

Cancer cells are normal cells which have defect in regulatory circuits that govern cell proliferation and homeostasis (Hanahan & Weinberg, 2000). Cancer treatment with chemotherapy often causes adverse reactions because normal cell is killed together with cancer cells too. An ideal anticancer agent is one which has a maximum capacity to inhibit tumor growth or to kill cancer cells, but causes minimum adverse health effects (Zhao et al., 2012). Nowadays, medical recommendations have confirmed that consumption of vegetables and fruits which are rich in flavonoids prevent the development of cancer (Mishra, Sharma, et al., 2013). Due to the presence of polyphenol aromatic rings in flavonoids, it has been found that flavonoids possess pro and antioxidant properties (Leung et al., 2007).

Likewise, recently studies have shown that anticancer properties of flavonoids may be due to their pro-oxidant properties (Habtemariam & Dagne, 2010; Liu et al., 2012). Additionally, some flavonoid compounds, like genistein, quercetin, and flavopiridol, were at the late phase of clinical trials for cancer treatment (Lazarevic et al., 2011). *In vitro* study, higher oxidative stress was observed in the cancer cells as compared to normal cells, making them more susceptible to be killed by a substance which enhanced reactive oxygen species level like flavonoids (Valdameri et al., 2011; Yuan et al., 2012). Therefore, a flavonoids acting as pro-oxidant or antioxidant is dependent on the concentration, type of cell and culture condition in which it is grown (Pacifico et al., 2010). Besides, recently article by George et al. (2017) noted that the most efficacious plant flavonoids, including luteolin, epigallocatechin gallate, quercetin, apigenin, and chrysin, contributed to the chemoprevention with a focus on protection against DNA damage caused by various carcinogenic factors.

Diabetes Mellitus (DM) is one of metabolic disorders characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both (Punthakee et al., 2018). Recently, epidemiological studies found that DM was the ninth major cause of death, and related to unhealthy behaviors, including overweight and obesity, sedentary lifestyle, and increased consumption of unhealthy diets containing high levels of red meat and processed meat, refined grains and sugar-sweetened beverages (Zheng et al., 2018). Likewise, earlier study has found that consumption of flavonoids rich diet regulate digestion of carbohydrates, secretion of insulin and uptake of glucose in insulin sensitive tissue by regulating several intracellular pathways (Hanhineva et al., 2010). Especially, the anti-diabetic effect of flavon-3-ols was reported that the function of mitochondria was also improved by maintaining its functional integrity in pancreatic beta-cells exposed to glucose toxicity (Erdman Jr et al., 2007). Also, rat model study by Nizamutdinova et al. (2009) showed that the seed coat of black soybeans was rich in delphinidin, cyanidin and petunidin. Soybean seed coat extract ameliorated insulin resistance by improved the insulin concentration in the serum, along with improving tissue glucose utilization. Similar to EGCG and ECG, naringin and hesperidin minimized the oxidative stress and hyperglycemia in male albino rats in which diabetes is induced by streptozotocin, by oral administration at the dose of 50 mg/kg for a period of 1 month (Mahmoud et al., 2012).

Liver is one of the major vital organs of the human body. All of biochemical processes such as growth, nutrient provision, supply of energy and reproduction, are related by liver function (Adewusi & Afolayan, 2010). There are many factors that can cause liver damage in everyday life. On the other hand, consumption of certain foods protects against liver damage. Hepatoprotective properties of plant-based foods are mostly attributed to bioactive compounds like flavonoids. Following these successes, several studies have been conducted to check the hepatoprotective activities of plant extract rich in flavonoids or individual flavonoid compounds (Madrigal-Santillán et al., 2014). Several flavonoids such as quercetin, rutin, catechin, naringenin, and venoruton have been reported for their hepatoprotective effect (Tapas et al., 2008).

Likewise, Dogan and Celik (2012) noted that Grapes and grape seeds were rich source of flavonoids like resveratrol, proanthocyanidin, anthocyanidins, epicatechins, and catechins. The hepatoprotective effect and antioxidant activity of grape seeds was observed in rats in which hepatitis was induced by oxidative stress and assessed by marker enzymes like GGT, ASAT, LDH, ALAT, SOD, GSH, MDA, GPx, and GST. This level of marker enzymes was significantly decreased in rats, which was fed ethanol along with grape seed. This showed that the adverse effects caused by the oxidative stress of ethanol were minimized by the consumption of grape seed. Also, a flavonoid named Silymarin has three structural components: silydianine, silibinin, and silychristine. These are extracted from the seeds and fruit of milk thistle *Silybum marianum* (Compositae) and have been reported to stimulate enzymatic activity of DNA-dependent RNA polymerase 1 and subsequent biosynthesis of RNA and protein which results in biosynthesis of DNA and cell proliferation resulting in liver regeneration in damaged livers (He et al., 2004).

Voluminous interventional and epidemiological studies have shown that consumption of whole grains can reduce the risks of chronic diseases, such as cardiovascular diseases, type II diabetes, obesity, and some cancers. Consequently, whole rice grain is becoming popular in western countries due to its health benefits, and is more gradually accepted in developing countries with the improvement of living standards (Shao et al., 2011). The colors of the whole grain rice range from white to red, and black (dark purple). Red and black (dark purple) pericarp have higher antioxidant activities than white pericarp (Min et al., 2011). The health benefits of whole grain are mainly contributed by one of its major constituents, the polyphenols. Polyphenols in rice grain can be classified into three subgroups: (1) phenolic acids, which is the most common secondary metabolites in cereal grains; (2) anthocyanins, which only exist in black or dark purple grains, as a large group of secondary metabolites, are water-soluble flavonoids, and may appear red, purple, or blue depending on pH (Jaiswal et al., 2012); and (3) proanthocyanidins, which mainly consist of catechin and epicatechin block unit in red rice and are considered to be the most effective antioxidants in nature (Gunaratne et al., 2013; Jaiswal et al., 2012; Min et al., 2011; Qiu et al., 2010).

Previously, the studies have shown that higher intake of white rice are associated with a significantly increased risk of Type 2 diabetes, as well as an elevated risk of glucose homeostasis disorder (Soriguer et al., 2013). According to Ti et al. (2015) and Meng et al. (2018), black rice has received increasing attention principally because of its low postprandial blood glucose response, which the phenolic compounds can inhibit the activity of intestinal alpha-glucosidase and pancreatic alpha-amylase (Ranilla et al., 2010). However, prior study found that anthocyanins extracted from black rice also played an important role in reducing hypertriglyceridemia and adverse effects of alcohol (Hou et al., 2010). *In vitro* study by Yang et al. (2011) showed that dyslipidemic rats were fed with high fat diets supplemented with anthocyanin extracted from black rice (AEBR), the platelet hyperactivity and body weight gain was significantly lower than in those fed with only high fat diet, suggesting that dietary intake of AEBR facilitates the maintenance of optimal platelet function in dyslipidemic rats induced by high fat diets.

According to Shao et al. (2018), the phenolic acids was distributed within the whole body, after become absorbed in the stomach and small intestine, with concomitant health benefits such as inhibition against oxidation of low-density lipoprotein cholesterol (Min et al., 2012). Besides, the phenolic acids from brown rice bran also showed putative breast and colon cancer chemopreventive properties (Hudson et al., 2000). Lately, Niu et al. (2013) has reported that the extract from red rice grain has potential anti-inflammatory effect in a dose-dependent manner; may be suggested that can improve holistic health and reduce risk factors in cardiovascular disease. Likewise, in 2013, cyanidin-3-glucoside has shown an improvement of hypercholesterolemia and hyperlipidemia associated to a reduction of hepatic lipogenic enzymes on hyperlipidemic rats (Um et al., 2013). These pigments have been reported to be effective in reducing cholesterol levels in the human body (Lee et al., 2008). In addition to Chen et al. (2006), the study indicated that the whole black rice grain, specifically the anthocyanins, cyanidin 3-glucoside and peonidin 3-glucoside, contributed to inhibiting the invasion and mobility ability of human hepatocellular carcinoma (SKHep-1) cells with a reduced expression of matrix metalloproteinase-9 and urokinase-type plasminogen activator.

Finally, from several studies have confirmed that colored rice act as a natural antioxidant and free radical scavenger which may increase oxidative stress and potentially damage biological molecules such as lipids, proteins and DNA (Finocchiaro et al., 2010). Antioxidants in colored rice may influence biological functions either individually or synergistically (Shao et al., 2018), meaning that foods containing antioxidants, one or more, may deliver greater health benefits than the sum of each antioxidant alone.

Metabolomics analysis in rice volatiles

Metabolomics is defined as the systematic analytical study of “all metabolites” (metabolome) which are biologically synthesized or degraded compounds in biological system (cell, tissue, organ, biological fluid, or organism) at a specific point in time. It can be used in non-targeted analysis, metabolite profiling or metabolic profiling is a term used to describe; and targeted analysis for quantify specific metabolites (Bino et al., 2004; Fiehn, 2002; Fukusaki & Kobayashi, 2005; Hall, 2006; Saito et al., 2006). Previously, the study has estimated that the plant kingdom contains more than 200,000 metabolites (Dixon, 2003). Volatile compounds is characterized by small-molecule metabolite because it has low molecular weight (less than 900 daltons) (Macielag, 2012); and their high volatility, low vapor pressure (≥0.01 kPa at 20 °C), and low water solubility (Herrmann, 2010).

Recently, Matthews et al. (2015) has shown that gas chromatography (GC) coupled with mass spectrometry (MS) is particularly well suited to the study of low molecular weight that can be made amenable to gas chromatography by chemical derivatization. GC is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary phase (column) and the mobile phase (carrier gas: He, N_2, or H_2) moves in a definite direction (McNair et al., 2019). Its applications include qualitative analysis in complex mixtures, determining solvent purity, analyzing organic synthesis products, monitoring water and air quality, and detecting explosives (Vitha, 2016); and, quantitative analysis of a large number of low-polarity compounds because it has high sensitivity, reproducibility and speed of resolution (Wilson & Walker, 2010). Nowadays, single technique analysis may not be enough in modern analytical chemistry. Consequently, GC is integrated with MS, which is an extremely valuable technique in which the molecules in a test sample are converted to gaseous ions that are subsequently separated through a capillary column according to boiling point and detected in a mass spectrometer according to mass-to-charge (*m/z*) ratio (Wilson & Walker, 2010). Analytically, the most recent applications are mostly oriented towards biochemical problems, such as proteome, metabolome, high throughput in drug discovery and metabolism (Hoffmann & Stroobant, 2007), and it is a very powerful technique when coupled to gas chromatography (Wilson & Walker, 2010). Data management may be considered as an importantly step of metabolomics study; consequently, various statistical methods used in volatiles studies are applicable to metabolomic data by considering the amount of each metabolite as a trait value. Principal component analysis (PCA), an unsupervised multivariate data analysis method (Oikawa et al., 2008).

In 2016, volatile compounds and 2AP precursors (proline and methylglyoxal) were assessed at seven different growth stages in two fragrant rice varieties Basmati-370 (BA-370) and Ambemohar-157 (AM-157) and compared with non-fragrant rice varieties IR-64. PCA showed that N-heterocyclic (2AP, 2-acetyl-1-pyrrole and indole) was the major distinguishing class between fragrant from non-fragrant rice varieties; and maximum number of volatiles compound were synthesized at seedling stage and decreased gradually at reproductive and maturity. This study showed that volatiles accumulation pattern through developmental stages was specific. The variation in volatile profiles were more within the developmental stages than between the cultivars. Hence, developmental stages were separated more clearly than cultivars via multivariate analyses by PCA (Hinge et al., 2016).

Although the 2-AP content of Jasmine rice has been determined, there is little information on the aroma active components that make Jasmine rice unique from other fragrant (Basmati and Jasmati) rice varieties. As the study by Mahattanatawee and Rouseff (2014), PCA showed that the Jasmine variety was characterised by the pattern of sulphur volatiles which had predominantly 2-acetyl-2-thiazoline. Likewise, Mathure et al. (2011) found that the volatile profiles of local varieties include Kamod, Raibhog, and Ghansal were closely associated with Basmati types via PCA. These rices could be promoted further to boost their popularity among consumers.

Besides, the difference of volatiles profiles in *indica* and *japonica* varieties were evaluated with PCA by Daygon et al. (2016). The PCA biplot of the sensory data in two types of aromatic rice varieties (*indica* and *japonica*) showed that the pleasant descriptors such as corn, grainy, sweet taste, dairy, grassy, floral, pandan, popcorn and sweet aroma were closely associated with *indica* varieties more than *japonica* varieties. Because of the biggest difference in rice volatiles was alpha-pinene which 20 times greater in *indica* than *japonica* varieties. This was followed by acetoin (six times greater in *indica* varieties), and the compounds limonene, 2,3-butanedione and 2-acetylpyrrole (twice the concentration in *indica* varieties). 2AP concentrations were on average 1.5 times greater in the *indica* varieties than the *japonica*. On the other hand, putative compounds with greater amounts on the average in the *japonica* varieties include dimethyl trisulfide, 2-methyl-2-undecanethiol, amylene hydrate, 2-methylbutanal, 2octyn-1-ol and 1-butanol.

Several studies have shown that PCA has obviously benefits to visualize the difference of interesting data. Cambodian rice were investigated by Concepcion et al. (2018), the authors showed that 157 compounds were emitted from nine Cambodian rice varieties. Based on the PCA loadings values, rice flavor was dominated by N-containing compounds (e.g., 2AP), aliphatic aldehydes, aliphatic ketones as well as 2-alkylfurans. Additionally, they also showed that the major variation in the amount of fatty acids is explained by PCA. The results indicated that there were statistically significant differences in the concentrations of two unsaturated fatty acids—oleic acid (C18:1n-9) and linoleic acid (C18:2n-6)—as well as of palmitic acid (C16:0). These fatty acids were the major free fatty acids produced during lipid hydrolysis in milled rice (Christie, 1973; Frankel, 1983, 2012; Lam & Proctor, 2003; Lam et al., 2001; Velasco et al., 2010).

Chapter III

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Title: Static headspace GC-MS analysis for determination of colored rice volatile profile

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Static headspace GC-MS analysis for determination of colored rice volatile profile

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Abstract

Thai white jasmine rice has been well known worldwide for its unique distinctive aroma. Many studies were conducted to characterize its aromatic profile. Several other varieties of Thai-colored rice can be volatile as well. Since the colored rice smell is generally not as strong as the jasmine rice, it is worth identifying fragrant compounds in the rice. The objective of this study was to investigate the proper preheated time and headspace incubation temperature to extract as many volatile compounds as possible from Thai-colored rice using the static headspace GC-MS approach. Red Hawm Rice (KDML105R-PSL-E-14) was used in this study. One gram of red rice in a 10-mL headspace vial was placed in a hot air oven at 80 °C at different preheated times (3, 4, 5, 6 and 7 h). The headspace oven temperature was altered from 80, 100 to 120 °C for 60 min. The GC oven was programmed at 40 °C for 2 min and ramped to 250 °C at 5 °C/min and finally held for 4 min. The results showed that a total of 22 volatile compounds were identified from the red rice including methyl acetate, 2-methylpropanal, 2,3-butadione, 2-butanone, 3-methylbutanal,2-methylbutanal, pentanal, 1-hydroxy-2-propanone, hexanal, furfural, 2-heptanone, heptanal, methyl n-hexanoate, benzaldehyde, 2-pentylfuran, nonanal, 2-hydroxy-5-methylacetophenone, 2,5-di-tert-butylphenol, methyl isomyristate, methyl n-hexadecanoate, methyl linoleate, and methyl oleate. The optimum condition was 5 h preheated time in the oven, followed by 120 °C headspace extraction temperature. More volatile compounds were identified at 5, 6 and 7 h compared to 3, 4 h incubation periods as some compounds were decomposed at equilibration time >5 h.

Introduction

Colored rice (*Oryza sativa* L.) is an unpolished rice that become more and more popular because of its vivid colors, which is a rich source of dietary fiber as well as antioxidants such as phenolic acids and anthocyanins (Walter et al., 2013). Anthocyanins found in Thai colored rice have been linked to a variety of health benefits such as high antioxidant activity, anti-hyperlipidemia, reduction of oxidative stress and anti-carcinogenic activity, etc. (Sivamaruthi et al., 2018). Supply is limited and demand is expanding as customers become more aware of its health benefits, so the price of colored rice is often set higher than normal white rice (Napasintuwong, 2020). Nevertheless, consumers nowadays are more concerned with consuming healthy foods and are willing to pay higher prices than in the past (De Magistris & Gracia, 2008; Krystallis & Chryssohoidis, 2005). In Thailand, domestic consumption of colored rice averaged 60,000 - 70,000 tons, with a rate of 2-3% increasing every year. In Europe, rice consumption increased from 4.7 kg per capita in 2005 to 5.5 kg in 2016. Colored rice has a selling price of 10-20 Euro/kg in Europe, which is 6-7 times higher than native European rice (Napasintuwong, 2020). Therefore, the colored rice market has the potential to grow both domestically and internationally.

Nowadays, Thailand has developed many new colored rice varieties that are both nutritious and appealing to customers. Several Thai-colored rice varieties can be volatile with its unique distinctive aroma. Red Hawm Rice (KDML105R-PSL-E-14), a well-known colored rice in Thailand, originated from the development of Jasmine Rice 105 (KDML105) , which has genes to produce aromatic substances. Therefore, it should be used as an initial agent in the study of aromatic compounds (*Thai RIce DB*, 2022).

However, they are only popular in the local community, perhaps because the odor is not well-known among the general population. To resolve this problem, characterization of volatile profiles in the colored rice cultivars is needed to search for the identity of Thai scent rice. In the past, rice fragrance analysis was commonly performed on white rice, such as Thai jasmine rice or Basmati rice. Little is known about how to extract the colored rice volatile chemicals. Since the colored rice smell is generally not as strong that of white fragrant rice, therefore, it is required to find a method to identify its volatile components. Gas chromatography is one of the most extensively applied hybrid chemical analysis techniques. (Rodinkov et al., 2020).

Presently, headspace analysis refers to a hybrid technique that involves gas extraction and subsequent detection of analytes in liquid and solid media. When compared to conventional gas-chromatographic analysis when samples are administrated directly into the injector, headspace analysis provides a number of benefits (Ioffe et al., 1984). In modern laboratories, fused silica capillary columns are utilized, which require a well-controlled sample inlet for optimal results and to prevent column overloading (Hübschmann, 2015). The selection of the proper column is a further factor to consider. It is also vital to choose the correct column polarity. There are numerous column types that have been utilized in rice volatile profiles in the past, for example: HP-5, DB-5, DB-624, BP-20, DB-wax, and RTX-5 (Bryant & McClung, 2011; Grimm et al., 2001; Lim et al., 2018; Mahattanatawee & Rouseff, 2014; Mathure et al., 2011).

The objective of this study was to investigate the proper preheated time and headspace incubation temperature to extract as many volatile compounds as possible from Thai-colored rice using the static headspace GC-MS approach. The findings of this study may serve as important resource of the optimized method to identify the volatile profile of colored rice in the future. This kind of use can be found in the next chapter, which is an analysis of Thai native-colored rice varieties.

Methods

Plant materials

Red Hawm Rice (KDML105R-PSL-E-14) was chosen as the rice sample in this study. It is derived from a natural mutation, which was always found in Khao Dawk Mali 105 (with the BADH2 allele) at the Surin Province Rice Research. When the rice seeds were planted, it was found that in one clump, the seed coat was reddish, containing starch for both glutinous rice and non-glutinous rice. Then the Phitsanulok Province Rice Research Center selects the varieties, produced outstanding rice cultivars resistant to brown planthoppers. Later, farmers near the Phitsanulok Rice Research Center brought the rice varieties to be planted and processed into red brown rice for sale until 1993. As a result, the Phitsanulok Provincial Rice Research Center brought new varieties to be selected. have become a species, KDML105R-PSL-E-14, which is a variety of rice that is widely planted today by using the name "Red Hawm Rice (*Thai RIce DB*, 2022)."

Rice samples were obtained from Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, as part of a collaborative project with Prof. Dr. Apichart Vanavichit and Dr. Siriphat Ruengphayak, the Rice Science Center of Thailand. It was kept at -80 °C until the analysis.

Sample preparation

The extraction protocol was adapted from Daygon et al. (2016). Briefly, one gram of uncooked red rice was weighed into 10-mL headspace vials. Ten µL of internal standard, 99% 2,4,6-trimethylpyridine (CAS Number: 108-75-8, Alfa Aesar, Heysham, England) was added into the sample. Then, rice samples were placed in an 80 °C hot air oven (WTC Binder Bd-53, Tuttlingen, Germany) at different incubation times for 3, 4, 5, 6 and 7 h before static headspace GC-MS analysis. All samples were prepared in triplicate for each preheated condition.

Static headspace GC-MS

The volatile profile of rice red was analyzed by static headspace extraction and separation by gas chromatograph-mass spectrometer (GC-MS). The rice sample vials were placed in a 7697A static headspace autosampler connected to a 7890B GC system and a 7000C Triple Quad mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The extraction temperatures of the headspace oven at 80, 100 and 120 °C for 60 min were investigated in order to select the best condition with the highest total peak of volatile compounds in the rice samples. The 1-mL headspace sample containing volatile compounds was collected at 140 °C and directly introduced into a GC-MS system. The temperature of GC inlet was 220°C with split ratio 20:1. The separation of volatile compounds was performed on a HP-5ms capillary column (5% phenyl/ 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent, CA, USA). GC oven was programmed at 40 °C for 2 min and ramped to 250°C at 5°C/min and finally held for 4 min. Ultrahigh purity Helium used as carrier gas was maintained at average velocity 35 cm/s. The MS was operated in electron impact (EI) mode with electron energy 70 eV. The temperature of MS interface, EI source and quadrupole were set at 250, 230 and 150 °C, respectively. Chromatogram and mass spectra were acquired using scan mode over a mass range of 33-400 m/z. The data processing i.e., peak integration, peak identification and peak deconvolution was carried out using Agilent MassHunter Qualitative Analysis software, version B.07.02. The identification of volatile compounds was done by comparing both their mass spectra (MS) and retention index (RI) with those contained in the National Institute of Standards and Technology library (NIST) 2011 database. The criteria acceptance for compound identification of mass spectrum was required matching score ≥70 and RI value difference of ≤20 units between the calculated RI and the database values for the same stationary phase. The RI value was calculated from the retention time of n-alkane series (C7-C30) (Supelco, Sigma-Aldrich, PA, USA) following the equation (Bianchi et al., 2007):

$$\mathrm{RI}(x) = 100 \times z + 100 \times \frac{\mathrm{RT(x)} - \mathrm{RT(z)}}{\mathrm{RT}(z+1) - \mathrm{RT}(z)}$$

where **RI (x)** is the retention index of the unknown compound x,
z is the number of carbon atoms of the n-alkane eluted before the unknown compound x,
z + 1 is the number of carbon atoms of the n-alkane eluted after the unknown compound x,
RT (x) is the retention time of the unknown compound x,
RT (z) the retention time of the n-alkane eluted before the unknown compound x,
RT (z + 1) is the retention time of n-alkane eluted after the unknown compound x.

Results

A total of 22 volatile compounds were identified from Red Hawm Rice using static headspace GC-MS technique (Table 4). Most of the detected volatile compounds were aldehydes and ketones. Fatty acid methyl esters, such as methyl linoleate and methyl oleate, were eluted from the column at the end. Changes in red rice volatile profiles were observed at different conditions including preheated times and headspace oven temperatures.

Effect of preheated time: It is noticeable that the longer time the samples were incubated in the hot air oven before the analysis by static headspace GC-MS, the more volatile compounds tended to be extracted. In order to provide better comparisons between each condition, percentages of the relative peak area of each volatile compound were calculated by dividing the peak area by the total peak area of all identified peaks in each chromatogram (Table 5). The total ion chromatogram (TIC) of each sample was used for peak area integration. Table 5 shows that there was no different in the eluted numbers of the compounds when using the preheated time for 3-7 h at 80 °C or 100 °C headspace oven temperatures. However, at 120 °C headspace incubation for 60 min, only 17 compounds were detected with the use of 3 h or 4 h preheated time as compared to 22 compounds identified from 5 h, 6 h and 7 h preheated times in the hot air oven (Table 5; Figure 8).

The 22 compounds are grouped into five groups: 1) aldehydes consisting of 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, pentanal, hexanal, furfural, heptanal, benzaldehyde, and nonanal; 2) ketones consisting of 2,3-butadione, 2-butanone, 2-heptanone, and 2-hydroxy-5-methylacetophenone; 3) esters include methyl acetate, methyl n-hexanoate, methyl isomyristate, methyl n-hexadecanoate, methyl linoleate, and methyl oleate; 4) furan contains 2-pentylfuran; 5) phenol contains 2,5-di-tert-butylphenol. Nevertheless, 5 out of 22 compounds (22.73%) compounds including 2,3-butadione, methyl n-hexanoate, nonanal, 2-hydroxy-5-

methylacetophenone and 2,5-di-tert-butylphenol were decomposed at equilibration time >5 h.

Effect of headspace oven temperature: The results showed that the higher the headspace oven temperature, the more the volatile compounds were extracted from the red rice. The most volatile compounds could be detected at 120 °C (17-22 compounds), followed by 100 °C (10 compounds) and 80 °C (6-7 compounds) respectively, with use of any preheated hot air oven time from 3-7 h (Table 5). The chromatogram of 120 °C headspace incubation temperature also illustrates the higher peaks rising from a baseline than chromatographic peaks at 100 °C (green line) and 80 °C (blue line) when using the same preheated time (80 °C for 5 h) (Figure 9).

Table 4 Volatile compounds from Red Hawm Rice detected by static headspace GC-MS

No.	Rt (min)	RI	Compounds	Identification	MW	Formula	CAS No.
1	1.882	*	Methyl acetate	RI	74	$C_3H_6O_2$	79-20-9
2	2.020	*	2-Methylpropanal	RI	72	C_4H_8O	78-84-2
3	2.155	*	2,3-Butadione	RI	86	$C_4H_6O_2$	431-03-8
4	2.206	*	2-Butanone	RI	72	C_4H_8O	78-93-3
5	2.714	*	3-Methylbutanal	RI	86	$C_5H_{10}O$	590-86-3
6	2.807	*	2-Methylbutanal	RI	86	$C_5H_{10}O$	96-17-3
7	3.222	*	Pentanal	RI	86	$C_5H_{10}O$	110-62-3
8	4.076	744	1-Hydroxy-2-propanone	MS, RI	74	$C_3H_6O_2$	116-09-6
9	5.170	801	Hexanal	MS, RI	100	$C_6H_{12}O$	66-25-1
10	6.014	832	Furfural	MS, RI	96	$C_5H_4O_2$	98-01-1
11	7.604	891	2-Heptanone	MS, RI	114	$C_7H_{14}O$	110-43-0
12	7.911	902	Heptanal	MS, RI	114	$C_7H_{14}O$	111-71-7
13	8.635	926	Methyl n-hexanoate	MS, RI	130	$C_7H_{14}O_2$	106-70-7
14	9.675	960	Benzaldehyde	MS, RI	106	C_7H_6O	100-52-7
15	10.654	992	2-Pentylfuran	MS, RI	138	$C_9H_{14}O$	3777-69-3
16	14.108	1104	Nonanal	MS, RI	142	$C_9H_{18}O$	124-19-6
17	20.113	1314	2-Hydroxy-5-methylacetophenone	MS, RI	150	$C_9H_{10}O_2$	1450-72-2
18	25.170	1513	2,5-Di-tert-butylphenol	MS, RI	206	$C_{14}H_{22}O$	5875-45-6
19	30.014	1725	Methyl isomyristate	MS, RI	242	$C_{15}H_{30}O_2$	5129-58-8
20	34.155	1927	Methyl n-hexadecanoate	MS, RI	270	$C_{17}H_{34}O_2$	112-39-0
21	37.351	2096	Methyl linoleate	MS, RI	294	$C_{19}H_{34}O_2$	112-63-0
22	37.460	2102	Methyl oleate	MS, RI	296	$C_{19}H_{36}O_2$	112-62-9

Rt: retention time

RI: retention index (calculated from the retention time of n-alkane series (C7-C30) (Supelco, Sigma-Aldrich, PA, USA) following the equation (Bianchi et al., 2007)); ***** cannot be calculated because the RT of the substance is outside the range of n-alkanes.

MW: molecular weight

Table 5 Average percentages and standard deviations (SD, in parentheses) of extraction of each volatile compound from Red Hawm Rice with regard to the total area at different preheated time (3, 4, 5, 6 and 7 h at 80 °C) in the hot air oven and different headspace incubation temperature (80 °C, 100 °C and 120 °C for 60 min).

Compounds	80 °C					100 °C					120 °C				
	3 h	4 h	5 h	6 h	7 h	3 h	4 h	5 h	6 h	7 h	3 h	4 h	5 h	6 h	7 h
Methyl acetate	66.90 (2.93)	57.52 (0.73)	49.15 (4.80)	50.55 (4.56)	57.35 (3.93)	ND	ND	ND	ND	ND	6.64 (0.45)	9.10 (0.65)	6.18 (0.11)	5.70 (0.38)	5.10 (1.64)
2-Methylpropanal	13.59 (0.45)	16.55 (0.39)	17.70 (2.71)	16.90 (2.42)	19.29 (4.03)	ND	ND	ND	ND	ND	5.94 (0.70)	6.81 (0.27)	4.08 (0.36)	3.97 (0.17)	5.69 (2.29)
2,3-Butadione	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.63 (1.23)	1.89 (0.55)	2.25 (1.28)	1.06 (0.17)	1.52 (0.77)
2-Butanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.43 (0.92)	2.09 (0.39)	0.85 (0.11)	1.20 (0.20)	1.99 (1.06)
3-Methylbutanal	10.71 (2.27)	12.87 (0.30)	13.01 (0.91)	13.00 (1.12)	15.26 (0.61)	43.01 (0.58)	43.22 (2.15)	44.62 (0.03)	46.82 (1.13)	42.41 (0.70)	6.01 (0.77)	7.05 (0.47)	4.21 (0.44)	4.13 (0.27)	6.57 (3.93)
2-Methylbutanal	ND	ND	10.13 (2.18)	9.27 (1.50)	ND	19.73 (0.83)	22.57 (0.88)	20.73 (0.90)	20.79 (1.59)	21.56 (0.80)	4.71 (1.02)	4.97 (0.99)	2.98 (0.51)	3.10 (0.98)	4.33 (1.85)
Pentanal	ND	2.48 (0.26)	ND	ND	ND	3.99 (0.15)	6.45 (0.43)	5.14 (1.25)	3.54 (0.86)	5.96 (0.43)	3.47 (0.40)	4.03 (0.58)	2.35 (0.40)	2.29 (0.65)	2.66 (0.26)
1-Hydroxy-2-propanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.12 (0.50)	6.26 (0.14)	3.88 (0.27)	3.59 (0.10)	2.99 (0.91)
Hexanal	5.53 (0.66)	6.29 (0.74)	6.39 (2.75)	6.61 (2.46)	4.87 (0.19)	15.86 (2.06)	13.05 (1.68)	13.88 (1.28)	13.38 (1.03)	13.86 (0.64)	7.98 (0.44)	8.18 (0.64)	5.13 (0.47)	4.44 (0.46)	4.24 (0.59)
Furfural	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.71 (1.27)	1.12 (0.16)	0.74 (0.15)	0.85 (0.31)	2.22 (1.19)
2-Heptanone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.36 (0.06)	0.51 (0.43)	0.63 (0.67)
Heptanal	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.29 (0.24)	0.20 (0.02)	0.12 (0.03)	0.17 (0.13)	0.09 (0.01)

Table 5 (cont.) Average percentages and standard deviations (SD, in parentheses) of extraction of each volatile compound from Red Hawm Rice with regard to the total area at different preheated time (3, 4, 5, 6 and 7 h at 80 °C) in the hot air oven and different headspace incubation temperature (80 °C, 100 °C and 120 °C for 60 min).

Compounds	80 °C					100 °C					120 °C				
	3 h	4 h	5 h	6 h	7 h	3 h	4 h	5 h	6 h	7 h	3 h	4 h	5 h	6 h	7 h
Methyl n-hexanoate	0.75 (0.10)	0.94 (0.05)	0.75 (0.10)	0.74 (0.11)	0.60 (0.17)	1.51 (0.11)	1.16 (0.28)	1.45 (0.20)	1.50 (0.07)	1.57 (0.20)	1.18 (1.48)	0.48 (0.03)	0.48 (0.30)	0.41 (0.14)	0.37 (0.13)
Benzaldehyde	ND	ND	ND	ND	ND	0.54 (0.06)	0.50 (0.09)	0.49 (0.01)	0.49 (0.03)	0.42 (0.03)	1.03 (0.08)	1.28 (0.15)	0.77 (0.09)	0.74 (0.03)	0.67 (0.09)
2-Pentylfuran	ND	ND	ND	ND	ND	1.08 (0.18)	0.78 (0.19)	0.85 (0.11)	0.87 (0.08)	0.79 (0.11)	0.84 (0.11)	0.76 (0.05)	0.47 (0.05)	0.43 (0.03)	0.33 (0.12)
Nonanal	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.33 (0.04)	0.23 (0.01)	0.22 (0.03)
2-Hydroxy-5-methylacetophenone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.15 (0.01)	0.13 (0.02)	0.12 (0.02)
2,5-Di-tert-butylphenol	ND	ND	ND	ND	ND	0.58 (0.005)	0.43 (0.11)	0.41 (0.02)	0.44 (0.03)	0.32 (0.03)	ND	ND	0.26 (0.06)	0.21 (0.01)	0.18 (0.01)
Methyl isomyristate	ND	ND	ND	ND	0.21 (0.04)	0.93 (0.20)	0.81 (0.10)	0.81 (0.09)	0.80 (0.05)	0.86 (0.04)	0.62 (0.03)	0.83 (0.04)	0.61 (0.01)	0.58 (0.03)	0.51 (0.05)
Methyl n-hexadecanoate	2.52 (0.09)	3.34 (0.15)	2.86 (0.80)	2.93 (0.72)	2.43 (0.28)	12.77 (1.16)	11.04 (0.39)	11.61 (0.73)	11.37 (0.97)	12.26 (0.27)	19.66 (1.53)	28.53 (1.05)	21.78 (1.08)	22.77 (0.92)	21.33 (2.77)
Methyl linoleate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.41 (0.73)	12.06 (0.57)	12.81 (1.04)	11.58 (1.63)
Methyl oleate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	29.73 (1.97)	ND	29.96 (1.75)	30.68 (1.64)	26.65 (2.51)

All samples were prepared in triplicate for each condition.

ND = not detected.

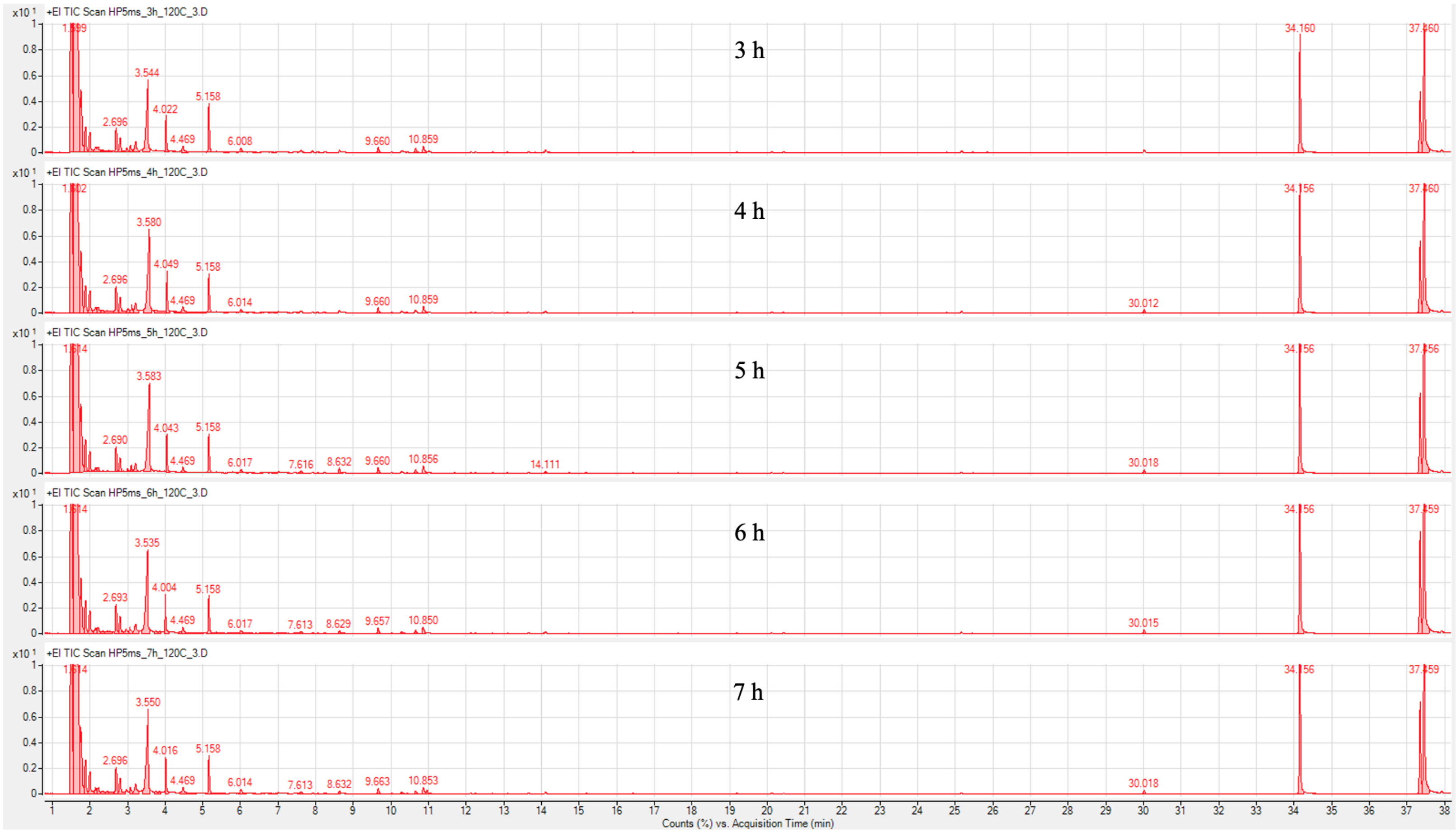

Figure 8 GC-MS chromatograms of volatile compounds from Red Hawm Rice samples with 3, 4, 5, 6 and 7 h preheated time at 80 °C in the hot air oven (followed by 120 °C headspace incubation temperature for 60 min).

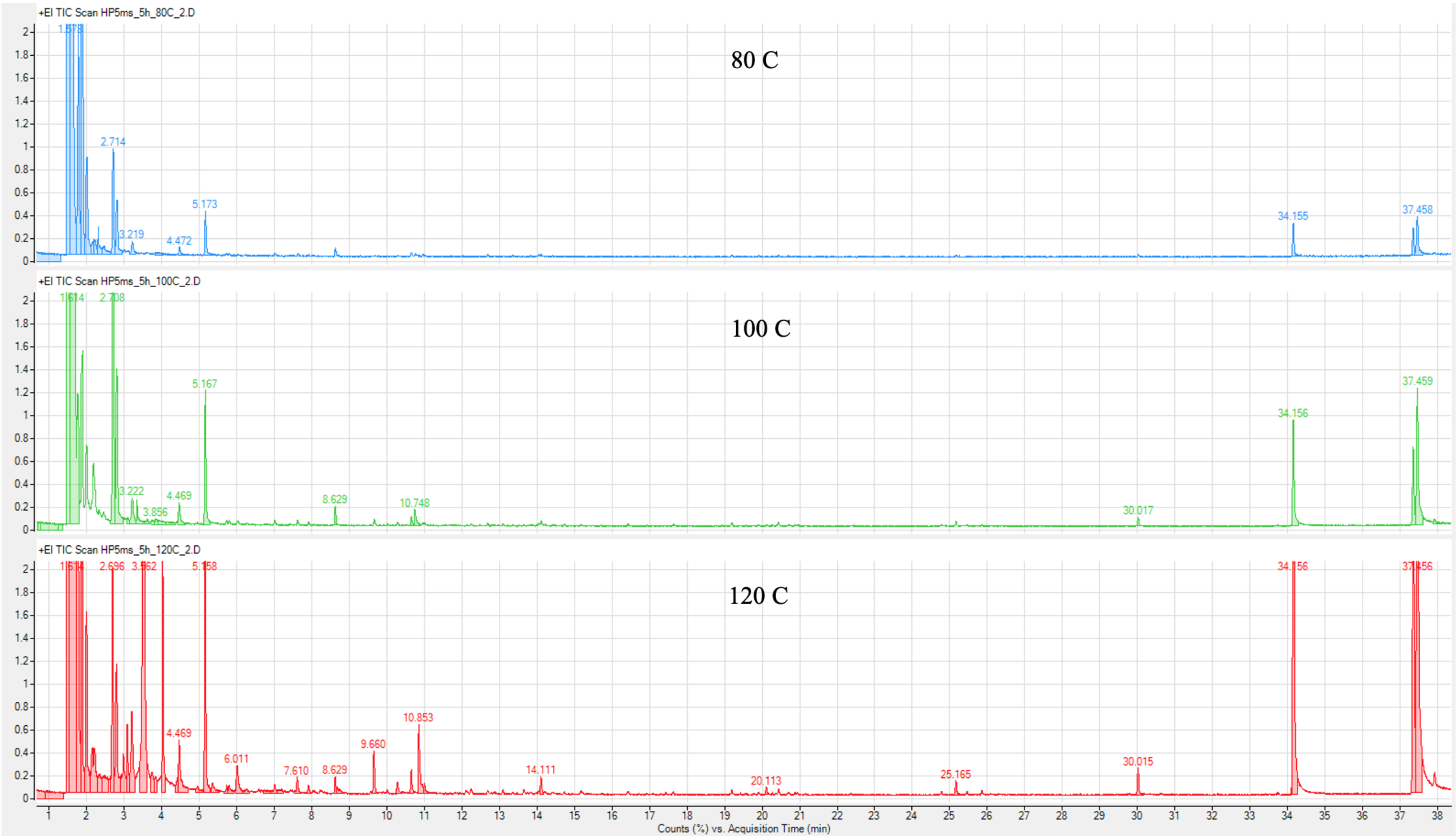

Figure 9 GC-MS chromatograms of volatile compounds from Red Hawm Rice samples at 80 °C (blue line), 100 °C (green line), and 120 °C (red line) headspace incubation temperature for 60 min. (All samples were previously preheated at 80 °C for 5 h in the hot air oven).

Discussion

Volatile profile of red rice in this study differed from that of white fragrant rice such as jasmine rice (KDML105) in many previous studies. For example, 2-acetyl-1-pyrroline (2AP), one of the key compounds responsible for desirable fragrant in the white jasmine rice (Hopfer et al., 2016; Sriseadka et al., 2006) was not detected from Red Hawm Rice. However, in the study conducted by Daygon et al. (2016) utilizing static HS, it was reported that the average concentration of 2AP was 1.5 times higher in the *indica* varieties (comprised of KDML105, PRD, RD6, and HNN cultivars) than in the *japonica*, and it was as much as four times lower in the Australian commercial cultivars Kyeema and Topaz. This addressed the need of the optimization method to identify the aromatic compounds in the colored rice. Despite not being 2AP, the compounds discovered this time have been reported to generate scents such as: The volatile chemical 2-methylpropanal was found in a wide variety of foods; it contributes those distinct "fruity," "almond," and "pungent" aromas and tastes (Chen et al., 2020). 3-methylbutanal was shown to be volatile in cooked black rice (Song et al., 2000), additionally, it contributes to the almond-like and cocoa-like fragrances and nutty flavor of cheese (Chen et al., 2020). Also, A total of 25 aroma-active chemicals were identified in Korean black rice in a study by Yang et al. (2008). 2AP, (E)-2-nonanal, nonanal, hexanal, and 3-octen-3-one all had a significant impact on the aroma of Korean black rice. When these compounds are coupled with other substances in minute proportions, the overall fragrance may also be distinctive.

Static headspace GC-MS is a tool that can be used to determine the volatile profiles of rice sample. According to our findings, numbers of identified compounds did not increase after 5 h preheated time in the hot air oven. The maximum number of volatile compounds were detected when the headspace incubation temperature was set at 120 °C. Thus, optimum condition to extract the most volatile compounds with the least degradation from Red Hawm Rice was 5 h preheated time in the 80 °C hot air oven, accompanied by 120 °C headspace incubation temperature for 60 min.

Equilibration time could be adjusted by increasing the amount of time that food samples are preheated (Hopfer et al., 2016; Sriseadka et al., 2006). Higher temperature in the headspace oven could cause more volatile compounds from food to enter headspace and accumulate until the equilibrium concentration is reached (Mathure et al., 2011; Sanz et al., 2001). These two steps resulted in a higher number of volatile compounds being released in a greater quantity. As a result, the quality of the GC separation will improve as shown by sharp chromatographic peaks. Overheating for long periods of time, on the other hand, does not always yield positive results. In this study, about 22% of the red rice volatile compounds were decreased in amount when using a preheated time in the hot air oven greater than 5 h. Excessive heat may cause degradation of some compounds. Since volatile compounds have a low boiling point, in consequence, exposing them to too much heat might cause it to decompose easily (Hopfer et al., 2016; Sriseadka et al., 2006). Eventually, the modified method for assessing volatile compounds in Red Hawm rice can be utilized to identify volatile compounds in Thai native-colored rice.

Conclusion

Volatile profiles of the colored rice can be identified by static headspace GC-MS approach. By determining the optimum condition of preheated time and headspace oven temperature, satisfactory information about volatile compounds can be obtained without the chemicals being degraded by excessive heat. In the future, the improved method for identifying volatile compounds in Red Hawm rice can be used to other Thai native-colored rice.

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Chapter IV

ARTICLE II (under review)

Title: Metabolomics Approach to Identify Key Volatile Aromas in Thai Colored Rice Cultivars

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Metabolomics Approach to Identify Key Volatile Aromas in Thai Colored Rice Cultivars

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Abstract

In addition to white jasmine rice, Thailand has many native-colored rice varieties with numerous health benefits and the potential to become a global economic crop. However, the chemical characteristics of aromatic substances in native-colored rice are still mostly unknown. This study aimed to identify the key volatile aroma compounds in Thai native-colored rice varieties, leading to its uniqueness in its ability to promote the consumption of nutritious rice both domestically and internationally. Twenty-three rice varieties in four categories: aromatic white, aromatic black, non-aromatic black, and non-aromatic red, were investigated (n=10 per variety). Paddy seeds from each variety were harvested from 10 randomly selected plants. Seed husks were removed before the analysis of rice volatile aromas by static headspace gas chromatography–mass spectrometry. Untargeted metabolomics was used to discover the key volatile compounds in colored rice. Forty-eight compounds were identified. Statistical analysis revealed that 38 of the 48 compounds significantly differed among groups at $p < 0.05$, 28 of which at $p < 0.001$, with the non-aromatic black and red rice containing much lower content of most volatile constituents than the aromatic black and white rice. Focusing on the aromatic black rice, the samples appeared to contain high level of both compound groups of aldehydes (3-methylbutanal, 2-methylbutanal, 2-methylpropanal, pentanal, hexanal) and alcohols (butane-2,3-diol, pentan-1-ol, hexan-1-ol).

Introduction

In addition to the well-known white jasmine rice, Thailand has numerous rice varieties with potential to become a worldwide economic crop. Consumers are currently interested in colored rice because of its health benefits, particularly its antioxidant effects, stronger than in white rice (Sansenya & Nanok, 2020; Walter et al., 2013). Thai native-colored rice such as riceberry, black glutinous rice, red rice, etc., have dark tones ranging from red, brown, and black due to the accumulation of proanthocyanin, anthocyanin, flavonoid, and phenolic acid compounds. Antioxidant activity, anti-hyperlipidemia, oxidative stress reduction, and anti-carcinogenic activity have all been related to the anthocyanins present in colored rice (Sivamaruthi et al., 2018).

Moreover, colored rice is high in fiber and protein, making it ideal as plant-based food. Black rice, according to Kushwaha (2016), has a higher protein content than other rice varieties. For a healthy diet and sustainable food production, increasing the consumption of plant-based diets and less animal-based foods is key (Langyan et al., 2021; Päivärinta et al., 2020). The global plant-based food market is predicted to grow from 29.4 billion USD in 2020 to 161.9 billion USD in 2030 (Statistica, 2022).

Currently, Thailand has developed novel colored rice types, both nutritious and appealing to customers. Although their aromatic properties have been established in several colored rice cultivars, information on the chemical characteristics of volatile substances in these colored rice varieties is still limited, particularly the compounds responsible for colored rice's distinct scent and flavor compared to white rice's. Hence, this study aimed to analyze the types of aromatic compounds in Thai native-colored rice varieties and determine key volatile compounds which could indicate biosynthesis pathways and genetic markers for improvement of Thai colored rice. The technology for analyzing the candidate compounds has tremendously come out over the years. Untargeted metabolomics, the hypothesis-generating tool (Schrimpe-Rutledge et al., 2016), is an emerging technique that combines high-resolution technology, like mass spectrometry or nuclear magnetic resonance, with advanced statistical analysis to extract the important compounds among a large number of metabolites in a biological sample. Metabolomics analysis of volatile organic compounds is applied in various research fields, notably medicine (Sukaram et al., 2022), food (Diez-Simon et al., 2019), and plant sciences (Mhlongo et al., 2022). In this study, static headspace gas chromatography–mass spectrometry (SHS-GC-MS) was employed for the analysis of the aromatic compounds in rice samples. This study aimed to identify the key volatile aroma compounds in Thai native-colored rice varieties, leading to their uniqueness in their ability to promote the consumption of nutritious rice both domestically and internationally.

Materials and Methods

Rice Plants

Twenty-three rice varieties in four categories: aromatic white, aromatic black, non-aromatic black, and non-aromatic red (Table 6), were planted at the Rice Science Center, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom Province, Thailand, during 2018's wet season (August 2018- January 2019). This investigation focuses on the aromatic chemicals found in natively colored rice. Not included is the Red Hawm rice from prior. Twenty-one-day-old seedlings were transplanted to the paddy field at 10 rows x 10 plants per row per variety, with 25 X 25 cm plant spacing and 50 cm variety spacing. Paddy seeds from each variety were harvested from 10 randomly selected plants.

Seed Preparation

The husk of paddy rice seed was removed by hand. One hundred and ten seeds per plants and 10 plants per variety from each field location were collected and stored at -80 °C before analysis.

Metabolomic Analysis

Sample Preparation

Rice sample preparation was done using the optimum condition previously established by Jindawatt et al. (2021), which specifically developed for the volatile analysis of colored rice. Briefly, 1 g of rice was placed into 10-mL headspace vials. Then, 10 µL 99% 2,4,6-trimethylpyridine (CAS No. 108-75- 8, Alfa Aesar, Heysham, England) was added into the vials as an internal standard. The vials were sealed and preheated in a hot air oven (WTC Binder Bd-53, Tuttlingen, Germany) at 80°C for 5 h before being transferred to the SHS-GC-MS in order to extract as many volatile compounds as possible.

Volatile Profile Analysis Using SHS-GC-MS

After equilibration, SHS-GC-MS analysis was carried out with a 7697A SHS autosampler coupled to 7890B GC system and 7000C QQQ MS (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5ms capillary column (5% phenyl/ 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent, CA, USA). A single quadrupole in scan mode was used for GC-MS analysis, which suitable for qualitative analysis or identification (an untargeted metabolomics). Samples were placed into a headspace autosampler oven and incubated again at 120°C for 60 min. Next, 1-mL headspace volatile was collected at 140°C and directly introduced into a GC-MS system. The temperature of the GC inlet was 220°C. Ultra-high purity helium (99.99%) was used as carrier gas at average velocity of 35 cm/s and a 20:1 split ratio. The initial oven temperature was set at 40°C for 2 min, ramped to 250°C at 5°C/min, and finally held for 4 min. The MS was operated in electron impact (EI) mode at 70 eV. The temperature of the MS interface, EI source, and quadrupole were set at 250, 230, and 150°C, respectively. Chromatogram and mass spectra were acquired using a scan mode ranging from 33-400 m/z.

Data Processing and Statistical Analysis

Rice volatile compounds were identified by comparing both the mass spectra and retention index (RI) against the National Institute of Standards and Technology library (NIST) 2014 library. The RI of the n-alkane series (C7-C30; Supelco, Sigma-Aldrich, PA, USA) was used to compute the RI values. A matching score ≥70 and a RI value difference ≤20 units between the calculated RI and the database values for the same stationary phase were required for compound identification. A pooled quality control (QC) sample was included every 10 samples. Peak picking, spectral deconvolution, and data alignment were performed using MS-DIAL 4.70 software (Tsugawa et al., 2015). Multivariate analysis, metabolite set enrichment analysis (MSEA), and analysis of variance (ANOVA) were performed with MetaboAnalyst 5.0 (Xia & Wishart, 2010).

Table 6 List of rice samples.

No.	Rice varieties	Code	Pericarp color	BADH2 allele
1	Basmati 370	BMT	white	aromatic
2	Khao Dawk Mali 105	KDML105	white	aromatic
3	Klamhom	KH	black	aromatic
4	LeumPua glutinous rice	LP	black	aromatic
5	UP_460_Chanohnai	UP_460	black	aromatic
6	UP_463_Pi-isu	UP_463	black	aromatic
7	UP_468_Pi-isu Maeradnoi	UP_468	black	aromatic
8	UP_469_Pi-isu Maekwangnuea	UP_469	black	aromatic
9	UP_470_Pi-isu Maekwangnuea	UP_470	black	aromatic
10	Niew Dam khaika glutinous rice	DKG	black	aromatic
11	Khao Hom Mae Phaya Tongdam	MTK	black	aromatic
12	Mu1309	Mu1309	black	aromatic
13	Mu2313	Mu2313	black	aromatic
14	Mu2550	Mu2550	black	aromatic
15	Riceberry 2 (#909)	RB2	black	aromatic
16	Niew Dam Chomaipai 49 glutinous rice	BSHMP	black	non-aromatic
17	Riceberry	RB	black	non-aromatic
18	Niew Dammo 37 glutinous rice	DM37	black	non-aromatic
19	Niew Dammuebueng glutinous rice	DMB	black	non-aromatic
20	Jao Hom Nin	JHN	black	non-aromatic
21	Khao Mednaifuy	MNF	black	non-aromatic
22	RD 69 (Tubtim Chumphae)	RUBY	red	non-aromatic
23	UP_417_Buetolasosobkhong	UP_417	red	non-aromatic

Results

Forty-eight volatile compounds were identified in 23 rice varieties (Table 7, Figure 10). A complete dataset is presented in Supplementary Table 1, which includes retention time (RT), RI, metabolite ID, CAS no., InChIKey, matching score, signal-to-noise ratio (S/N), EI spectrum, and peak area. Metabolite set enrichment analysis was carried out to observe the patterns of the main chemical class sets by MetaboAnalyst software. In the MetaboAnalyst 5.0 database, 33 out of the 48 chemicals identified had a PubChem CID (compound ID number) match, as shown in the overview of aroma compound sets in Figure 11. Fatty aldehydes, aldehydes, and fatty esters were the most common volatile chemical classes found in the rice samples.

Prior to multivariate statistical analysis, data were normalized using log transform and pareto scale for volatile chemical profiling. Due to the complication of the obtained data, PCA may not be distinguished; therefore, The partial least squares-discriminant analysis (PLS-DA) was employed to help explain. PLS-DA is a multivariate projection method utilized to describe the connection between dependent variables (Y, the volatile profiles) and independent factors (X, the rice samples). PLS-DA aims at finding the variables and directions in the multivariate space which discriminate the established classes in the set. PLS-DA score plot shows different volatile profiles among the four rice types (R^2 = 0.76 and Q^2 = 0.68, see

Supplementary Figure S2 for the output of permutation test) (Figure 12). Start the comparison by dividing the group by pericarp to facilitate consumption promotion. Investigate the connection between the pericarp and aromatic components of the various cultivars. White and black rice patterns, and black and red rice samples, are the opposite. The volatile compound pattern in black rice is similar. It can be seen that the black circle is aromatic black rice and the gray cross is non-aromatic black rice. They are mostly overlapping. Similarly, with white rice and red rice, white rice is represented by the light blue circle that will overlap with red rice to form a red cross, indicating a similar pattern of volatiles. Therefore, it may be concluded that the volatile profile of red rice tends to resemble that of white rice. However, the aromatic and non-aromatic black rice profiles are remarkably similar.

When considering each colored rice sample group independently, the volatile compounds present in black aromatic rice varieties are grouped together. From the top view, the volatile components of UP460, UP463, UP468, UP469, and UP470 are close to each other and positioned slightly isolated from DKG, MTK, Mu1309, Mu2313, Mu2550, and RB2 (Figure 13A). As for the volatile components of non-aromatic black rice cultivars, the RB aroma profile is related to MNF. However, it is quite different from that of BSHMP, located close to DM37 and DMB (Figure 13B). The red rice volatile profiles RUBY and UP417 are plotted separately in the PLS-DA scores plot (Figure 13C). Overall, the heatmap in Figure 14 shows the different patterns of volatile chemicals derived from the various colored rice groups. Statistical analyses by ANOVA followed by Tukey's HSD post-hoc test showed significant differences at $p < 0.05$ in 38 of the 48 compounds among the four rice groups, 28 of which at $p < 0.001$ (Table 7). From statistical analysis in Table 7, the primary volatile chemicals discovered in the black rice samples as compared to the four categories of rice are shown in Figure 15, which include 2-methylpropanal, 3-methylbutanal, and 2-methoxyphenol. In addition, fold-change values were calculated to identify which volatile compounds are more abundant between the two groups (Figure 16). When compare between aroma rice (aroma black vs. aroma white rice), the results illustrate that white rice has a higher concentration of several volatile components while there are only two substances, 2-methoxyphenol and butane-2,3-diol, that are higher in the black fragrant rice (Figure 16A). On the contrary, when comparing solely the two types of black rice (aroma vs. non-aroma), aroma black rice exhibits higher levels of many volatile compounds than the non-aroma rice (Figure 16B). This study did not detect 2AP in aromatic white rice. This may be because analytical methods have been modified to focus on finding as many substances as possible.

Table 7 Volatile compounds detected in aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice samples.

No.	RT (min)	Metabolite name	Formula	CAS No.	p-value*	f-value
1	1.829	methyl acetate	$C_3H_6O_2$	79-20-9	NS	-
2	1.961	2-methylpropanal	C_4H_8O	78-84-2	<0.0001	26.49
3	2.641	3-methylbutanal	$C_5H_{10}O$	590-86-3	<0.0001	8.90
4	2.746	2-methylbutanal	$C_5H_{10}O$	96-17-3	NS	-
5	2.818	4-(dimethylamino)-3-hydroxybutanoic acid	$C_6H_{13}NO_3$	3688-46-8	NS	-
6	3.107	pentane-2,3-dione	$C_5H_8O_2$	600-14-6	NS	-
7	3.151	pentanal	$C_5H_{10}O$	110-62-3	NS	-
8	3.232	acetic acid	$C_2H_4O_2$	64-19-7	NS	-
9	3.310	formyl acetate	$C_3H_4O_3$	2258-42-6	<0.001	5.69
10	3.731	1-hydroxypropan-2-one	$C_3H_6O_2$	116-09-6	NS	-
11	4.390	pentan-1-ol	$C_5H_{12}O$	71-41-0	<0.001	6.77
12	5.075	hexanal	$C_6H_{12}O$	66-25-1	<0.0001	12.5
13	5.568	butane-2,3-diol	$C_4H_{10}O_2$	513-85-9	NS	-
14	5.698	4-methylpyrimidine	$C_5H_6N_2$	3438-46-8	<0.0001	-
15	5.927	furan-2-carbaldehyde	$C_5H_4O_2$	98-01-1	<0.0001	12.11
16	5.984	1-(5-methyl-1H-pyrazol-3-yl)propan-2-amine	$C_7H_{13}N_3$	1025087-55-1	<0.0001	11.21
17	6.628	3,3-dimethyl-4-(methylamino)butan-2-one	$C_7H_{15}NO$	123528-99-4	<0.01	3.4781
18	6.901	hexan-1-ol	$C_6H_{14}O$	111-27-3	<0.0001	11.8
19	7.505	heptan-2-one	$C_7H_{14}O$	110-43-0	<0.0001	12.26
20	7.799	heptanal	$C_7H_{14}O$	111-71-7	<0.0001	9.42
21	8.116	2,6-dimethylpyrazine	$C_6H_8N_2$	108-50-9	<0.0001	27.28

Note: Gray grid = more prevalent in black rice, light blue grid = more prevalent in white rice.

Table 7 (cont.) Volatile compounds detected in aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice samples.

No.	RT (min)	Metabolite name	Formula	CAS No.	p-value*	f-value
22	8.514	methyl hexanoate	$C_7H_{14}O_2$	106-70-7	<0.0001	24.69
23	9.539	benzaldehyde	C_7H_6O	100-52-7	<0.0001	20.15
24	10.307	2-propylpropanedioic acid	$C_6H_{10}O_4$	616-62-6	<0.05	3.9392
25	10.881	octanal	$C_8H_{16}O$	124-13-0	<0.0001	13.18
26	11.563	methyl 5-methylhexanoate	$C_8H_{16}O_2$	2177-83-5	<0.0001	26.48
27	11.582	methyl heptanoate	$C_8H_{16}O_2$	106-73-0	<0.0001	8.81
28	11.976	3-hydroxy-4,4-dimethyloxolan-2-one	$C_6H_{10}O_3$	79-50-5	<0.01	5.73
29	12.117	2-phenylacetaldehyde	C_8H_8O	122-78-1	<0.05	3.9562
30	13.515	2-methoxyphenol	$C_7H_8O_2$	90-05-1	<0.0001	393.51
31	13.977	nonanal	$C_9H_{18}O$	124-19-6	<0.01	9.31
32	14.598	methyl octanoate	$C_9H_{18}O_2$	111-11-5	<0.0001	23.57
33	15.038	methyl pyridine-3-carboxylate	$C_7H_7NO_2$	93-60-7	<0.01	4.32
34	15.150	1-methylpyridin-1-ium-3-carboxylate	$C_7H_7NO_2$	535-83-1	<0.0001	10.20
35	16.165	methyl 2-phenylacetate	$C_9H_{10}O_2$	101-41-7	<0.0001	9.34
36	16.286	naphthalene	$C_{10}H_8$	91-20-3	<0.0001	11.89
37	16.962	decanal	$C_{10}H_{20}O$	112-31-2	<0.01	7.046
38	17.324	2,3-dihydro-1-benzofuran	C_8H_8O	496-16-2	<0.0001	7.94
39	17.496	methyl nonanoate	$C_{10}H_{20}O_2$	1731-84-6	<0.0001	14.87

Note: Gray grid = more prevalent in black rice, light blue grid = more prevalent in white rice.

Table 7 (cont.) Volatile compounds detected in aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice samples.

No.	RT (min)	Metabolite name	Formula	CAS No.	p-value*	f-value
40	17.732	3-ethyl-4-methylpyrrole-2,5-dione	$C_7H_9NO_2$	20189-42-8	<0.0001	29.20
41	19.973	1-(2-hydroxy-5-methylphenyl)ethanone	$C_9H_{10}O_2$	1450-72-2	<0.0001	24.58
42	25.311	methyl 10-methylundecanoate	$C_{13}H_{26}O_2$	5129-56-6	<0.0001	11.75
43	29.865	methyl 12-methyltridecanoate	$C_{15}H_{30}O_2$	5129-58-8	NS	-
44	32.394	6,10,14-trimethylpentadecan-2-one	$C_{18}H_{36}O$	502-69-2	<0.0001	19.17
45	34.001	methyl hexadecanoate	$C_{17}H_{34}O_2$	112-39-0	<0.001	7.21
46	37.165	methyl (9Z,11E)-octadeca-9,11-dienoate	$C_{19}H_{34}O_2$	13058-52-1	NS	-
47	37.185	methyl (10E,12Z)-octadeca-10,12-dienoate	$C_{19}H_{34}O_2$	21870-97-3	<0.01	5.66
48	37.296	methyl-octadec-9-enoate	$C_{19}H_{36}O_2$	112-62-9	<0.0001	11.20

Note: Gray grid = more prevalent in black rice, light blue grid = more prevalent in white rice.

* P-value is determined by ANOVA among the four groups of rice (aroma white, aroma black, non-aroma black, and non-aroma red rice) of each compound; NS: not significant ($p>0.05$).

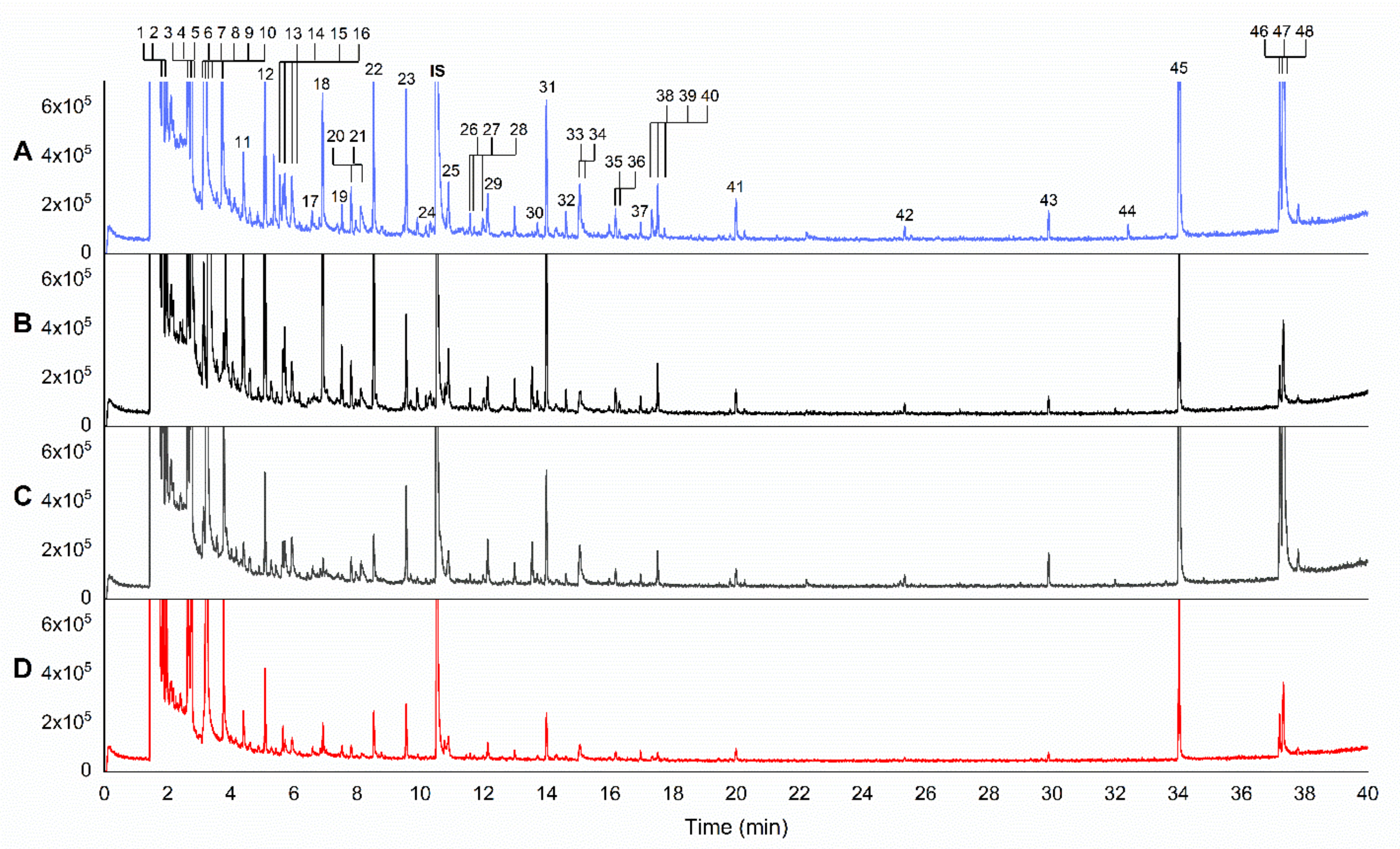


Figure 10 Representative chromatograms. **(A)** aromatic white rice (Basmati 370), **(B)** aromatic black rice (Klamhom), **(C)** non-aromatic black rice (Riceberry), **(D)** non-aromatic red rice (RD 69 Tubtim Chumphae); IS = internal standard.

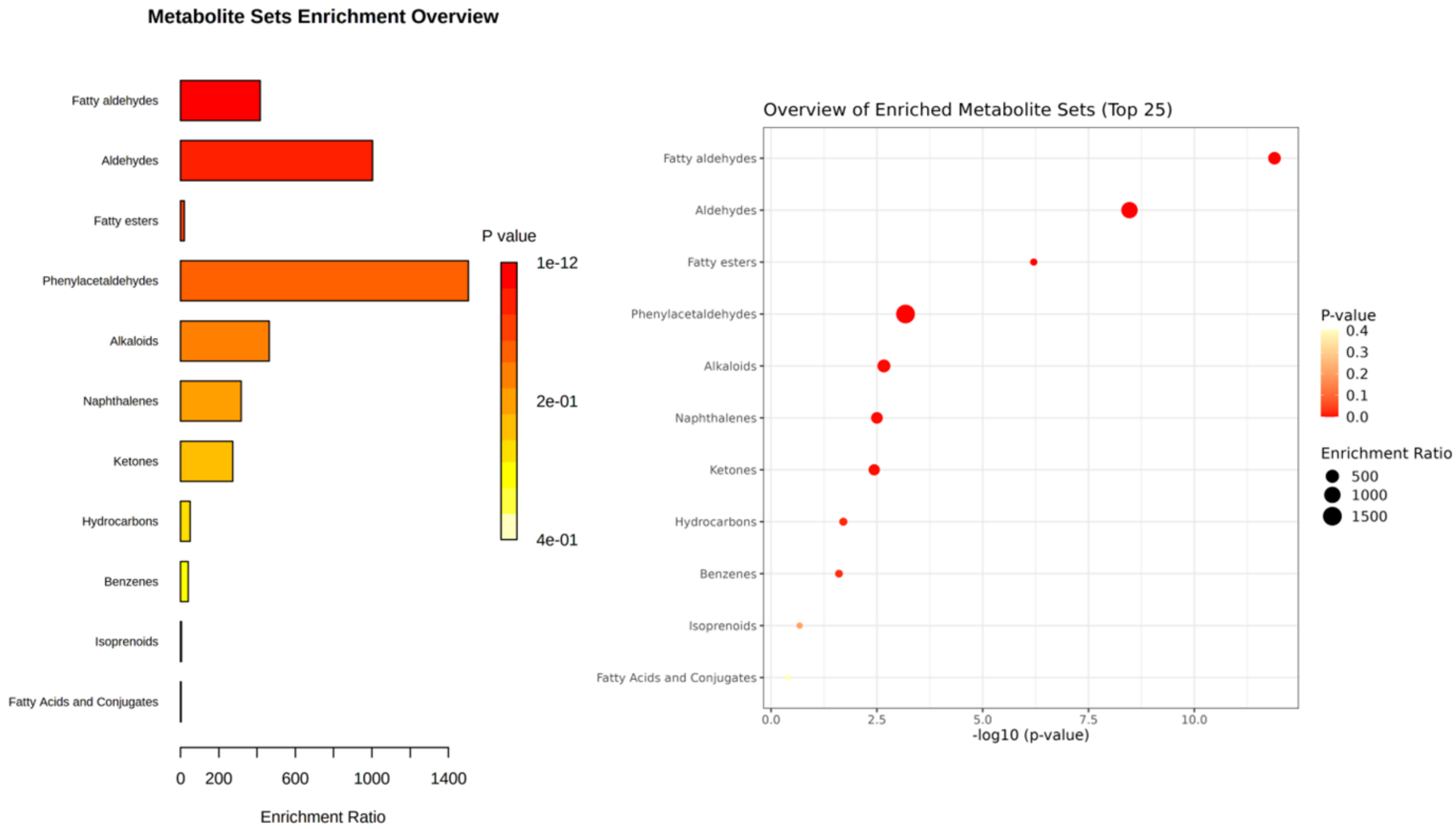


Figure 11 Enrichment of rice aroma compound groups classified by sets of main class chemica

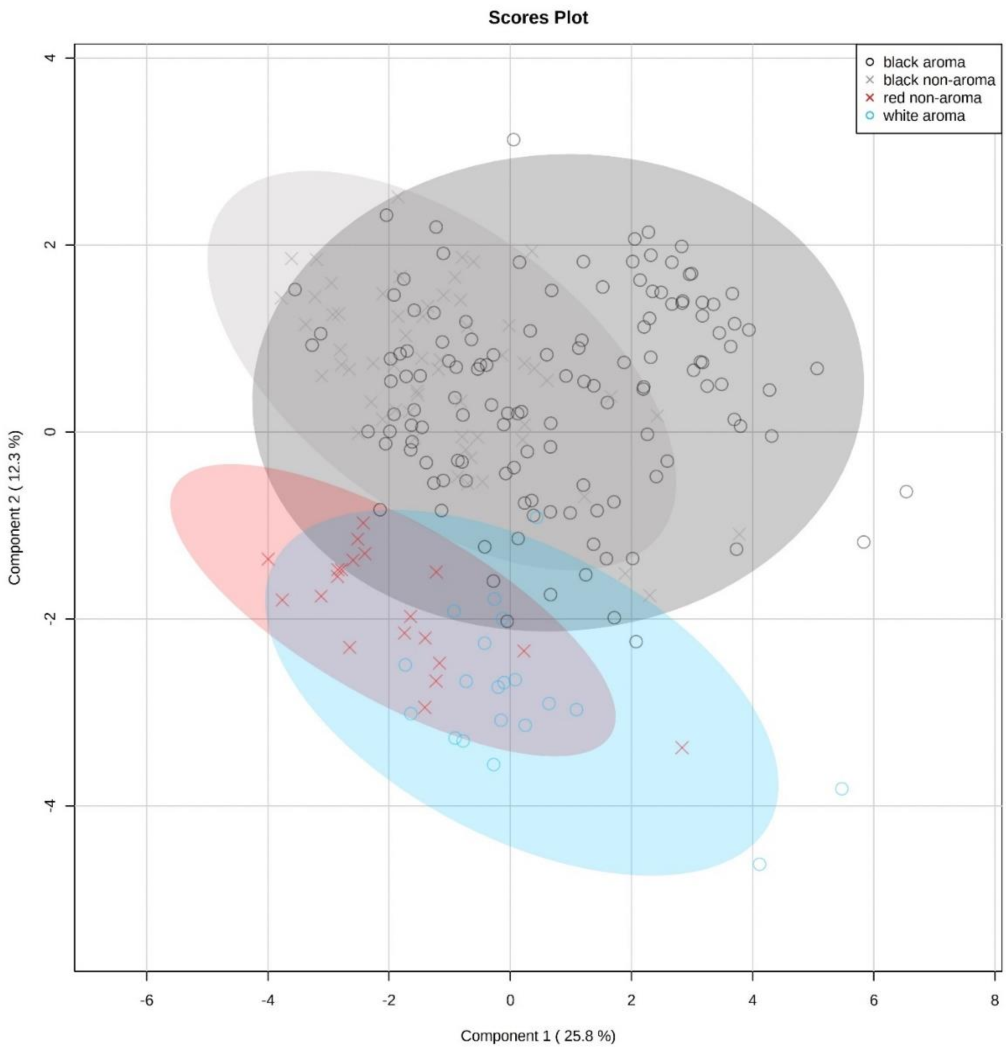


Figure 12 Partial least squares-discriminant analysis score plot of volatile profiles of aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice samples.

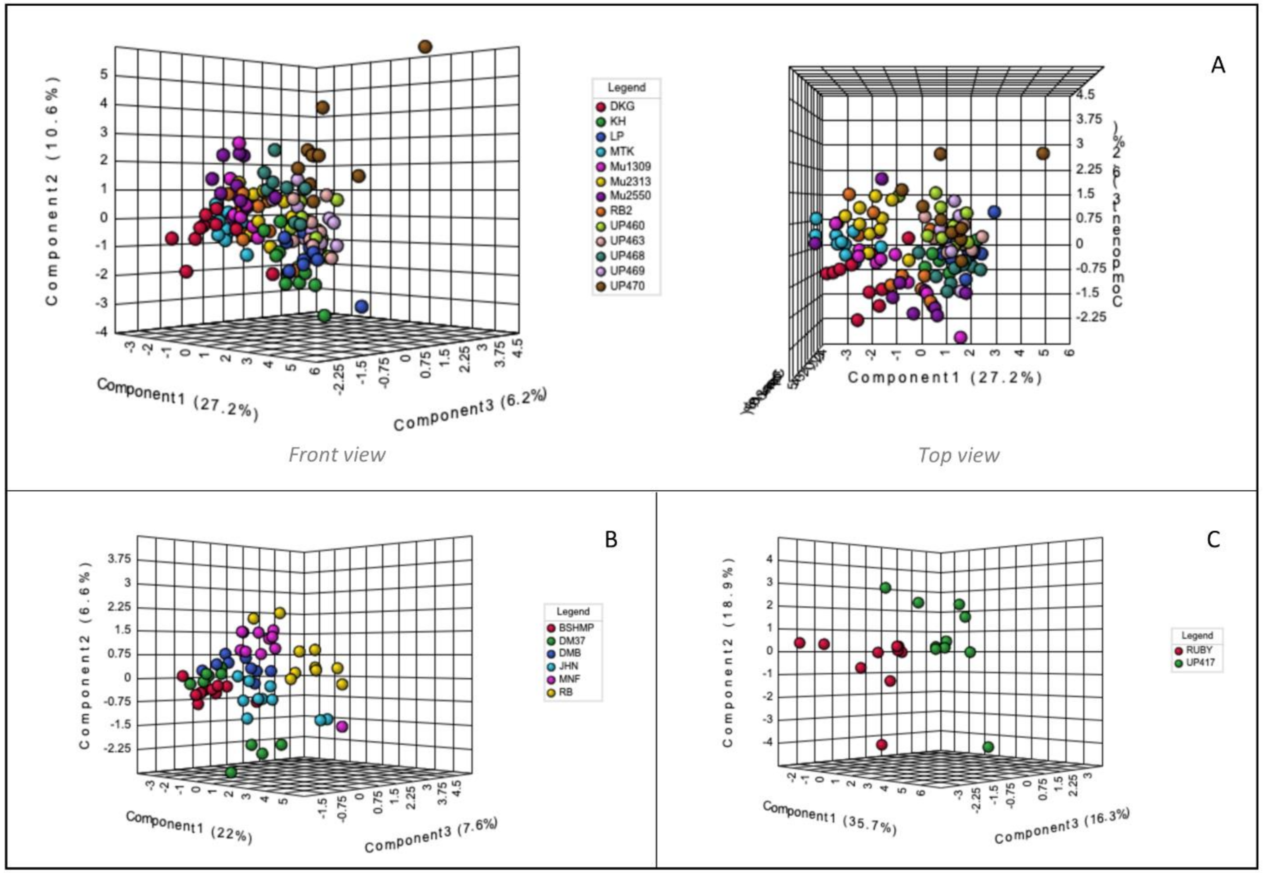


Figure 13 Partial least squares-discriminant analysis of Thai colored rice volatile profiles. **(A)** aromatic black rice, **(B)** non-aromatic black rice, **(C)** non-aromatic red rice.

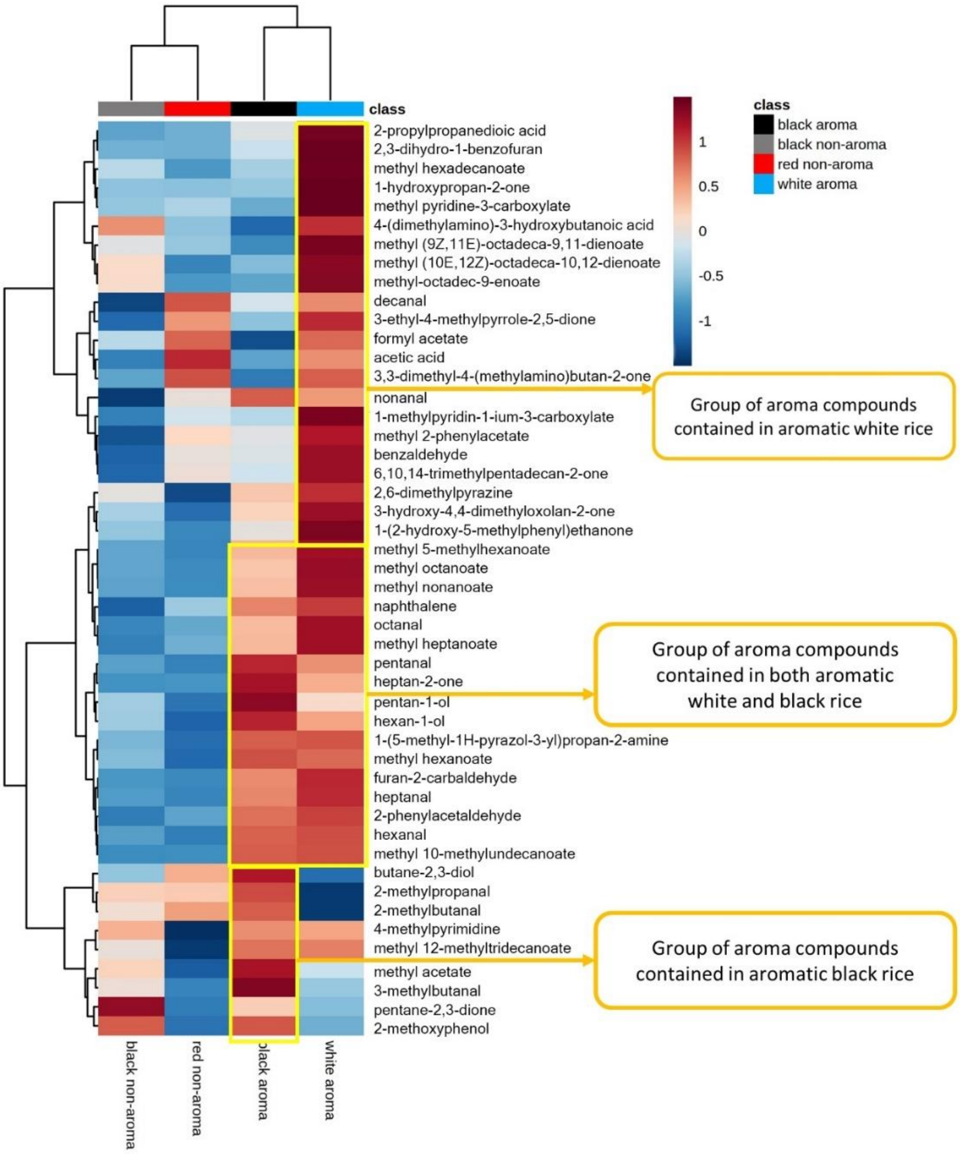


Figure 14 Heatmap of aroma compounds identified in aromatic white, aromatic black, non-aromatic black, and non-aromatic red rice samples

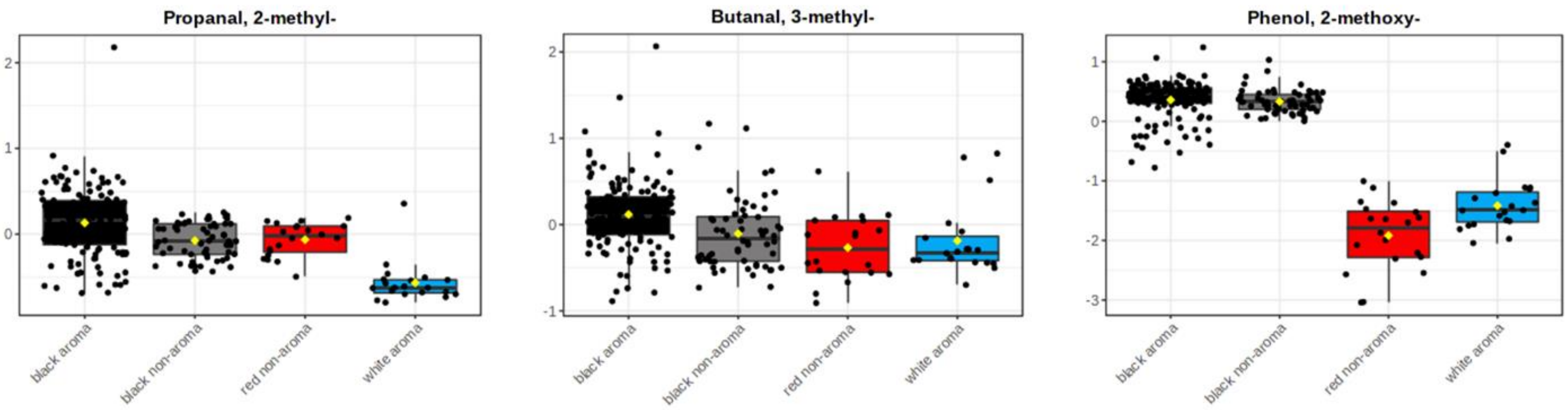
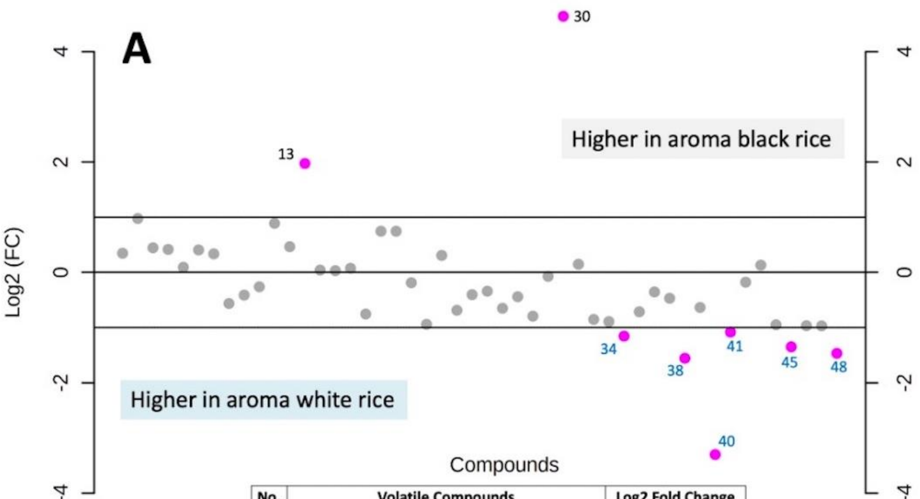
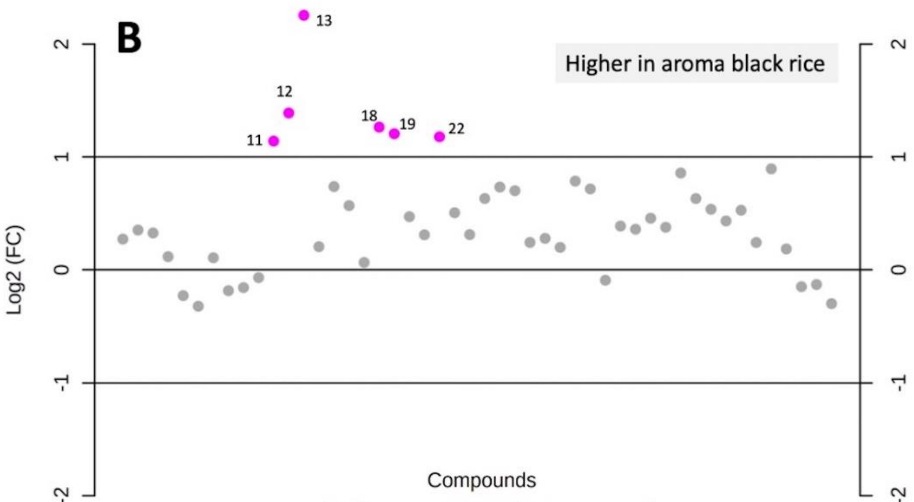


Figure 15 Box and whisker plots of principal aroma compounds identified in aromatic black rice samples.



No.	Volatile Compounds	Log2 Fold Change
30	2-methoxyphenol	4.6388
40	3-ethyl-4-methylpyrrole-2,5-dione	-3.2977
13	butane-2,3-diol	1.9724
38	2,3-dihydro-1-benzofuran	-1.5546
48	methyl (Z)-octadec-9-enoate	-1.4653
45	methyl hexadecanoate	-1.3483
34	1-methylpyridin-1-ium-3-carboxylate	-1.1534
41	1-(2-hydroxy-5-methylphenyl)ethanone	-1.0806



No.	Volatile Compounds	Log 2 Fold Change
13	butane-2,3-diol	2.2562
12	hexanal	1.3901
18	hexan-1-ol	1.2646
19	heptan-2-one	1.2068
22	methyl hexanoate	1.1795
11	pentan-1-ol	1.1409

Figure 16 Fold-change analysis of the volatile compounds abundance between **(A)** aromatic black rice and aromatic white rice samples, **(B)** aromatic black rice and non-aromatic black rice samples.

Discussion

Volatile Components in Thai Colored Rice Cultivars

The results showed that the main volatile found in the black rice was 2-methoxyphenol. Although both 2-methoxyphenol and butane-2,3-diol showed large fold-changes when compared between the two aroma rice groups (black vs. white, Figure 16), levels of butane-2,3-diol were not significantly different among the four rice groups as revealed in Table 7. 2-methoxyphenol levels in both aromatic and non-aromatic black rice were significantly higher than in white and red rice ($p < 0.001$), with the highest f-value (393.94) and the highest VIP score (> 2.0). This finding agrees with previous research by Yang et al. (2008), who found that 2-methoxyphenol is the primary component underlying black rice's uniqueness, and that it also contributes to the aroma of smoked and roasted foods like bacon and coffee (Schranz et al., 2017). The food and perfume industries employ it for aromatization, and it can be found in a wide variety of plant life. 3-methylbutanal was highly present in aromatic black rice as compared to other rice types. This compound was also reported as volatile in cooked black rice (Song et al., 2000), and it has been noted that it contributes to the almond-like and cocoa-like aromas, nutty flavor of cheese (Chen et al., 2020). 2-methylpropanal, a volatile chemical present in numerous foods, This volatile substance, which has been linked to fruity, almond, and pungent flavors (Chen et al., 2020), was found in lower concentrations in white rice than in black and red rice (Figure 15).

The aromatic rice samples with BADH2 genotype, volatile compounds detected in both white and black aromatic rice unique to non-aromatic rice in this study were methyl 5-methylhexanoate, methyl octanoate, 4-methylpyrimidine, methyl hexanoate, methyl nonanoate, hexanal, methyl 10-methylundecanoate, heptan-2-one, octanal, hexan-1-ol, naphthalene, furan-2-carbaldehyde, 1-(5-methyl-1H-pyrazol-3-yl)propan-2-amine, pentan-1-ol, nonanal, and 2-phenylacetaldehyde (Figure 14). Nevertheless, several volatile components in white fragrant rice samples were found at higher levels than in aromatic black rice samples. These aroma compounds include 3-ethyl-4-methylpyrrole-2,5-dione, previously observed in pandan leaves (Cheetangdee & Chaiseri, 2006) 2,6-dimethylpyrazine, that gives a bread-like aroma (*FooDB Version 1.0*, 2022) 1-(2-hydroxy-5-methylphenyl)ethanone and 3,3-dimethyl-4-(methylamino)butan-2-one, with a sweet floral fragrance (*FooDB Version 1.0*, 2022; Koksal et al., 2015) benzaldehyde and methyl 2-phenylacetate, a methyl ester with an almond-like smell (*FooDB Version 1.0*, 2022) 2-propylpropanedioic acid, found in honey (Tian et al., 2018) 1-methylpyridin-1-ium-3-carboxylate or trigonelline, found in roasted coffee (*FooDB Version 1.0*, 2022; Heo et al., 2020) and the characteristic tobacco-like herbaceous odor of methyl pyridine-3-carboxylate or methyl nicotinate (*FooDB Version 1.0*, 2022; Rao et al., 2007).

In addition, many rice-related aromatic compounds were found at higher amounts in white rice samples. These compounds were 2,3-dihydro-1-benzofuran, contained in the rice husks (Tian et al., 2021), 3-hydroxy-4,4-dimethyloxolan-2-one, formerly observed in cooked rice (Jinakot & Jirapakkul, 2018) and 6,10,14-trimethylpentadecan-2-one, the major volatile substance of red rice (Sukhonthara et al., 2009), found in high concentrations in both white and red rice samples in this study. Fatty aldehydes such as decanal as well as fatty acid methyl esters including methyl-octadec-9-enoate, methyl hexadecanoate, and methyl (10E,12Z)-octadeca-10,12-dienoate were also identified. Unsurprisingly, when only the black variety is considered, aroma black rice contains more volatile compounds than the non-aroma rice (Figure 16B). Volatile substances that have been reported pleasant smells include a buttery, creamy scent from butane-2,3-diol (*FooDB Version 1.0*, 2022), a fruity and floral-like smell from heptan-2-one (Verma & Srivastav, 2020) and a sweet, fresh flavor from methyl hexanoate (*FooDB Version 1.0*, 2022). Hexanal and hexan-1-ol contribute to a green scent in rice (Choi & Lee, 2021; Verma & Srivastav, 2020) and pentan-1-ol is described a fusel oil-like odor(Verma & Srivastav, 2020), which might contribute to the unpleasant smell of the black rice.

Key Volatile Compounds

The heatmap patterns shown in Figure 14, which summarize quantitatively various volatile components detected in the four categories of rice samples, show that the non-aroma group (black and red) had a much lower content of most volatile components than the aroma group (black and white). Interestingly, the heatmap also clearly shows that each rice category has its own uniqueness in terms of major volatile components. The non-aroma black rice showed high content of pentane-2,3-dione, 2-methoxyphenol and 4-methylpyrimidine while the non-aroma red showed high content of acetic acid, decanal, 3,3-dimethyl-4-(methylamino)butan-2-one. On the other hand, the aroma black appeared to contain high content of some aldehyde components, specifically of 3-methylbutanal, 2-methylbutanal, 2-methylpropanal, pentanal, hexanal, and some alcohol components, mainly of butane-2,3-diol, pentan-1-ol, hexan-1-ol. By comparing with the white-aroma rice, these main black-aroma constituents are in only the minor components of the white-aroma rice (Figure 14).

Conclusion

The key volatile aromas in Thai native-colored rice cultivars were identified using SHS-GC-MS untargeted metabolomics approach. 2-methylpropanal was the most distinctive volatile in colored rice (black and red rice). 2-methoxy phenol was mainly found in both aromatic and non-aromatic black rice, while 3-methylbutanal was the major compound in aromatic black rice. However, it should be noted that all the volatile constituents were detected in all the four rice categories but in different accumulated contents. Research on volatile profiles may be utilized to advocate for eating different types of colored rice for better health and economic growth.

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CHAPTER V

CONCLUSION OF THESIS

To summarize key findings of both research studies in the thesis, colored rice is an unpolished rice that has grown in popularity because to its vivid colors, which are a rich source of dietary fiber and antioxidants include phenolic acids and anthocyanins. The proper incubation time and temperature to extract the volatile compounds in Thai-colored rice using the static headspace GC-MS approach. The optimum condition was 5 h preheated time in the oven, followed by 120 °C headspace extraction temperature. For the analysis of volatile compounds in native colored rice, this study found the main volatile substances 2-methoxyphenol, 3-methylbutanal, and 2-methylbutanal. In the future, samples of purple or black rice may be used as a representative of optimum conditions. Besides red rice, it should help to select the appropriate conditions more efficiently. Validate extraction method using static headspace GC-MS with 2-3 types of spike key volatile with different volatility. It will increase the confidence in applying the method to other test samples in the future. This new finding will lead to improvements in Thai colored-rice breeding so that the native-colored rice in Thailand will be commercially valuable in the future.

However, 2AP, the key contributes to rice aroma, did not clearly detected by the optimized in this study. There are two main possible explanations. The first reason is that 2AP is heat labile and might have been degraded by the preheated process. For the untargeted metabolomics analysis point of view, the goal was to identify as many metabolites (volatile compounds) as possible. So, we tried to get a lot of volatile compounds and might have lost the 2AP. Another possible explanation is regarding to the parameters that were set in the data processing step. Metabolomics data processing steps include deconvolution, peak picking, and data alignment. This step is critical prior to multivariate statistical analysis. S/N ratio ≥3 was generally used for a noise cut-off for peak picking process, and it could be greater than the 2AP chromatogram signal. From this thesis, the findings of volatile profiles may be used to promote the consumption of colored rice varieties for optimal consumer health and to increase the country's market value.

SUPPLEMENTARY DATA

Supplementary Table 1 A complete dataset that includes retention time (RT), RI, metabolite ID, CAS no., InChIKey, matching score, signal-to-noise ratio (S/N), EI spectrum, and peak area

Supplementary Table 1

No.	Aver RT(min)	Ave RI	Quant mass	Metabolite ID	Formula
1	1.829	640.68	43.2	methyl acetate	$C_3H_6O_2$
2	1.961	646.95	39.17273	2-methylpropanal	C_4H_8O
3	2.641	675.47	44.21203	3-methylbutanal	$C_5H_{10}O$
4	2.746	679.79	57.19853	2-methylbutanal	$C_5H_{10}O$
5	2.818	682.81	58.16667	4-(dimethylamino)-3-hydroxybutanoic acid	$C_6H_{13}NO_3$
6	3.107	694.67	79	pentane-2,3-dione	$C_5H_8O_2$
7	3.151	696.65	44.14667	pentanal	$C_5H_{10}O$
8	3.232	699.07	60.1	acetic acid	$C_2H_4O_2$
9	3.310	703.83	60.1	formyl acetate	$C_3H_4O_3$
10	3.731	726.27	43.2	1-hydroxypropan-2-one	$C_3H_6O_2$
11	4.390	760.93	55.13077	pentan-1-ol	$C_5H_{12}O$
12	5.075	797.15	72.2	hexanal	$C_6H_{12}O$
13	5.568	815.72	45.2	butane-2,3-diol	$C_4H_{10}O_2$
14	5.698	820.47	94.0698	4-methylpyrimidine	$C_5H_6N_2$
15	5.927	829.15	96	furan-2-carbaldehyde	$C_5H_4O_2$
16	5.984	831.07	57.04047	1-(5-methyl-1H-pyrazol-3-yl)propan-2-amine	$C_7H_{13}N_3$
17	6.628	854.79	60.06808	3,3-dimethyl-4-(methylamino)butan-2-one	$C_7H_{15}NO$
18	6.901	864.86	56.15278	hexan-1-ol	$C_6H_{14}O$
19	7.505	887.16	43.15714	heptan-2-one	$C_7H_{14}O$
20	7.799	898.06	70.07609	heptanal	$C_7H_{14}O$
21	8.116	908.53	108.0554	2,6-dimethylpyrazine	$C_6H_8N_2$
22	8.514	921.65	74.10102	methyl hexanoate	$C_7H_{14}O_2$
23	9.539	955.15	106.0614	benzaldehyde	C_7H_6O
24	10.307	980.27	60.02821	2-propylpropanedioic acid	$C_6H_{10}O_4$
25	10.881	999.07	84.05306	octanal	$C_8H_{16}O$
26	11.563	1021.27	74.03287	methyl 5-methylhexanoate	$C_8H_{16}O_2$
27	11.582	1021.95	54.96667	methyl heptanoate	$C_8H_{16}O_2$
28	11.976	1034.69	71.04681	3-hydroxy-4,4-dimethyloxolan-2-one	$C_6H_{10}O_3$

Supplementary Table 1

No.	Aver RT(min)	Ave RI	Quant mass	Metabolite ID	Formula
29	12.117	1039.30	91.07306	2-phenylacetaldehyde	C8H8O
30	13.515	1084.78	124.025	2-methoxyphenol	C7H8O2
31	13.977	1099.88	57.12347	nonanal	C9H18O
32	14.598	1120.89	74.05056	methyl octanoate	C9H18O2
33	15.038	1135.94	106.0079	methyl pyridine-3-carboxylate	C7H7NO2
34	15.150	1139.55	67.96154	1-methylpyridin-1-ium-3-carboxylate	C7H7NO2
35	16.165	1173.95	91.05605	methyl 2-phenylacetate	C9H10O2
36	16.286	1178.02	127.9975	naphthalene	C10H8
37	16.962	1200.98	57.03519	decanal	C10H20O
38	17.324	1213.87	120.0364	2,3-dihydro-1-benzofuran	C8H8O
39	17.496	1220.10	74.07265	methyl nonanoate	C10H20O2
40	17.732	1228.49	139.0385	3-ethyl-4-methylpyrrole-2,5-dione	C7H9NO2
41	19.973	1309.18	150.0386	1-(2-hydroxy-5-methylphenyl)ethanone	C9H10O2
42	25.311	1518.81	74.00948	methyl 10-methylundecanoate	C13H26O2
43	29.865	1718.57	74.06219	methyl 12-methyltridecanoate	C15H30O2
44	32.394	1839.00	43.01667	6,10,14-trimethylpentadecan-2-one	C18H36O
45	34.001	1919.06	74.1	methyl hexadecanoate	C17H34O2
46	37.165	2085.60	55.1	methyl (9Z,11E)-octadeca-9,11-dienoate	C19H34O2
47	37.185	2087.09	81.10619	methyl (10E,12Z)-octadeca-10,12-dienoate	C19H34O2
48	37.296	2093.06	55.15072	methyl-octadec-9-enoate	C19H36O2

Supplementary Table 1

No.	MS fragment pattern (m/z)	CAS no	InChIKey	R match	S/N average
1	43, 74, 42, 59, 44, 45, 41	79-20-9	KXKVLQRXCPHEJC-UHFFFAOYSA-N	932	759.90
2	43, 41, 72, 39, 42, 38	78-84-2	AMIMRNSIRUDHCM-UHFFFAOYSA-N	863	97.71
3	44, 43, 41, 58, 39, 57, 71, 42	590-86-3	YGHRJJRRZDOVPD-UHFFFAOYSA-N	918	435.53
4	41, 57, 58, 39, 43, 86, 55	96-17-3	BYGQBDHUGHBGMD-UHFFFAOYSA-N	859	3691.63
5	58, 42, 44, 88, 59, 147, 33, 43	3688-46-8	NXDDNODAJKZARA-UHFFFAOYSA-N	832	177.29
6	43, 57, 42, 100	600-14-6	TZMFJUDUGYTVRY-UHFFFAOYSA-N	755	46.37
7	44, 58, 41, 57, 43, 39, 42, 45	110-62-3	HGBOYTHUEUWSSQ-UHFFFAOYSA-N	873	38.04
8	43, 45, 60, 42, 41	64-19-7	QTBSBXVTEAMEQO-UHFFFAOYSA-N	916	1072.95
9	43, 75, 85, 101, 47, 117, 58	2258-42-6	ORWKVZNEPHTCQE-UHFFFAOYSA-N	798	4443.94
10	43, 74, 42, 45, 44	116-09-6	XLSMFKSTNGKWQX-UHFFFAOYSA-N	885	1443.16
11	42, 55, 41, 70, 43, 57, 39	71-41-0	AMQJEAYHLZJPGS-UHFFFAOYSA-N	859	26.66
12	44, 56, 41, 43, 57, 39, 45, 72, 82	66-25-1	JARKCYVAAOWBJS-UHFFFAOYSA-N	942	23.98
13	45, 43, 57, 47, 44, 46	513-85-9	OWBTYPJTUOEWEK-UHFFFAOYSA-N	790	130.66
14	94, 40, 53, 67, 39, 52, 79, 38	3438-46-8	LVILGAOSPDLNRM-UHFFFAOYSA-N	851	78.11
15	96, 95, 39, 38, 37, 67, 40, 97, 42	98-01-1	HYBBIBNJHNGZAN-UHFFFAOYSA-N	864	29.98
16	96, 44, 95, 42, 39, 41, 45, 97, 81, 54	1025087-55-1	OHGJHBXVKFERPR-UHFFFAOYSA-N	750	8.35
17	44, 43, 42, 71, 41, 55, 70, 45, 86, 39, 100	123528-99-4	QPKSAEVZZQMSER-UHFFFAOYSA-N	794	15.58
18	56, 43, 41, 55, 39, 69, 84	111-27-3	ZSIAUFGUXNUGDI-UHFFFAOYSA-N	845	178.74
19	43, 58, 71, 41, 39, 59, 42, 99, 114	110-43-0	CATSNJVOTSVZJV-UHFFFAOYSA-N	803	46.05
20	70, 41, 44, 43, 55, 57, 42, 39, 81, 96	111-71-7	FXHGMKSSBGDXIY-UHFFFAOYSA-N	825	22.22
21	108, 42, 40, 39, 38, 41, 67, 109, 37	108-50-9	HJFZAYHYIWGLNL-UHFFFAOYSA-N	835	41.97
22	74, 87, 43, 59, 99, 55, 41, 101, 42, 71	106-70-7	NUKZAGXMHTUAFE-UHFFFAOYSA-N	882	305.64
23	77, 106, 105, 51, 50, 78, 52, 74, 107, 39	100-52-7	HUMNYLRZRPPJDN-UHFFFAOYSA-N	903	246.45
24	60, 44, 73, 41, 43, 45, 55, 42	616-62-6	VQDJODAWOFNASI-UHFFFAOYSA-N	842	9.76
25	43, 44, 41, 56, 84, 57, 55, 42, 69, 100	124-13-0	NUJGJRNETVAIRJ-UHFFFAOYSA-N	897	19.02
26	74, 43, 87, 69, 41, 9, 101, 55, 95	106-73-0	XNCNNDVCAUWAIT-UHFFFAOYSA-N	724	25.40
27	74, 87, 43, 113, 55, 101, 59, 41, 39, 75	106-73-0	XNCNNDVCAUWAIT-UHFFFAOYSA-N	759	5.30
28	71, 43, 41, 57, 55, 39, 72, 56	79-50-5	SERHXTVXHNVDKA-UHFFFAOYSA-N	725	18.06

Supplementary Table 1

No.	MS fragment pattern (m/z)	CAS no	InChIKey	R match	S/N average
29	91, 92, 120, 65, 39, 63, 51, 89, 121, 50	122-78-1	DTUQWGWMVIHBKE-UHFFFAOYSA-N	895	101.01
30	109, 124, 81, 53, 52, 51, 39, 50, 63, 110	90-05-1	LHGVFZTZFXWLCP-UHFFFAOYSA-N	821	73.12
31	57, 41, 43, 56, 44, 55, 70, 98, 69	124-19-6	GYHFUZHODSMOHU-UHFFFAOYSA-N	859	78.91
32	74, 87, 43, 41, 55, 57, 127, 59, 115	111-11-5	JGHZJRVDZXSNKQ-UHFFFAOYSA-N	806	25.49
33	106, 78, 137, 51, 136, 50, 107, 105, 77, 52	93-60-7	YNBADRVTZLEFNH-UHFFFAOYSA-N	926	51.04
34	106, 78, 137, 44, 51, 50, 136, 79, 42, 52	535-83-1	WWNNZCOKKKDOPX-UHFFFAOYSA-N	880	7.95
35	91, 150, 65, 92, 89, 59, 63, 39, 90, 151	101-41-7	CRZQGDNQQAALAY-UHFFFAOYSA-N	857	38.43
36	128, 129, 127, 51, 64, 102, 126, 63, 77, 75	91-20-3	UFWIBTONFRDIAS-UHFFFAOYSA-N	788	30.94
37	43, 41, 57, 55, 44, 70, 56, 68, 71	112-31-2	KSMVZQYAVGTKIV-UHFFFAOYSA-N	753	6.94
38	120, 91, 119, 92, 39, 89, 63, 65, 121, 51	496-16-2	HBEDSQVIWPRPAY-UHFFFAOYSA-N	759	32.17
39	74, 87, 55, 43, 41, 59, 141, 129, 143, 57	1731-84-6	IJXHLVMUNBOGRR-UHFFFAOYSA-N	818	49.58
40	53, 139, 67, 68, 124, 96, 110, 94, 95, 111	20189-42-8	CUBICSJJYOPOIA-UHFFFAOYSA-N	787	19.70
41	135,150, 107, 77, 43, 136, 51, 151, 79, 39	1450-72-2	YNPDFBFVMJNGKZ-UHFFFAOYSA-N	850	67.11
42	74, 87, 57, 41, 43, 55, 69, 143, 59, 75, 214	5129-56-6	XPVCTJYIICVJOE-UHFFFAOYSA-N	741	15.97
43	74, 87, 43, 55, 41, 199, 57, 143, 59, 75	5129-58-8	FLESKWMKPOBWDE-UHFFFAOYSA-N	795	54.30
44	43, 58, 71, 57, 59, 41, 55, 69, 85, 95	502-69-2	WHWDWIHXSPCOKZ-UHFFFAOYSA-N	694	8.60
45	74, 87, 43, 55, 41, 143, 75, 57, 69, 227, 270	112-39-0	FLIACVVOZYBSBS-UHFFFAOYSA-N	903	1926.32
46	67, 81, 95, 79, 55, 82, 96, 68, 109, 69, 262	13058-52-1	KVIWYYOMPLJRMC-OCBXPSTGSA-N	893	34.62
47	67, 81, 95, 55, 82, 79, 96, 68, 294, 54	21870-97-3	KMXSXYSNZMSDFK-UQGDGPGGSA-N	802	228.94
48	55, 69, 74, 83, 97, 41, 96, 87, 43, 84, 222, 264, 296	112-62-9	QYDYPVFESGNLHU-KHPPLWFESA-N	866	335.31

Supplementary Table 1

		Class	Blank	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma
		File type	Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Spectrum reference file name**	**EI spectrum**	**Blank**	**BMT_1**	**BMT_2**	**BMT_3**	**BMT_4**	**BMT_5**	**BMT_6**	**BMT_7**	**BMT_8**
1	BMT_9	29.1:34523	12595.86	2120320	4022418	3537319	1849103	2523384	4324584	2297588	4057372
2	BMT_10	29.1:23814	2988.972	126124	123359	156265	146061	139210	144109	166602	134621
3	BMT_1	29.2:143343	8854.098	2048825	601968	596656	605592	705291	804483	954113	698230
4	BMT_1	29.3:151024	2238.208	1311870	1000651	1613490	1333583	1376528	550085	1141675	1037882
5	BSHMP_1	29.1:2583 3	432.9984	28799	760925	1183199	1069862	1068594	57032	7923	4525
6	BMT_6	30.1:204 32	1750.808	2138	714	616	669	1107	3273	664	1425
7	BMT_2	29.2:9644 3	5189.124	409399	51306	99483	105472	93268	118175	30220	71499
8	BMT_1	29.2:3312 3	134.4884	6229736	4374542	19223	5268546	31759	759	5509775	4882345
9	BMT_10	29.2:72423	466.6143	32506	4443323	4486257	5700525	4907696	4725940	5605076	4867373
10	BMT_1	29.2:38139	2601.178	1793670	939772	37724	7677	1336522	26602	1032801	855975
11	BMT_1	29.2:1976 3	1440.991	134040	58837	68721	63992	53123	62550	60176	70268
12	BMT_1	29.3:523 31	558.9857	146640	65773	66545	64842	54056	62835	60044	72324
13	BMT_1	30.2:556 31	1352.826	236071	24303	34774	25442	25306	195	33045	21449
14	BMT_1	32.1:803 34	822.7706	248380	59963	105143	69325	44601	27762	62316	79453
15	BMT_1	30.9:273 34	176.933	252570	86275	78778	87060	79917	74353	66628	68439
16	BMT_1	33:120 37.1	670.6593	7574	4817	1330	4209	5347	2433	3668	5065
17	BMT_3	29.1:888 30	227.8461	71656	4752	20010	11101	19450	10799	19725	19291
18	BMT_1	29.2:11099	1359.278	334621	66302	95863	83878	67999	62982	79149	88186
19	BMT_1	30.2:345 31	1242.432	78690	22281	24610	19197	18007	16746	22032	23008
20	BMT_1	29.2:3765 3	1028.067	48915	17514	14330	14411	10537	14017	11424	11022
21	BMT_1	31.1:415 32	60.25156	187436	29131	26547	24776	31058	24895	19204	17383
22	BMT_1	29.2:7678 3	291.5629	427952	116202	159738	90706	98734	112105	100079	108151
23	BMT_1	29.2:1011 3	693.6786	392290	141274	127607	128483	116378	120770	105836	99095
24	BMT_1	29.2:1048 3	58.9946	32872	7453	9740	7717	9177	3879	3896	5407
25	BMT_1	29.1:3563 3	155.5682	36463	13822	12393	12441	9699	14127	10069	11168
26	BMT_1	29.2:1392 3	287.2936	66509	19071	17761	13064	14500	14903	12379	11580
27	BMT_1	29.1:589 32	779.2449	11494	5762	4868	4805	4818	6039	5246	4082
28	BMT_1	37:278 38.3	331.7184	66815	7630	8469	6799	3980	7445	6124	4019

Supplementary Table 1

		Class	Blank	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma
		File type	Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Spectrum reference file name**	**EI spectrum**	**Blank**	**BMT_1**	**BMT_2**	**BMT_3**	**BMT_4**	**BMT_5**	**BMT_6**	**BMT_7**	**BMT_8**
29	BMT_1	29.1:1289 3	1876.34	209479	75601	73739	61284	65551	59734	61821	51775
30	BSHMP_10	30.2:235 37	0	10266	2558	878	2425	1210	1219	1141	1552
31	BMT_1	29.1:5648 3	1641.944	132962	41742	34290	41929	32538	43676	25024	23689
32	BMT_1	29.2:1569 3	755.8695	74567	16652	15730	10860	12316	16503	12035	11725
33	BMT_1	30.2:308 32	54.71814	249869	128751	121819	55739	66078	137948	29149	89785
34	BMT_1	29.1:634 30	54.67401	15514	3958	4480	3898	3840	3474	2619	2717
35	BMT_1	30.1:50 32.2	949.8409	112860	27504	21586	18743	20952	23890	13682	19105
36	BMT_1	29.1:467 29	576.2521	48327	15266	11344	8831	10601	11099	8976	7894
37	BMT_1	29.1:988 31	762.771	13100	3622	3239	3480	3622	2209	514	2274
38	BMT_1	29.2:631 32	171.7302	150620	41426	31573	26510	18810	16534	8410	8046
39	BMT_1	29.1:1150 3	456.8582	137286	37883	28765	25859	24559	33386	20125	18340
40	BMT_1	29.1:1 30.2:	63.99809	31753	6060	5693	4303	3733	2421	1004	1786
41	BMT_1	31.1:177 32	174.6236	139096	39429	41056	43061	37647	36661	30057	28253
42	BMT_1	29.8:63 32.2	1101.084	30367	10035	9441	6714	5140	6249	8133	3907
43	BMT_1	29.2:688 29	841.2583	53961	18418	25177	14094	15559	19862	15939	12544
44	BMT_1	29.9:167 30	1557.963	14277	7743	5472	6217	6086	6243	4668	5182
45	BMT_1	29.2:12074	3242.613	1583578	535312	699339	408727	381224	445323	523361	364594
46	BMT_10	29.1:2216 3	971.9097	54520	34605	47239	37549	35950	32901	32418	29701
47	BMT_1	29.2:2136 3	260.6558	111079	55912	82663	62320	60934	64851	57270	56391
48	BMT_1	29.2:7969 3	986.3108	349650	120343	174196	126748	114443	136068	138762	115324

Supplementary Table 1

	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**BMT_9**	**BMT_10**	**KDML_1**	**KDML_2**	**KDML_3**	**KDML_4**	**KDML_5**	**KDML_6**	**KDML_7**	**KDML_8**	**KDML_9**
1	3655534	3100914	2513910	3472612	2694896	1805566	1857192	2880761	1984607	3293160	2853619
2	137060	143981	134631	348103	157313	160314	143502	155235	137771	150886	183654
3	2145309	865567	465696	1573021	566728	634562	621750	672676	684703	621008	706142
4	1109714	1222860	1141559	3002897	1006521	1030878	914786	1019862	979239	550141	1121418
5	3016	8155	241587	38607	136022	105125	126626	212921	180662	82635	169721
6	363	482	1005	778	7010	461	1879	1462	282	1178	114
7	451293	69	220683	40941	83549	107244	109994	97931	88539	100300	97776
8	5112911	5776453	514	4279515	1569	985	3093	1532	552	694	103721
9	21226	5820418	7008579	4538647	4212473	4689573	4164341	4973198	5039464	4436832	5324898
10	1058336	1034342	31888	967023	70875	38159	76139	75795	19479	37795	54870
11	56459	69606	109081	57105	49053	50897	47198	47848	869	61456	52043
12	48282	64775	142820	87176	47460	61490	51855	42925	40481	56915	42899
13	39206	21153	2487	20591	1084	1725	966	491	1356	1149	86408
14	75296	34635	186697	56988	46297	34143	61793	52056	35678	20730	67987
15	64893	72846	241095	100511	81135	87236	81870	69815	72956	78749	79251
16	4800	3481	6307	5883	3656	2824	4040	4375	3082	2784	3444
17	2514	2006	12904	23783	9824	6448	7884	3034	2247	9202	2560
18	65496	79700	114589	38597	40678	35069	26980	28376	24252	36399	39572
19	18767	21585	65885	28282	17940	20721	14112	15452	21848	17710	13917
20	10104	10139	35946	13776	9761	11189	7986	6972	6945	9175	7366
21	23470	21864	177506	48347	30327	18239	23488	32375	18152	14006	26649
22	104758	96313	451333	222566	136615	187208	119175	110991	87651	156616	125323
23	89297	93627	496938	202281	138591	150587	142116	121187	116881	119251	115536
24	5386	4663	7030	10829	7972	5352	9058	4217	6285	9578	5946
25	8605	9589	29276	11869	10449	10770	7467	6929	10499	9637	5170
26	11958	11595	78841	24084	20642	25236	17002	18728	16406	19666	17073
27	643	5455	14530	8329	6997	3833	5347	6761	967	7289	5305
28	5373	6550	68637	12254	11583	11626	10550	7584	7675	6002	9536

Supplementary Table 1

	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma	white aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**BMT_9**	**BMT_10**	**KDML_1**	**KDML_2**	**KDML_3**	**KDML_4**	**KDML_5**	**KDML_6**	**KDML_7**	**KDML_8**	**KDML_9**
29	46686	49624	237441	100265	63599	65568	60588	59877	53384	48131	55826
30	769	417	8282	2545	2130	852	656	1805	1017	479	1393
31	20648	24494	92889	34242	27673	30907	17286	17118	21178	21690	18414
32	14939	10439	111094	29726	24452	30996	20709	18609	15894	28711	18461
33	4531	73468	293908	127850	84769	60816	69998	79368	66863	90365	67796
34	2861	861	16858	2642	3239	3187	1950	2635	2832	1083	2762
35	15393	15489	200395	41131	36294	43706	22294	31647	31233	33725	29163
36	7404	8014	72735	23261	12683	11847	8862	11083	10842	11000	7904
37	1312	1744	11031	3176	1595	3287	2564	2505	1375	2049	1516
38	8172	7190	100062	26908	23913	17692	14723	3909	9807	8119	7745
39	22720	20629	194013	41261	33479	43873	28589	30828	29752	40649	31578
40	486	805	31406	4634	4199	3228	2242	2898	2055	3066	397
41	29119	24953	211365	65404	65838	88156	56943	52960	63785	47426	44965
42	9318	5305	35830	11412	7309	4245	7151	6433	5273	5529	14249
43	14535	13639	77340	38805	29493	38858	40103	30244	25308	30368	39427
44	4053	4426	8978	1562	4721	4301	3451	4656	4166	3057	5141
45	477118	281158	5462945	5616387	2801235	6030291	4020985	3031776	3012215	3527249	3702743
46	38938	31585	271366	128123	127536	274073	214461	170525	177213	167461	170227
47	71011	46816	523997	246073	266665	535198	431427	327637	330921	325744	308625
48	148378	98137	843415	795197	684598	1613836	1220933	816592	887321	948036	934382

Supplementary Table 1

	white aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	KDML_10	KH_1	KH_2	KH_3	KH_4	KH_5	KH_6	KH_7	KH_8	KH_9	KH_10
1	2461057	3370495	5626685	4429574	4133717	3898642	5874127	4071638	4668308	4546352	3810160
2	130620	247457	345399	291882	295756	321952	335654	313095	316567	300875	338855
3	630872	607867	1019732	776741	876388	1008924	1034084	999241	1014304	858744	1285655
4	787761	1000516	960564	743919	1385604	852478	791039	744958	761959	725440	1013924
5	49124	253571	2244	119595	289228	712	575685	308857	934	214274	246541
6	162	12924	1245	4358	1690	870	686	3597	1030	2546	2675
7	31962	288464	297017	235085	241543	202376	223258	191759	199451	149185	176338
8	5281265	4528	890	1802	7201	8670	9766	511	2262	707	6766
9	5360000	7709257	107	5169	5321344	373	233	209227	1479	6337732	6738559
10	1038017	48075	35370	42976	12164	36407	64851	42462	34110	74103	22224
11	42499	407527	434448	359028	371480	294722	322631	264580	265632	216330	207316
12	37669	270552	298094	226816	250328	192118	210912	174610	180220	132379	127331
13	11060	4716	15107	15651	1357	2658	3806	2952	3012	3161	1891
14	37998	203569	134764	92040	101283	103301	54342	71695	90588	31432	87771
15	57931	199270	103627	90989	82938	97980	90832	77428	84164	68379	77200
16	2120	8601	6221	5340	2414	1812	5380	4211	4416	4349	2579
17	1127	17517	20803	26073	1796	1033	11577	13891	8619	1996	1570
18	24241	724607	540297	389065	417722	294089	427611	242082	235718	204785	180738
19	12716	151397	127884	77683	84115	75402	86386	56534	69193	41038	46096
20	7131	58187	26242	21369	19068	20970	23955	20035	20527	10978	18027
21	15951	121170	30597	27751	15796	23157	20256	24684	25431	11712	19326
22	76801	871380	573515	543268	484975	369009	406145	316693	386808	250805	200930
23	91305	267260	130643	114333	90528	103944	92595	88521	88870	59009	69783
24	2934	21957	29343	45163	18302	3985	17978	20372	16928	1951	4947
25	8146	45407	20403	18319	14048	17678	15193	18055	19078	11784	16769
26	12093	59681	27970	24896	17470	18116	18713	17263	18240	11681	10775
27	1671	10677	7225	8698	5692	7304	8097	7049	1161	5587	3459
28	3973	32974	10727	11073	6095	4612	5479	8144	4593	4312	4951

Supplementary Table 1

	white aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**KDML_10**	**KH_1**	**KH_2**	**KH_3**	**KH_4**	**KH_5**	**KH_6**	**KH_7**	**KH_8**	**KH_9**	**KH_10**
29	47340	158983	65029	57405	62195	65092	56266	50515	53843	46445	58087
30	740	175889	89807	61280	55102	66181	46847	40455	54903	40136	43153
31	20015	236340	102027	82521	68579	117463	104225	118896	119055	71270	99091
32	10602	58340	23000	23100	18609	17638	18346	13950	16952	10873	11375
33	56665	97152	26397	66749	55204	48764	63448	47642	53385	55148	32099
34	2587	8239	3555	2623	1924	2089	2659	2110	2168	1004	2053
35	26007	105966	42564	44473	26193	29828	24987	25408	30256	25418	21880
36	8737	62193	24114	28637	9073	14149	12619	12103	18184	7934	8107
37	1582	12407	4417	3827	2677	3860	3519	3012	4562	2511	1772
38	4658	25996	12580	5257	4387	5223	4770	5249	3008	1818	926
39	18376	117712	45106	37776	34977	35223	32896	27370	33306	18362	21285
40	1134	2316	658	343	99	67	427	625	92	487	81
41	46850	81154	42274	38906	30246	35170	35750	35259	25322	22315	26978
42	4067	21793	10353	8698	7235	7363	7101	4628	6060	5929	6965
43	23039	32415	21101	16124	16301	9799	15331	12009	11807	10629	16271
44	3962	7476	4264	3699	2486	4183	4485	2894	1894	1630	1720
45	2493764	396457	351561	282830	237340	222373	189925	177942	201511	161905	197147
46	149085	175	24743	24140	24633	24873	18467	24159	27159	21563	29360
47	277767	29848	44562	47768	45923	42535	32517	44167	43867	37862	47393
48	582393	42814	70602	82865	73922	64634	45681	67816	64748	50369	88653

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**LP_1**	**LP_2**	**LP_3**	**LP_4**	**LP_5**	**LP_6**	**LP_7**	**LP_8**	**LP_9**	**LP_10**	**UP460_1**
1	4606097	6409573	5668075	3590930	7922578	6407584	4684016	6651369	5083198	4661315	4846026
2	405805	436887	436825	395910	321102	455929	426967	460516	457778	435847	306246
3	1158458	1359719	1376277	1297131	1193030	1236137	1513473	1468483	1442548	1502751	2770366
4	968198	1256078	1295998	1001011	874855	1152833	1136898	1228599	1335978	1199704	2296061
5	3985	6607	1405	2266	2217	2251	349	3529	1825	2680	1907466
6	13694	3029	5442	1019	1724	2401	410	4702	1544	483	1254
7	464364	235775	212057	286175	287052	241767	333390	317349	278727	4669	377029
8	4455	3406	2889	5139	3456	3226	1160	3455	3935	3212	4991243
9	679	429	6349474	1021	2542	62	139	3543	472	657	30028
10	107605	17038	17389	4323	101489	11575	16495	6614	27667	16005	1312624
11	546	235892	220980	318313	301659	251870	325705	348320	257191	268032	208915
12	461726	261471	204851	300042	266638	234418	301678	271318	1147	200926	210184
13	25518	33776	1482	18420	9500	27425	3015	21107	36658	10139	37953
14	163878	120120	100871	51861	53672	60938	94326	109014	83823	77793	131023
15	356433	208776	178801	178870	120825	129724	193723	156151	145677	144271	118376
16	8673	8196	7471	7522	6828	5395	7746	7570	6736	3619	6083
17	1720	1905	1502	2755	3162	1782	2875	2566	2514	2622	1879
18	685326	267446	191987	617241	312549	184412	305346	339390	293811	342898	253862
19	258145	95297	85935	123531	131865	105038	124525	134193	96797	96639	101098
20	49468	18097	13869	14459	12911	10714	15439	14462	11176	9746	22939
21	109118	27069	17654	22597	14646	13542	20122	17377	15957	12634	42573
22	1018195	415946	263933	352087	578674	321592	343659	506311	205197	232064	544342
23	368817	149156	140560	144006	113881	118691	138832	138474	109236	105548	174040
24	4200	1164	6733	1032	6594	3448	3471	3774	4834	3415	1287
25	38948	18909	8529	11061	11442	7792	14817	13283	11002	8735	15825
26	62460	21154	16138	14949	22385	20257	14855	20043	10817	11573	28942
27	11549	6323	4395	7426	8233	5541	5718	7575	875	1370	7255
28	30502	15714	14253	16927	6279	6129	12963	7204	9639	12433	10048

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**LP_1**	**LP_2**	**LP_3**	**LP_4**	**LP_5**	**LP_6**	**LP_7**	**LP_8**	**LP_9**	**LP_10**	**UP460_1**
29	253429	109700	107127	95002	88379	94885	104894	110535	106968	83137	143041
30	99074	52924	47924	55325	49462	46266	41957	55252	39915	38807	81824
31	161224	60937	29677	28743	26301	19822	33684	27378	25791	28585	56713
32	80732	25741	15053	14956	21931	18412	14714	18434	10869	11155	25264
33	61270	59534	36754	26108	66766	43947	30820	39602	23609	9076	65959
34	5118	2299	2367	1920	712	1180	359	2329	1158	71	3889
35	74382	22523	19024	13597	26177	10979	14815	18336	14714	13635	58740
36	47027	17165	13731	14071	13419	13312	14017	13393	10254	11620	20831
37	7683	3687	2405	1568	1047	1915	2324	2207	1514	2026	1881
38	133887	50255	35636	36041	31148	21870	21169	17449	15583	12543	39798
39	150545	42407	26054	21093	25756	25567	18827	22854	19656	23278	60875
40	1552	352	961	429	57	84	731	167	0	99	299
41	100042	69383	64940	56797	48986	49393	58844	56578	55136	36074	40652
42	25692	12157	16543	6543	8957	7699	6003	8369	7401	9398	13732
43	74213	55102	57345	31697	36780	54430	29321	33924	23854	30364	31396
44	7229	4046	4725	2173	3374	3927	3638	1079	2873	1685	4318
45	1002000	806910	748613	373408	490078	875596	377993	382081	266756	580784	534256
46	91	67063	74348	47293	45072	53536	40123	38073	37164	43438	43772
47	87198	126310	132922	87128	84762	108708	76086	64142	67352	78354	79768
48	172303	214680	182511	127986	114983	113100	104071	106217	97042	149704	128191

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP460_2**	**UP460_3**	**UP460_4**	**UP460_5**	**UP460_6**	**UP460_7**	**UP460_8**	**UP460_9**	**UP460_10**	**UP463_1**	**UP463_2**
1	4834511	5631916	4884649	4927657	4921408	4458867	4363166	4911850	3755567	4762536	4025801
2	282541	343298	366726	352223	352305	316240	434985	311923	359467	305671	362879
3	834522	1186600	1235766	1120746	1171852	1127950	1379716	1054814	1232497	1078599	1065757
4	762521	2354486	954903	2501528	879104	864200	1094360	794213	933634	804335	932331
5	522285	88153	711	2095897	2607	2066	2028	1069	260290	4545	3812
6	586	866	670	2058	1560	688	2165	1604	2583	4554	8198
7	3738	46780	189416	181588	143083	165074	150472	119492	172917	248785	172490
8	1395	3955117	741	1585	1801	999	6464	4321	8145	2640	3841
9	2454	12344	1511	7636654	1591	67	1618	158250	173263	20004	1095
10	38274	1227609	26597	19285	22254	15975	31498	27368	32599	16024	9019
11	183535	204632	209810	192794	148557	205122	162864	151004	173307	239240	204245
12	106916	145134	151383	153506	97123	147487	92566	90029	143625	255050	164515
13	21234	13536	18631	65987	35448	10795	4967239	7008	295357	2874	23944
14	95606	100733	112692	129940	73843	90023	211783	77463	89844	119258	135439
15	67731	72596	84631	89395	74989	72940	128143	56575	80110	118517	113708
16	5018	5255	5695	5538	3339	4364	4518	3623	924	8512	5225
17	507	2112	2124	2439	2348	883	1522	1555	3652	1569	4349
18	216858	231713	207554	224711	137288	216890	148898	171870	164675	182901	148089
19	47899	67161	75477	68267	45942	57058	45202	41130	49642	106772	69941
20	11719	11797	12015	13864	11166	12987	12676	9321	9761	19610	10988
21	29768	22587	24569	26307	25255	22077	26703	20889	20597	67318	35997
22	235990	345156	308133	344636	205852	205393	207955	254250	185116	536740	263931
23	95361	93048	116159	112918	98926	89560	103596	72297	81103	162765	114853
24	1834	14754	13179	3987	865	10036	2269	2235	437	2772	7483
25	9717	5485	9818	13492	9796	15622	11297	4209	8484	15713	10477
26	14563	14841	15021	15133	15279	11712	12981	12656	10092	34435	18479
27	4565	6711	1676	6159	4874	6286	1541	719	4195	10151	6103
28	13339	8791	10941	7762	6594	9804	4819	8716	7136	17113	14295

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP460_2**	**UP460_3**	**UP460_4**	**UP460_5**	**UP460_6**	**UP460_7**	**UP460_8**	**UP460_9**	**UP460_10**	**UP463_1**	**UP463_2**
29	78833	77432	99287	92611	81018	69497	91737	60162	59415	137125	88547
30	78366	70698	55736	67218	67156	61213	78522	57249	41576	76971	66629
31	39728	35418	33829	41846	41301	48452	45340	26863	34756	48192	38161
32	13508	17317	17133	14768	14079	12475	13538	14323	8937	28669	14792
33	45522	63319	58477	64238	39797	48944	43257	41001	30491	28453	33759
34	2705	1271	1996	2557	1051	1115	1188	1141	1562	2661	2352
35	27651	23049	24994	23994	28835	16770	27631	21586	14655	56832	23976
36	9770	9754	11072	11984	9775	5096	9295	8014	7677	20187	14789
37	1846	2533	1994	2597	1938	2957	2119	985	1665	3252	2956
38	22177	14267	15052	19289	11598	5978	2996	5464	5261	21365	11645
39	29759	31732	34202	30301	39126	21157	32520	31886	19835	65589	31833
40	124	0	570	73	153	122	59	27	293	1523	942
41	28284	27676	37742	33305	25436	33465	22200	19770	24094	41243	37821
42	8221	7976	7616	6846	8942	5981	7839	8581	8312	15381	12454
43	20238	21574	28249	19323	22087	17832	24894	17075	21224	53191	47243
44	3642	2637	3859	347	1853	2296	3080	872	1969	6796	3542
45	273187	346369	359580	339645	242077	253439	425584	299831	265609	539972	545773
46	27263	31622	49024	30663	30504	28674	36001	34217	32416	46921	63721
47	51140	55890	98503	55223	56960	57128	69900	61102	56699	88732	113614
48	60343	65092	128766	72980	64810	67199	125522	82243	67580	94523	131693

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP463_3**	**UP463_4**	**UP463_5**	**UP463_6**	**UP463_7**	**UP463_8**	**UP463_9**	**UP463_10**	**UP468_1**	**UP468_2**	**UP468_3**
1	6311004	3989409	4562433	5098362	4956040	3449730	3757267	3500750	3184864	3561420	2212641
2	256461	322464	359537	464671	285545	444356	340998	295044	219925	323706	295387
3	728479	1102770	980471	1575981	1019548	1539510	1049320	1043101	859491	978278	1117333
4	753942	949357	1001716	1251097	924120	1209045	869263	994642	1089525	930161	691766
5	2162	3360	4243	1658	161299	2534	711	3580	338696	216519	409815
6	751	3065	2945	2442	970	1891	2411	514	5028	6053	950
7	93933	138308	137827	255743	162805	178892	115948	82078	138065	130760	139314
8	823	5276	380	3428	1447	2664	1763	1517	4576	1280	6973
9	4367	465	0	14948	7298171	368	485	11531	5999848	4975798	109146
10	23694	21258	20479	44131	26727	6633	11490	14630	54549	44337	23666
11	135747	155910	153573	261835	175915	165668	118055	102526	72988	71309	92506
12	84452	122506	115772	249450	144384	153905	92034	59873	87346	75780	109243
13	2384	27693	1294	972	74487	1098	291318	6241	5891	354055	847
14	79244	117399	100497	96101	78022	82465	117746	92724	98908	145207	110787
15	61570	82445	106192	131692	61996	107992	88998	66524	117729	93607	142319
16	7037	5953	5752	6585	3111	4500	5995	3403	6993	6573	4525
17	4096	3660	2737	2513	1139	3385	5691	3120	8325	45900	3600
18	99876	134964	136783	156758	114830	105991	96229	69645	60664	73095	90680
19	55749	57883	60157	109471	59637	76331	42369	39304	40638	25876	25131
20	6858	9018	9541	12896	8348	8800	7305	5781	19531	15774	17449
21	24622	23128	30291	36389	26077	24285	40937	18303	44144	38836	60555
22	172695	248235	259785	265576	213929	221975	191996	96687	243420	207173	141269
23	70601	90944	103978	128952	81449	111314	73702	56396	180132	146137	162687
24	3851	3666	1600	5282	6878	1024	2578	3059	9494	10333	3236
25	6286	8506	9579	12670	9406	9392	6195	3701	22049	15655	23038
26	9118	15454	16516	16555	12227	12978	11700	5659	30927	22394	14905
27	3258	5938	5846	5153	4201	4112	5251	1219	9406	9104	3153
28	12549	9416	10786	10010	7128	10091	6720	5092	8369	7520	5171

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP463_3**	**UP463_4**	**UP463_5**	**UP463_6**	**UP463_7**	**UP463_8**	**UP463_9**	**UP463_10**	**UP468_1**	**UP468_2**	**UP468_3**
29	67166	74999	93632	63369	78365	92758	69710	51296	99183	87322	82206
30	69443	58842	67627	57565	53793	41144	46347	54840	9367	10139	7975
31	17089	20864	27687	33771	22672	23006	12929	14665	70556	44358	58269
32	10532	14866	17732	15643	15073	12831	12629	7298	29098	20457	16178
33	72404	27716	53814	41008	23936	28395	25653	19492	80914	86858	36423
34	993	1677	2250	1856	1294	1754	1072	552	4267	3233	3131
35	10508	24827	24393	15254	8867	14162	16411	9745	54894	38079	28784
36	10793	8522	10412	9762	11370	9522	7413	5799	23074	14465	13425
37	1356	2044	2865	1912	1870	1053	1786	121	3562	3189	3044
38	9129	3883	5912	4596	5876	1677	1090	1247	29137	18430	19663
39	15202	29370	23857	25816	20687	22729	21772	13005	63219	34832	28908
40	171	222	145	1000	509	204	282	0	2900	3267	1740
41	19693	31831	16167	34693	20793	27011	16952	15386	59402	57211	53574
42	8802	9897	9406	7672	5827	7911	11335	8331	11932	12480	7585
43	24790	33805	52808	32139	27612	44826	33641	16080	36459	25785	16480
44	996	2149	2063	5061	3384	537	736	492	5012	9179	6420
45	337808	362984	966790	365689	345994	450453	409782	222178	785890	858658	536651
46	28484	34778	76940	49870	38209	54003	48070	25160	46900	63442	44324
47	51096	66529	137609	83772	67175	107686	83434	44979	79539	109955	81674
48	32515	65075	172616	90527	75342	126273	94941	37181	156133	166834	132734

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP468_4**	**UP468_5**	**UP468_6**	**UP468_7**	**UP468_8**	**UP468_9**	**UP468_10**	**UP469_1**	**UP469_2**	**UP469_3**	**UP469_4**
1	2568890	3896074	2486746	3254544	3267747	3351144	1886289	5484054	4688937	4478161	3916517
2	378760	275530	300308	333093	254660	279570	275647	326601	367697	363181	390856
3	1419549	1281827	1086459	1198799	1037251	1088367	1003596	909788	1081055	1211467	1215244
4	992097	805806	1028063	1236990	1851629	737985	1996395	951989	1023022	977207	1180959
5	682192	702092	323945	207469	238102	230149	80025	671445	460	1699	850349
6	1836	6093	3168	3751	2094	2224	90	3807	1709	670	3831
7	160536	186505	165822	117329	143553	108926	114943	142068	182537	165226	139808
8	2265	1233	639	4958	2598	4290	4868	3538	2636	3315	3851
9	809	15770	4884790	5655332	5393331	6707440	5340407	376034	3047	395	1282
10	59315	68118	30877	40881	23460	18096	1192285	51327	27992	22579	6045
11	74580	102858	84105	61335	81913	58960	63638	167024	198367	207830	164337
12	101848	135382	114916	514	105344	56449	76028	115603	176839	148807	120239
13	1920	3502	66079	133881	80023	1372	37766	50402	23376	35504	18911
14	164721	85348	94671	100450	87836	50911	41861	63127	75971	116614	71431
15	123883	102366	72988	83434	71447	87774	64640	84671	82660	86956	83535
16	3828	5690	3207	3979	3873	2775	1368	7314	2432	4526	5651
17	38300	5168	29457	30055	29698	3454	25299	2631	1988	2281	2146
18	58967	54689	73374	45530	73391	30141	36882	272351	250025	153277	242739
19	30134	33473	33177	18886	37319	16510	22895	72911	95799	86665	65212
20	13016	14812	12005	10415	11645	8215	7420	13367	12957	13116	9802
21	25890	28937	14449	17552	20565	14894	13432	24897	33262	31676	26747
22	159716	276697	109811	129595	126459	108585	72192	438682	277431	175681	262080
23	177962	152881	106470	118324	93631	105228	75197	119440	123899	120083	99995
24	15699	26446	13882	9790	16053	3515	11079	5985	5406	3161	1868
25	10789	14309	8734	11803	7639	13692	5695	9543	8663	9393	6865
26	19294	25507	8678	17202	9556	12801	8690	20991	12769	13646	15062
27	6471	6949	3483	5602	1274	4518	3994	6027	4885	3725	5948
28	5014	6862	5741	6302	5588	4451	4126	17319	14612	10509	8205

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP468_4**	**UP468_5**	**UP468_6**	**UP468_7**	**UP468_8**	**UP468_9**	**UP468_10**	**UP469_1**	**UP469_2**	**UP469_3**	**UP469_4**
29	80942	92343	70755	74741	62170	56953	52919	101455	110686	112681	82712
30	12693	10331	13533	11181	13724	5863	4885	84771	72441	93864	77566
31	34701	40581	25959	34533	16867	24652	14479	26503	22010	36243	20986
32	21754	25299	9295	16915	9833	15508	7377	26934	11299	12076	19355
33	48216	70161	70376	86680	97609	50293	43864	45483	58428	45043	26826
34	2187	2862	2300	2387	2364	712	1381	2454	2614	1310	1929
35	23835	28394	15120	16703	16295	23607	9515	32451	30548	31474	17647
36	11144	13697	8677	11975	9876	8841	4090	16543	13778	13150	11124
37	3059	1802	1587	1907	2075	1780	100	2435	1584	3002	2048
38	11329	27426	15604	10181	16229	5858	7996	26985	20717	11735	13597
39	28355	50553	16670	27331	16511	22330	11734	41034	23711	25415	31064
40	401	648	317	1705	856	911	836	278	0	269	79
41	49846	53168	36554	37555	32771	34967	34280	26241	33517	34481	27619
42	10061	12019	5064	8561	7967	8871	9461	12439	9409	9424	10244
43	23699	51271	18380	25514	25435	18253	21686	30403	30412	26087	30587
44	3449	5562	6787	5597	3979	3774	460	2636	4259	2176	2368
45	1130529	1239132	384308	1623051	1403616	822889	721349	443823	321159	294170	501191
46	87304	92585	32208	87438	80520	59977	54321	0	40877	41355	60958
47	171304	166553	63410	162682	138042	106668	97112	59428	72311	73151	117756
48	262122	423424	80097	327895	234237	263847	150079	53928	79049	79537	134808

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP469_5**	**UP469_6**	**UP469_7**	**UP469_8**	**UP469_9**	**UP469_10**	**UP470_1**	**UP470_2**	**UP470_3**	**UP470_4**	**UP470_5**
1	5010717	3965223	4996701	3140806	4897084	5087135	5080181	4397445	3638096	3825965	4201834
2	341162	490749	482791	432919	575366	506597	324653	403921	343020	390441	361079
3	1252744	1766623	1829274	1426374	1915427	1821928	835978	1441837	1041431	1113535	1094379
4	1668457	1566216	3011374	1278017	1643781	1481443	712665	2935050	2214264	1804008	2554864
5	241149	3913	181856	2278	941	1294	62312	2245696	103339	81709	78425
6	885	1734	1696	808	492	3860	5177	218	550	5388	798
7	117754	143515	108565	180720	157895	159175	115501	5102	84789	108844	98054
8	997	4417	329	3376	6333	1359	1514	5109552	3323	1126	1534
9	6598172	351	6627510	490	102	8233016	5080207	5372074	5730214	5557750	5497598
10	14489	19827	76756	44745	36649	37243	44656	888825	53431	28432	43651
11	114985	158035	116719	171258	151721	140406	78179	67901	58736	72355	50136
12	93785	110822	88280	136834	108532	141311	66821	58390	54261	68030	47294
13	1882	18553	25662	20237	4462	5552	2204	98799	69000	2147	1189
14	87853	85427	90397	73310	120848	51613	79946	63071	42029	37175	21174
15	68028	72566	66715	78101	113678	150041	75801	90852	75736	70627	59012
16	6250	3880	2410	3243	5601	7094	3402	2014	5180	5935	5090
17	962	1771	1313	1207	907	3129	18716	40301	13305	16646	13395
18	120199	130015	108052	153786	159551	123984	59618	48572	50632	55244	37358
19	46961	68969	44967	74168	49401	60097	24773	29478	27080	23441	18348
20	9934	10622	9462	11913	10896	9756	12296	14125	9688	11310	10242
21	18409	30469	21252	18713	30451	21928	28877	18914	19284	15758	13819
22	297374	163800	252859	125646	211367	293205	167918	232655	102799	103722	109875
23	82115	110736	83743	93883	112518	87549	131987	134903	121388	106877	105389
24	3457	2030	4674	3157	1634	4569	8646	13535	5921	12772	4385
25	8444	12458	5730	11427	8427	7333	11888	13871	9851	10556	6945
26	16281	12094	14292	7567	9358	19941	20847	23177	11572	11562	14148
27	6227	4859	427	3508	934	1241	5620	6736	3840	3647	3202
28	7465	10128	8985	8100	8242	8488	7843	6985	6739	5414	4835

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP469_5**	**UP469_6**	**UP469_7**	**UP469_8**	**UP469_9**	**UP469_10**	**UP470_1**	**UP470_2**	**UP470_3**	**UP470_4**	**UP470_5**
29	80666	145574	121053	80256	109979	72552	88248	89084	82646	68152	69389
30	60922	71349	48681	75118	59621	43220	24132	26149	18041	16536	19153
31	37858	50391	36248	57708	35723	33923	37087	72853	38951	46450	35988
32	17158	15150	18967	7657	15596	18178	15429	27466	11559	8955	13229
33	54682	37172	47492	19486	38146	39530	62612	51910	37624	41061	22401
34	1794	1503	830	1065	2077	2565	2993	3874	2473	2764	2216
35	24726	26472	13277	16628	31999	15658	52704	38586	35271	20433	28359
36	13112	11383	9977	10852	10277	13479	13088	11645	8911	8502	5533
37	754	2081	1294	2686	1806	1599	2440	3140	3035	2061	1635
38	6467	6222	8514	7361	4915	3149	7679	4942	3567	1737	1705
39	30969	28050	39547	19624	28547	67629	48674	80686	28552	31906	39723
40	442	249	0	57	96	398	3730	1666	1934	1863	478
41	22251	25378	17491	21079	22395	27440	19803	17918	16426	16225	13725
42	7432	6534	9372	5105	11126	16049	17469	23631	10962	10997	9989
43	25506	22953	26672	18040	34368	57602	34659	43293	30541	40743	26947
44	2270	3039	1053	795	963	505	7088	9144	6521	6863	8268
45	385439	373999	939859	245199	1053132	3480111	491679	1307826	477100	751922	519054
46	36233	45095	73566	37264	68615	84671	22562	60616	42176	48990	31910
47	60915	83832	136151	64958	118546	153954	44978	110099	73053	90353	58499
48	82145	99004	213213	87528	268877	708532	47405	144635	86277	120351	72415

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP470_6**	**UP470_7**	**UP470_8**	**UP470_9**	**UP470_10**	**DKG_1**	**DKG_2**	**DKG_3**	**DKG_4**	**DKG_5**	**DKG_6**
1	3311538	6123904	2979336	3468396	3400442	3190032	3463745	444	2299076	2707296	2064676
2	340305	461915	381443	1793825	421809	148757	208174	166890	143693	136731	167386
3	1010578	4114374	1116845	7457650	1313981	1606965	970265	522752	385427	425742	447647
4	2263250	3008655	1944399	14923010	2468250	1357505	1489901	1137982	1102367	941923	1157947
5	21088	3072	132163	149210	6862	1085544	1384668	11884	48198	941038	1147853
6	467	343	318	293	57	3087	1268	846	734	784	2402
7	90689	377506	76043	89888	66254	806297	24371	4839	35144	30320	45277
8	10660	6871674	594	1049	6778451	5250159	5103890	4607752	23320	128637	13065
9	6028010	1990	6742751	7956212	7061685	23449	5253169	4660480	4822768	5172613	5357280
10	35592	825365	3098	81911	1025565	1433579	1638353	1271102	19479	1268861	33654
11	61802	75493	50961	52981	67750	89561	63563	60070	45823	41719	42747
12	57529	52902	44632	42161	55917	54438	27973	23539	22475	25745	24691
13	38522	48974	31853	3912256	58150	62935	47647	19009	2712	821	1177
14	46563	84079	26810	148152	64205	109440	76218	81437	46556	27065	29104
15	61003	92235	65421	1846124	66441	79976	49125	42116	50522	46009	48986
16	2304	6476	1092	17757	2264	3087	2984	4218	1542	2164	2117
17	14701	4974	4626	4354	3631	2898	19018	20366	21045	16711	21520
18	55256	48876	33189	38072	44572	111136	71945	66189	41425	34332	47472
19	21707	30403	20699	21309	17811	41289	18397	17776	13195	15953	16666
20	9890	13868	7194	12584	9263	11957	5279	4668	5490	5791	6413
21	17513	26779	14535	25497	10512	33632	23502	24428	20729	12065	10665
22	89053	215062	57227	98443	87284	322511	116493	97395	63254	80679	57437
23	98623	121375	93951	144849	101387	91356	49737	47692	39461	42973	41978
24	5934	4106	5069	1055	4902	9031	9287	7588	4985	2915	4404
25	8181	10361	7245	10620	6744	9966	4652	4725	3446	3035	4177
26	8442	16137	7318	11179	9541	26294	8125	8474	5802	9173	6730
27	3933	6008	1045	5718	646	5790	4157	1537	2677	1770	2149
28	4410	4069	4278	6526	2693	9625	10118	6799	8977	3995	4790

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**UP470_6**	**UP470_7**	**UP470_8**	**UP470_9**	**UP470_10**	**DKG_1**	**DKG_2**	**DKG_3**	**DKG_4**	**DKG_5**	**DKG_6**
29	68051	65511	70192	314865	66333	89748	74752	46677	39037	37952	40568
30	16872	18746	15911	23664	13345	72690	42755	40695	41709	37716	40843
31	33208	47723	29816	50502	29648	36426	10369	9673	12303	12857	15097
32	11186	15011	4627	11553	29086	25796	8727	7106	5766	8634	5422
33	30209	33859	12692	33703	21674	63493	54698	76867	19938	51168	39036
34	1858	1600	2988	1864	1570	3811	972	206	1367	1974	1002
35	18065	27506	16482	25447	17833	37177	16837	13012	13354	13419	12187
36	6233	5927	6186	5509	4821	45487	12749	14150	11324	12070	10684
37	1956	2436	954	2582	2238	2690	1142	1643	1104	1094	878
38	917	585	210	754	165	31437	19808	10233	6290	4600	8027
39	29159	51301	17996	40415	29224	44342	13293	12474	11275	15198	9652
40	134	326	311	1737	882	1171	312	565	329	779	385
41	14490	11359	10794	8523	13042	33057	27414	21302	27038	26032	20084
42	8844	16477	7690	14304	12124	15064	11512	7314	5355	5332	2847
43	19477	36057	25680	31071	29801	59936	44543	20634	15121	31008	22981
44	5146	3291	5777	6120	3354	1581	1500	1026	2023	2605	2344
45	329920	952070	444309	591283	603240	1039411	744628	389242	297204	1585662	289825
46	26431	38638	30891	40800	49714	60811	75877	52012	42930	125438	48057
47	47642	70851	62889	67667	80670	91450	140827	89070	68824	243003	86758
48	54257	100809	74749	83766	112638	208531	219552	137547	96699	546954	115802

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**DKG_7**	**DKG_8**	**DKG_9**	**DKG_10**	**MTK_1**	**MTK_2**	**MTK_3**	**MTK_4**	**MTK_5**	**MTK_6**	**MTK_7**
1	3510485	3745092	3807822	3227989	3117055	2889148	3966504	1934555	2641061	1830895	2936037
2	146788	168982	179807	189557	148360	136161	163078	148792	195182	178669	180612
3	430755	608015	516775	565439	1010108	702262	550061	968309	664214	888273	2198140
4	948455	1327143	1172247	1042915	1157756	844462	1020159	989172	1306423	995398	1081493
5	915394	37614	1240731	3143	6655	2619	6177	3632	976056	760167	2565
6	255	1079	238	646	256	288	828	706	521	561	76
7	22810	39259	30731	5766	24921	31285	17525	1658	28000	19100	565893
8	5131695	60069	6260201	5830467	5165899	4562375	4258952	5071173	5213217	5403286	5438276
9	5422510	5898208	6571541	6083644	5442869	4842327	4509490	4443577	5301580	5085606	19284
10	1233408	1350355	1292004	1195274	1292268	1128572	1003692	1039918	1067703	1013803	967027
11	42381	47400	41744	41762	40542	26765	29942	28348	32342	25968	23824
12	21664	25451	24799	22691	38875	17585	21722	24563	20160	19567	18256
13	1465	28654	2388847	27038	10247	19630	12868	27355	1966	5378	10930
14	53630	29732	108684	62039	63237	32250	24163	35222	53509	21497	30112
15	40952	47707	56366	44548	96948	51967	44161	52382	53931	45794	41900
16	1918	3125	2600	1085	7089	2850	3593	2528	2091	2531	2243
17	2215	21590	3183	3631	2293	4432	1933	2285	873	807	5533
18	34940	39743	41750	34843	34608	22090	17289	17589	24528	19528	20699
19	13614	14060	18359	9796	16993	12212	10115	8602	7565	10402	12956
20	3807	9196	5421	5825	11626	5115	5515	6293	6154	5366	4361
21	14154	13270	9341	9387	24147	13541	16836	13220	8626	10573	6818
22	59353	61182	94198	73748	170815	74012	62962	69757	81368	45159	67287
23	34412	40783	34792	35790	114414	55168	56175	62380	59683	46569	47091
24	1158	1848	1286	2698	2208	4420	2379	1854	3159	2669	1759
25	3426	6413	3953	6132	8839	4721	3788	1882	3710	4488	4102
26	6136	5467	6592	5958	24038	10957	9604	11671	10063	6766	8419
27	2487	1087	3389	577	3227	2038	3786	3440	3396	2789	139
28	3584	3652	2385	5065	12796	8480	9995	6929	4689	5757	4306

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**DKG_7**	**DKG_8**	**DKG_9**	**DKG_10**	**MTK_1**	**MTK_2**	**MTK_3**	**MTK_4**	**MTK_5**	**MTK_6**	**MTK_7**
29	34332	47261	37424	36453	77680	38777	40099	41027	49452	44218	35231
30	32899	38197	34660	36471	82261	62772	54144	55888	51808	61767	46107
31	9950	21741	16990	16489	32758	13589	15412	12927	13813	17170	14766
32	7601	3819	5671	7255	22883	8868	7393	8113	9285	8337	7607
33	52731	60769	47962	37874	135043	113083	170947	39055	71943	73495	79470
34	667	1289	153	997	3386	2138	943	1192	1575	1216	1250
35	10430	12681	13321	10943	50855	26359	18370	18383	17400	15772	15949
36	9827	13768	10762	9723	24575	10025	8595	9920	6754	9178	9655
37	1277	2203	1865	1085	3964	1183	2424	1543	1811	3042	2036
38	4318	1258	3274	2010	13159	8399	5242	2688	1144	2495	2277
39	13519	10641	11161	14031	51481	20210	14072	18614	20012	14138	12919
40	475	238	230	286	814	815	200	155	508	229	197
41	22289	20956	17773	19453	32315	28833	19606	23365	13526	21412	17221
42	5059	5129	6990	10041	12985	11116	4385	3485	4509	2740	3708
43	28086	27823	22912	22380	33476	24083	14942	13592	17817	8267	10401
44	1281	2091	2034	518	2157	2049	2154	579	766	2886	1998
45	594362	528918	550316	752964	1737155	1256735	618272	598320	1190005	365502	514964
46	64506	54399	68114	74111	100783	103359	61295	64041	91920	41430	46088
47	120525	106040	130305	149952	186672	201315	114132	115771	170149	68588	93020
48	222674	188937	211933	306310	237281	231658	112042	128137	266888	75442	92420

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**MTK_8**	**MTK_9**	**MTK_10**	**Mu1309_1**	**Mu1309_2**	**Mu1309_3**	**Mu1309_4**	**Mu1309_5**	**Mu1309_6**	**Mu1309_7**	**Mu1309_8**
1	2732575	3238526	2392450	2693769	2963685	3353071	2280726	2785952	1898435	2907088	2765946
2	168712	153110	168337	209029	208153	184395	256585	249125	228567	296944	262193
3	993254	722519	762729	968543	665212	703369	891045	869770	817213	990913	943161
4	1009525	1026213	898804	2565943	2068763	2003564	1642533	2329154	2273420	2312971	2423402
5	748137	759231	4907	1859678	19991	1440318	25660	4554	10488	7697	3788
6	398	192	300	996	1051	1060	2963	332	175	762	1097
7	56612	1045	3055	19871	76452	35157	82264	73042	63862	76965	64668
8	5390595	5547020	6072469	4555954	1222	4016561	3718	154736	256995	0	130047
9	5680152	5545594	6351066	4564061	4920016	4082350	4993702	5950798	5764856	4924271	5877881
10	934856	1043326	1085452	1082468	64933	902137	23484	1193411	30318	13802	28698
11	29578	26515	29245	1120	36051	1045	35388	54752	33038	36135	36000
12	20920	14523	15714	48658	32442	26873	33599	54255	25676	37785	41265
13	5082	9702	7031	21871	53249	41330	5291	23346	1667	78109	26396
14	42027	38102	25262	76198	70822	59816	83640	69923	44992	73659	24789
15	49062	33320	39909	92632	70878	47471	62889	87478	64027	55506	65485
16	1825	1029	1444	4673	3808	4412	1809	3421	2844	4467	2355
17	3319	1692	1299	14949	17615	8486	17891	3626	10157	20884	598
18	18800	17511	16592	38494	36243	21277	31534	51673	27172	25726	26744
19	9293	8111	4504	19781	14666	7703	12300	14280	11014	15803	11628
20	5604	3385	4790	15327	10265	7796	8436	11673	9506	8664	8475
21	10487	7492	10158	17067	16110	8229	15243	11822	7876	6952	9545
22	75082	42977	40106	181002	121192	88254	85806	192545	62133	89539	93373
23	49927	34009	40656	139443	89415	80053	91910	98936	79138	76179	73449
24	1410	910	1744	6738	6942	1263	8281	7397	6142	2657	3154
25	4832	3634	1593	12142	7708	6358	6752	9066	8116	8129	4845
26	11659	5681	3971	15763	10367	11138	10044	13634	6192	11843	9479
27	4247	1995	173	4256	4726	4093	4092	4789	3435	2263	699
28	7058	5041	7344	8499	5730	7726	7523	4731	5854	3664	5212

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**MTK_8**	**MTK_9**	**MTK_10**	**Mu1309_1**	**Mu1309_2**	**Mu1309_3**	**Mu1309_4**	**Mu1309_5**	**Mu1309_6**	**Mu1309_7**	**Mu1309_8**
29	35685	30174	28351	86723	52784	54797	61549	73691	46203	52205	48419
30	53564	58251	45027	96141	97798	72851	72102	64653	69341	58011	74390
31	16380	9544	10044	48714	51432	40149	33001	46753	33489	31024	44533
32	7421	2896	3705	13428	9486	10265	8430	14279	7934	14786	6220
33	101015	84630	63609	61013	62062	94374	47844	71866	34182	54373	50305
34	1695	502	1279	3483	2889	1534	1111	3092	1219	2500	1511
35	17476	12072	20359	33773	30568	17414	19412	23126	14360	14416	22050
36	8100	5670	5648	24099	14428	11083	9912	12570	9517	11136	11634
37	2258	1401	1902	3958	2368	2353	2648	2897	2764	2335	1180
38	1107	241	795	7617	4643	2638	4158	3574	757	216	339
39	17147	6994	7704	23661	26511	23045	19892	23462	17013	29181	18166
40	0	351	122	858	925	473	0	295	99	134	0
41	17930	12735	15539	25234	29302	21807	21936	24501	18033	19784	19030
42	5494	6786	5156	9567	10173	7675	4784	5202	3716	5644	4386
43	11265	15413	10392	17488	17745	17379	16040	18101	11254	25209	16277
44	1576	1102	741	1520	1988	1588	1033	2050	897	854	201
45	567111	434772	269712	641713	705670	791511	521527	569802	332786	2327516	992230
46	55706	46468	36156	32703	65527	58349	51156	45398	34352	123888	63554
47	97791	85569	63138	58338	118971	102231	94394	81790	61022	231648	123294
48	117146	81256	61045	78474	188701	141413	123088	128486	83428	496244	206251

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Mu1309_9**	**Mu1309_10**	**Mu2313_1**	**Mu2313_2**	**Mu2313_3**	**Mu2313_4**	**Mu2313_5**	**Mu2313_6**	**Mu2313_7**	**Mu2313_8**	**Mu2313_9**
1	2561750	1593154	3532537	3493226	2439100	3011632	3758177	3125755	3454745	2069747	3074158
2	217034	225735	204148	195812	281552	232601	245534	238617	232540	204918	213927
3	808810	1373945	1323335	670036	808702	2120200	749183	1602525	676552	668735	1727841
4	2529174	2388723	1491939	1420444	1397475	1610099	1882343	1473623	1742684	1370470	1371771
5	3295	2519	43389	35625	222551	6325	43595	1264019	36563	51474	1098468
6	304	237	3110	1646	517	2008	9	221	353	637	1181
7	14650	2768	1908	9582	85164	981113	37936	327937	45583	5632	525853
8	213093	5821238	5609068	4609304	4338	4477830	164741	5347959	5474504	5641528	5721449
9	5085424	6078966	5890466	4675937	5839001	4143	5324737	30231	5803916	5878780	67267
10	1107972	1262554	751283	777106	22793	715501	724524	738753	34218	953177	741676
11	34894	28221	45236	39661	49440	34399	39466	31978	35323	30492	26850
12	19538	18795	40684	27673	35878	23365	28443	19894	21534	17412	17003
13	15098	12884	73224	44325	782	20977	26423	24648	23383	76186	15402
14	39379	56624	15218	34419	45866	25072	26221	33483	28766	28643	11453
15	51671	55124	47481	44538	50282	35821	34521	31400	22986	29680	28554
16	625	3173	5595	3402	2718	2219	3261	1997	2550	2334	2067
17	1934	2895	2853	2296	2552	1489	3527	2809	3909	3039	3354
18	23804	19306	54304	41773	53478	30187	40072	30067	24205	26140	25502
19	7217	12179	17862	11200	14711	13230	17583	11099	12817	6971	7723
20	7460	6695	38540	20385	23269	15027	17867	13026	10478	15062	12688
21	9462	16498	9814	4283	9421	5902	8565	6610	5608	13230	6016
22	65441	46445	166859	90925	69784	102600	74115	61306	41410	32131	38187
23	63054	54625	97232	80400	88618	64963	74920	60381	57836	53271	48732
24	1292	1723	2077	9598	5571	8644	2766	5673	3351	2205	1641
25	4496	4863	17789	11044	10530	4836	10590	6738	6636	8298	6357
26	6654	4924	33860	12892	11000	21165	13021	17309	9394	6373	7575
27	2189	836	7889	5684	4053	5476	4838	6288	3005	2076	2858
28	4199	5922	8065	7852	7748	2815	3930	3005	4259	3237	1809

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Mu1309_9**	**Mu1309_10**	**Mu2313_1**	**Mu2313_2**	**Mu2313_3**	**Mu2313_4**	**Mu2313_5**	**Mu2313_6**	**Mu2313_7**	**Mu2313_8**	**Mu2313_9**
29	52026	53211	47101	42946	49861	39368	32156	40097	45672	33326	32773
30	56629	53487	57653	56853	63519	43505	52315	49254	60635	54690	40739
31	23624	27158	86745	45757	38798	27348	34699	23389	21234	26363	19896
32	5875	4358	26209	11991	10576	17151	11770	12397	11885	4621	6311
33	35899	26552	71416	74800	50679	64316	84914	48705	3373	11807	48288
34	1102	189	3621	2917	2028	2654	1563	1865	1588	1087	1380
35	18885	11691	27237	25355	17073	18788	22166	17038	16029	12184	16531
36	6582	7317	30041	13216	16810	6807	9878	10177	9251	4699	6104
37	1656	1137	5155	4040	3732	2429	2289	2092	1952	2675	1756
38	0	73	4795	2699	2987	1085	452	238	67	753	165
39	16297	10187	80198	31254	25722	47440	27059	37802	23311	16611	16080
40	138	0	839	1383	311	283	589	301	28	402	295
41	16265	17688	16247	12445	15028	9121	10536	8097	10633	8876	9173
42	5958	6088	10014	11116	6758	9704	5862	6174	5424	3338	5305
43	17843	12031	20259	20602	12287	27439	19489	13790	17491	10555	15790
44	554	612	3240	2010	2107	1449	2360	2049	800	1053	871
45	771340	469010	514909	548517	317289	611989	548744	357965	1155718	332560	548622
46	65320	49182	28920	37658	26729	36360	34063	25513	76046	29430	38860
47	113728	91510	47445	66780	49809	64774	53679	46177	131091	52439	74285
48	186632	120876	54701	84795	54330	79410	67871	50727	241243	61175	109421

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Mu2313_10**	**Mu2550_1**	**Mu2550_2**	**Mu2550_3**	**Mu2550_4**	**Mu2550_5**	**Mu2550_6**	**Mu2550_7**	**Mu2550_8**	**Mu2550_9**	**Mu2550_10**
1	2886974	3823676	2777630	2021996	3988805	2766917	2890309	2801609	2178417	2523384	2550153
2	220430	287580	234458	259544	411211	259407	289950	313692	363229	209164	286911
3	1705397	1013374	785939	2710519	1635504	1004241	1093892	1295720	1372404	609747	1118898
4	1370241	1955346	1496430	1856508	1162238	1805442	2093073	2283860	2590590	1385304	2015776
5	2912	1488183	22426	1459626	815706	28518	9554	68620	8681	10642	20181
6	367	1231	438	275	426	1682	1135	857	1327	190	117
7	1102348	70442	78114	5152	149474	71424	65113	72264	79332	780	48425
8	5668375	83200	28160	4616228	1895	5662	125565	1715	557	4828935	152763
9	1095	5467320	4920405	4244	417	5055460	5504491	5932318	7311295	4963114	6637161
10	668350	1194416	30054	1362686	65546	42379	1307658	23011	1485768	920613	1258727
11	31424	45935	49205	32445	53549	33802	31166	35256	38796	30343	32751
12	18125	46762	48671	28256	64358	29968	30745	32165	40123	16026	28030
13	7896	29672	1371	10602	18560	843	6932	2680	13483	32113	27209
14	10853	29636	42672	69857	94593	69689	70168	23663	84917	17888	67611
15	26689	82506	77645	70897	92491	66894	69282	65939	79602	25680	55631
16	1402	4670	4003	3434	5265	4576	3967	3064	3108	2222	1904
17	809	16165	11898	952	16433	5282	1652	6474	9794	2306	4340
18	23174	32019	24546	18786	27783	23593	20028	19203	18419	16724	16326
19	10598	19738	14252	14652	11628	9472	9335	8240	11698	7265	7988
20	11578	11314	8927	10098	12237	9665	6275	8192	8910	7914	5133
21	5336	20739	24063	17251	14085	6668	18822	12869	21882	5485	13442
22	38922	215886	107145	70599	146187	107671	89318	73641	62617	31259	67949
23	45655	123651	108601	84341	132791	83816	87037	93504	99562	42550	74150
24	1991	4217	4913	7533	5920	1826	1516	2210	4112	1416	1454
25	5279	8178	10466	6038	8704	7193	5971	7903	7714	2404	4230
26	8990	26011	14877	10887	18506	13535	10560	11072	9227	2925	9591
27	434	5653	4338	3206	6244	5298	3506	3666	3978	1723	4074
28	3467	8230	11517	2894	6790	3169	6619	6887	5839	1655	4699

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**Mu2313_10**	**Mu2550_1**	**Mu2550_2**	**Mu2550_3**	**Mu2550_4**	**Mu2550_5**	**Mu2550_6**	**Mu2550_7**	**Mu2550_8**	**Mu2550_9**	**Mu2550_10**
29	26614	90217	69082	66249	74535	68663	72837	73806	75359	32908	62808
30	37892	42814	13353	57084	41971	58019	29486	24487	20514	49336	24902
31	18695	30235	41627	26372	34462	27185	20552	27800	30883	9895	15895
32	7032	25418	17244	9880	17746	13976	11348	8947	6896	3909	9784
33	37272	96945	41335	36618	74679	42440	69043	62679	34178	30781	47861
34	1492	2769	3091	2383	3176	1495	1876	2012	1538	1240	286
35	13898	34823	32265	25416	31378	27108	16830	22771	26067	21359	22544
36	5548	22373	10876	10370	13714	9395	10675	11978	9391	6148	6329
37	1360	2977	2719	2119	3352	2538	900	921	2311	1457	1075
38	0	8927	11812	1991	8386	298	879	634	541	0	337
39	18674	47204	29183	19466	28557	20897	21905	18277	13395	4936	16035
40	72	1984	2051	68	1549	144	358	1035	728	362	553
41	7942	36261	52855	24386	38474	20167	30168	24629	27793	7851	22696
42	8394	18069	10498	8829	13592	5058	6644	6463	4712	3292	12324
43	17360	177843	94010	89860	175978	44246	78532	119366	85284	19951	143556
44	400	6132	3692	2649	5534	3286	3585	3681	3653	125	2230
45	794720	4298662	4328565	2213213	5599838	2487238	4364134	4705578	3345749	582888	7829225
46	56554	178830	211924	0	268840	125645	194532	222490	174258	61604	369051
47	106766	316859	408396	253722	528464	239215	381479	409340	352743	113529	720088
48	128682	857706	1030123	563744	1319676	698903	1100081	1119337	902756	177312	1727477

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**RB2_1**	**RB2_2**	**RB2_3**	**RB2_4**	**RB2_5**	**RB2_6**	**RB2_7**	**RB2_8**	**RB2_9**	**RB2_10**	**BSHMP_1**
1	2608340	3309235	2688806	1950605	1848967	3370537	2381652	2668042	2648140	1833871	3499856
2	240049	236568	267459	272016	252245	247850	245801	240443	230784	204330	296982
3	633383	771026	743012	813261	2121317	911496	873348	764214	851845	845327	627340
4	2252455	2022760	2123563	2113359	2022839	1434539	2159213	1871020	1722113	1277553	2189615
5	1678921	33416	16853	47504	13171	30997	20131	3825	3347	955107	243738
6	531	1017	1882	727	658	879	720	532	822	78	1323
7	101308	83180	74230	66555	872284	73407	78076	41019	3095	292061	96617
8	6798097	1475	6006	16	4084372	2194	27575	5148509	4652472	5476001	870
9	7126575	5084651	5028887	5780866	21106	5269253	5427910	5413788	4923528	5724247	6189653
10	105259	35610	28354	37108	1692086	13114	1543383	1471119	1287837	1511780	61312
11	111384	71614	56653	47693	46595	49469	42217	47433	39749	48555	143207
12	97768	52378	41897	30713	28988	32634	32567	28402	25235	20356	84755
13	73758	710	43185	4777	14398	1091	56474	24671	20448	39612	82730
14	149742	47368	78018	49381	59950	19934	44145	30234	34601	37889	163695
15	228390	86762	83538	87025	76844	68166	75418	70012	56754	50308	84616
16	11041	5388	4966	3575	3884	5453	4979	5190	3451	2526	7008
17	4564	7742	17250	7614	4008	11554	20042	13917	5933	1346	12342
18	215400	100899	91146	61163	54422	54058	55712	55922	46325	80269	242042
19	47952	30723	22023	14597	17194	16028	17384	11499	12773	14798	68382
20	34027	14359	9810	6945	8681	9347	8039	12432	8408	7411	15385
21	56555	14549	20729	14501	29818	11193	10973	13047	10862	14990	88480
22	347906	140477	105841	82968	77142	78719	77170	71231	63645	53303	279047
23	262723	93732	84064	81738	83595	72540	67500	67036	58205	45553	122638
24	7797	7283	9804	9764	6594	3299	6521	4876	2333	3279	11290
25	26486	9422	8000	7096	7602	8170	6904	9688	8493	8845	10716
26	30202	10689	6874	5222	6323	7160	6514	5623	4232	3950	17631
27	5870	3733	2614	2472	2747	2797	2775	4510	448	1734	6420
28	39136	11591	7764	6174	6366	5782	8186	6549	2896	3281	10123

Supplementary Table 1

	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**RB2_1**	**RB2_2**	**RB2_3**	**RB2_4**	**RB2_5**	**RB2_6**	**RB2_7**	**RB2_8**	**RB2_9**	**RB2_10**	**BSHMP_1**
29	220719	71109	65098	68982	62506	58603	62213	52531	46767	40169	88052
30	248040	73871	77486	73070	65831	76243	61720	62419	48808	49767	95405
31	151196	56730	41458	22964	33907	32543	19829	69134	50013	49677	30930
32	28624	9429	7205	5765	4781	5230	5585	7263	2799	2866	19166
33	126693	78954	68306	40185	42735	61402	58793	54119	49474	37439	79854
34	4011	1932	1306	548	196	442	847	500	674	659	2026
35	84624	27542	20239	18836	14919	17315	14352	16048	11705	12221	34953
36	41877	16470	7892	10444	7754	10237	9057	8836	6082	5222	33331
37	9134	2826	2199	2900	2228	2524	927	2668	1628	1937	3035
38	15405	8490	5573	3067	1054	669	595	1407	114	250	22517
39	37711	11204	9572	7843	4558	5515	8001	10621	6289	3230	35539
40	2657	900	744	363	64	66	100	234	134	311	259
41	56366	28815	26945	30648	19115	19774	19666	16278	18099	12934	26576
42	20872	8528	7927	4955	4423	4977	6501	4264	2769	3501	9489
43	45282	20841	28637	19788	17597	17228	34029	12469	10237	10390	19806
44	7020	1094	981	1413	833	1286	1148	1045	469	2629	1836
45	1183240	649408	588543	348303	315734	435842	3565884	309199	198238	147169	422378
46	40377	40997	46550	37398	29974	19662	41643	20673	18504	9377	27504
47	65692	75721	83797	68100	51645	40265	76095	33352	34334	16145	44527
48	106390	102787	109233	75800	53895	40640	394450	49548	31779	13649	43678

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**BSHMP_2**	**BSHMP_3**	**BSHMP_4**	**BSHMP_5**	**BSHMP_6**	**BSHMP_7**	**BSHMP_8**	**BSHMP_9**	**BSHMP_10**	**RB_1**
1	3649033	4283196	3170957	3641461	2768093	4038561	4068148	3600266	2552603	1977646
2	245291	248924	317172	273971	302826	282187	297520	263554	281801	234561
3	687326	829644	882259	918946	874607	987886	980018	888442	874697	652113
4	702318	800983	826603	801658	831215	843011	884055	850763	790044	2199569
5	189332	288705	498710	410955	461121	466828	384032	495183	292437	3409
6	616	8142	5430	323	4790	487	5227	6336	7695	1274
7	103881	109316	104631	85199	92362	95022	128104	90417	99756	75330
8	853	654	556	1511	884	769	847	676	1147	4989910
9	516	101741	84	17511	76538	6443598	41745	6897194	7037008	5076827
10	47076	48399	42181	11442	42162	39845	46466	40448	46726	22563
11	122687	124300	2254	102933	115906	112617	109389	104543	90658	52968
12	62811	72686	73973	57542	53911	68256	66656	47835	68281	52463
13	1175	3363	31950	3015	3406	42039	4511	2267	1450	47982
14	97537	40233	76651	116411	119429	98978	98973	33968	87853	180577
15	62003	50685	52503	53044	49110	49804	56331	45639	49768	184795
16	2441	3940	1846	4265	1923	2790	2068	4498	2644	4595
17	15990	20378	7406	13280	12242	7663	13126	13927	17239	38463
18	133511	119864	167032	107704	131596	97538	112357	111230	83523	48032
19	34039	37802	43569	29860	24392	33799	37748	29746	28731	18877
20	11168	8993	8296	8191	7631	7241	9731	5810	5566	33472
21	23756	24863	26696	22644	22729	15022	12300	12207	13543	66142
22	133958	114214	112007	108182	77443	92326	105093	84124	62909	103582
23	80410	69090	68088	73431	67725	62229	66431	53987	50702	270970
24	18907	16961	9529	10603	7383	7587	7455	14034	12235	3776
25	8319	5700	3794	5153	5414	4791	6290	4773	5221	25639
26	10452	7381	7753	8297	5071	6854	6843	6259	4676	23797
27	4499	3151	2774	3304	2374	2805	2695	849	1111	4472
28	14434	11283	8448	3751	7983	6155	6135	4529	5761	30004

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	BSHMP_2	BSHMP_3	BSHMP_4	BSHMP_5	BSHMP_6	BSHMP_7	BSHMP_8	BSHMP_9	BSHMP_10	RB_1
29	50273	45168	53143	49394	50519	47884	49075	38712	37352	234461
30	75080	73584	54900	59889	60151	56966	57051	52677	58257	164776
31	27253	20483	11888	17911	24108	14990	17345	12373	14789	107802
32	9795	4499	8278	6346	5953	6418	4861	5675	3116	29389
33	85209	77572	68149	79823	48148	38134	70505	56419	32906	189248
34	1875	1160	1634	1731	1158	781	1570	1051	726	5291
35	27713	27767	19729	18623	12074	14667	17191	12955	13110	67362
36	13832	12405	9102	11844	10742	9091	8711	7772	5539	3384
37	2795	1340	1279	1741	2100	1326	1392	536	966	8616
38	18376	8252	8380	5394	5730	5999	2699	4886	1485	5514
39	21398	12906	11852	16280	8975	9165	11048	11493	12732	88890
40	665	80	0	237	275	0	245	0	27	4972
41	28480	18510	22697	21687	21213	17962	15461	16293	18322	61453
42	8941	4863	5080	4693	2455	3172	3774	4349	5365	22134
43	19549	17935	16256	21176	13034	13140	17111	11738	14546	64576
44	2672	1744	711	1839	2738	277	524	1873	929	6014
45	255975	406271	266053	398776	217404	230730	282288	198475	261349	1950477
46	25537	42966	28133	42832	27772	23336	30103	21108	34520	89162
47	44732	72406	56338	75953	46928	46236	51332	36924	61372	163904
48	52977	80045	51737	114184	61689	59400	55184	40611	95196	406282

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	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	RB_2	RB_3	RB_4	RB_5	RB_6	RB_7	RB_8	RB_9	RB_10	DM37_1
1	2708462	2412261	2362396	1918923	3172928	2086981	2623282	2523915	2165169	4018509
2	256550	266335	274435	289067	281588	311395	307710	281386	290764	188420
3	1712749	764497	1005760	871679	1025001	3032552	1130563	1041990	1061805	1753702
4	1655139	2037812	2141117	2105615	1581563	2041459	2037873	1774785	1942250	1436935
5	1214920	8805	1617124	1559346	2440	4296	7715	3129	2344	1100713
6	776	187	503	1167	572	363	997	368	481	1700
7	974886	28899	1444	18843	80701	383127	4830	1057	15169	906730
8	3222812	3692815	4380395	4069903	1019	4413159	4112863	4135976	4483402	3065282
9	24070	3684556	4577152	4287735	4068193	20794	50644	4360551	4716830	59851
10	1034447	1230913	1120072	1058141	21139	1055762	966475	985383	1101986	892788
11	30053	26985	26982	34043	32550	27210	30308	28040	23902	47038
12	18376	20719	19443	18049	25982	16193	21897	14745	16163	23460
13	78522	17903	12647	8038	5432	4417	1835	13258	20428	7239
14	75944	95353	37790	98019	72588	76402	54879	52223	60004	53583
15	54569	65667	65575	57812	54207	63995	64391	49291	45408	27455
16	4696	3067	2794	1929	2201	3905	2514	2809	2716	916
17	4467	4638	20709	3706	17259	1231	4142	6775	3950	1377
18	21676	22199	20839	19001	17800	15314	16871	16426	15993	43110
19	10682	7574	7364	14986	8015	5185	7080	7299	3164	17060
20	10373	10318	9523	8905	7917	8739	8884	6511	7687	5758
21	14334	13979	15289	20614	10443	11051	12229	12571	13060	12174
22	36639	41640	34780	31957	35404	34605	43638	32182	24557	61616
23	88108	89256	88405	89788	83334	80676	80906	60267	61065	48614
24	814	1650	1772	3135	1037	2239	564	989	1097	2149
25	9128	6720	7705	8268	6960	7208	5945	3863	6534	5456
26	6993	8256	6218	6775	7664	7862	7139	5747	4595	6847
27	2085	2210	2793	3230	2307	377	3003	1351	1819	2670
28	6362	4123	5359	5124	3780	5755	4773	2801	3157	5375

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	RB_2	RB_3	RB_4	RB_5	RB_6	RB_7	RB_8	RB_9	RB_10	DM37_1
29	76541	78028	73176	70903	67333	62762	68360	60585	60701	47307
30	50729	56117	47457	50405	46996	36796	43458	37141	32868	37805
31	30478	32185	37534	32349	27694	31030	28734	23791	31975	11378
32	5885	7367	6727	9124	6247	5606	6938	6932	3351	5980
33	103154	80929	81067	60510	84398	58660	68075	58405	55717	63291
34	1075	2163	388	1257	1502	1509	695	597	312	123
35	19263	16806	19224	15686	17150	14267	16467	11933	14945	7648
36	495	635	129	232	120	364	447	481	224	16000
37	2950	2684	1116	4267	2116	1688	1996	682	1663	2046
38	3454	983	1099	64	60	265	234	187	68	18885
39	19467	22080	16144	17484	17336	23066	21088	15562	9822	10627
40	429	99	326	0	0	32	97	140	93	235
41	13334	24341	24518	21414	20339	18106	17476	12124	15590	23566
42	8286	3761	4676	5213	3577	5633	7722	5431	3538	6145
43	26007	20569	16816	21991	21033	30300	23490	18519	15099	43296
44	2223	1100	3304	440	1808	1440	1072	287	852	725
45	792104	872065	480768	672157	717624	3129020	1197862	599584	617694	632882
46	55121	70775	48358	3	65334	79093	84186	51848	56679	65896
47	115258	127619	92336	115990	109730	142173	145155	97784	107328	113737
48	208217	251939	140441	228262	207873	744621	338188	185779	212365	185588

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	DM37_2	DM37_3	DM37_4	DM37_5	DM37_6	DM37_7	DM37_8	DM37_9	DM37_10	DMB_1
1	4008800	2897400	4052700	3572777	3043700	4341017	3000017	3538730	3260200	3393176
2	180514	236235	204246	189978	181344	222001	215104	198107	179968	235922
3	553101	615630	535411	1392134	520818	569664	552791	611648	451043	657302
4	1347679	2063330	621849	1393251	1235522	762600	910164	1628456	1041742	573059
5	128421	56791	56959	1093782	51795	50917	11105	8828	7322	395810
6	7496	413	5638	489	3702	2884	2993	329	349	2158
7	69143	41273	42947	827597	38594	6698	44398	12305	34475	170678
8	3213	967	3590	4127900	192	4514	455	4762205	4852964	1165
9	4275752	5334418	4536949	23450	4560682	4683470	5840180	4885695	5142716	8664
10	25557	8589	8498	854280	15217	12788	7351	879428	1077521	35661
11	58988	41085	51441	44530	39516	50858	38545	43725	31152	106259
12	39854	25066	17987	18456	15218	19368	13623	22177	12382	104552
13	3814	22440	3365	9714	1161	1972	1690	24028	0	34433
14	82595	76271	42089	31152	37837	62601	27113	47258	26713	83259
15	45217	33507	22952	19756	18676	19795	24317	20503	21709	150433
16	3956	2429	1917	1960	305	2633	2021	861	1953	7521
17	15570	12848	14775	2679	1927	11647	14988	6417	5457	2251
18	57079	35774	38757	41915	28515	37119	26180	42209	25372	94957
19	25963	12176	15642	19400	14424	19439	9961	17333	9438	74455
20	7375	6001	5556	5184	5001	6592	4543	3666	6063	29101
21	16771	13854	11593	8006	8227	8081	13286	9550	4733	47848
22	106828	60989	54394	64934	36290	50866	25706	54922	25355	328406
23	58072	54653	37468	44363	36388	39677	35117	36339	25855	175584
24	8056	3334	3404	3874	1495	2689	237	5641	2825	6688
25	5017	3075	3524	4049	3351	4831	3059	3059	4475	19167
26	11531	5170	3734	4889	4268	4815	3313	4042	2129	44724
27	3807	3249	1723	710	1084	1865	942	51	1389	7984
28	6847	5288	4539	3575	3677	3738	3793	3471	3340	19936

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	DM37_2	DM37_3	DM37_4	DM37_5	DM37_6	DM37_7	DM37_8	DM37_9	DM37_10	DMB_1
29	42607	57713	32937	33054	28601	26932	27763	36423	21278	145225
30	43287	38470	45481	30656	36439	41678	28175	26591	26658	114205
31	20399	10828	12302	9152	11489	17959	7999	7770	27560	62584
32	9140	7277	6692	7656	5313	6215	2292	4479	4025	41153
33	77597	46362	55641	45452	39205	54618	26365	32364	40305	87521
34	922	954	1196	1564	1451	597	482	585	100	6857
35	8946	7774	9169	5458	3667	4993	5152	5147	5370	57508
36	9515	8898	10971	10198	7252	9553	8632	5984	3274	48851
37	1833	1369	1291	2437	587	918	1009	807	1729	3946
38	15201	15116	8491	6788	5902	2574	1323	2180	1136	29303
39	20741	11719	11619	13736	10012	10938	3608	6754	8464	119093
40	723	441	459	57	148	181	0	0	0	2899
41	34876	25236	18556	20540	17913	15853	11609	17445	11385	38499
42	8670	6923	4651	4987	3579	3603	3154	4122	6512	24151
43	53201	54907	24170	31058	33266	24761	36023	36595	20977	55511
44	1400	1791	1363	1469	1962	1341	405	929	314	3878
45	1499126	1135815	492999	469399	761374	438878	510590	751808	805695	2740259
46	150611	117216	63986	63476	91298	61009	64541	82257	89128	122046
47	274816	230873	113022	115458	173887	99824	120991	161755	163132	217060
48	497778	412183	199163	172335	367553	176027	204198	359563	381927	571712

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	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	DMB_2	DMB_3	DMB_4	DMB_5	DMB_6	DMB_7	DMB_8	DMB_9	DMB_10	JHN_1
1	4551772	4565108	2995499	3812988	5187883	3199767	4457858	3336827	2733840	2754393
2	209548	225475	228843	217662	208012	225428	271612	180501	206861	232637
3	589753	702775	641291	742982	746683	769380	833650	613741	775663	823038
4	1304200	611720	1175132	1207614	563414	594919	749209	692978	689973	2463162
5	293795	425098	378449	472310	298974	1532	433951	430066	298188	1860129
6	1570	3328	2820	820	3022	3833	5408	2543	186	1494
7	60009	86887	6643	55324	62582	61009	62515	53031	32450	104136
8	749	1957	62	1238	1379	1298	2580	3787	835	5034966
9	5019086	157	5578301	3368	5544547	25077	6962779	5969930	6592847	5378198
10	57952	73536	40150	20893	21904	17857	17313	41888	16310	69972
11	54213	69289	52735	44528	57495	41910	54608	37753	35029	76101
12	38292	57138	27739	27079	39110	24013	39416	21109	19608	90367
13	2385	2023	3163	5913	4517	1953	10520	2041	3917	78167
14	57675	62078	78012	34492	26536	71658	86567	38121	54509	94059
15	48900	50174	45665	43029	56526	49915	63192	37070	40269	141474
16	1958	3631	1753	2725	3195	2673	3474	1838	1406	3643
17	4617	4152	5203	3225	11697	569	1248	1085	1778	10463
18	42289	42538	59001	34349	31933	24900	51139	25336	17751	90712
19	17986	28758	17021	10117	22712	13899	16595	11540	9728	34661
20	6812	9917	5612	5669	6144	5412	7206	5595	4174	26161
21	13583	15095	22748	16253	13438	10687	15225	10168	9433	27069
22	91271	120307	61691	63064	82160	58746	66604	52781	43958	306955
23	57852	59628	55796	55282	46481	47171	53842	33602	39060	157954
24	3502	6444	5083	2194	4267	2266	2648	2743	2251	13370
25	6405	7536	4869	5598	2825	5873	7875	3422	4358	13986
26	8877	11136	5745	8410	8179	7306	7291	5986	3967	23674
27	3343	2934	1687	3899	2979	3332	2475	324	2000	7044
28	7914	10319	5968	5298	5168	5911	5407	5132	5934	14032

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	DMB_2	DMB_3	DMB_4	DMB_5	DMB_6	DMB_7	DMB_8	DMB_9	DMB_10	JHN_1
29	55740	56233	53900	50094	40735	44266	50742	38625	36595	119506
30	43896	56905	37652	32393	43981	33786	39000	23959	24680	38260
31	17115	26240	10757	10545	19330	14156	17015	14339	13270	53620
32	9193	9040	6479	7317	7970	8091	4707	5405	3509	26701
33	87386	41237	50588	17867	76899	29180	48545	18465	19396	93385
34	2496	1902	1600	962	1276	1242	1307	1334	624	3387
35	14912	15901	6313	7490	10951	8797	9500	9772	9974	44363
36	11830	11940	8481	8535	8306	7872	7162	5867	5135	27513
37	894	2000	491	979	494	2106	1536	367	1133	3570
38	7902	11941	5952	3527	1751	6842	2786	1337	1915	9781
39	21078	28894	10672	17610	16983	19015	10256	14830	11174	29340
40	481	196	0	0	397	0	177	116	216	2425
41	22900	24477	23273	23872	20832	22757	21718	17627	17647	38331
42	7813	6756	3223	5016	5579	4564	2306	3869	3842	8741
43	27132	34835	16814	19014	25347	21136	18791	16692	19790	34932
44	3328	1276	1582	1657	364	2400	1434	414	391	3784
45	551252	1402107	385191	431977	1151358	1016349	413870	504315	540472	768958
46	42319	96631	42704	50001	91781	84766	36358	59851	61872	40011
47	89368	192827	77594	92249	178954	179806	76719	115879	106319	79276
48	153334	434788	115208	154439	412452	421139	124223	219367	266752	140076

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	JHN_2	JHN_3	JHN_4	JHN_5	JHN_6	JHN_7	JHN_8	JHN_9	JHN_10	MNF_1
1	2568401	2060376	2305573	2647789	2120272	2488511	2671351	2429565	1264691	3537904
2	285023	251276	281430	295982	267147	304773	284892	266919	234454	170674
3	896945	1003494	1114178	1161634	1254249	1322948	1212775	2874422	2305810	454932
4	2511158	2521941	2937583	3054118	2551961	2625038	2648753	2442635	1931477	1364863
5	22287	1960014	20748	19064	19905	5009	5271	3231	2657	1008623
6	460	548	1419	2117	858	221	999	333	536	76
7	14856	4223	20360	53376	1479	15983	6409	499707	1148800	39508
8	4772100	5410706	4749424	59905	5305709	5683604	5721695	6143479	5887718	51974
9	5047611	5507341	4755291	5983546	5269139	5852647	5913367	23701	20012	4915047
10	1446488	1328613	1477018	1493045	1388040	1517881	1623688	1466873	1322948	28616
11	60788	38821	46278	50738	37221	35645	39558	917	29079	37427
12	57172	29760	35771	48820	34298	30982	43801	32432	22876	25537
13	64899	122578	98857	64834	47454	43131	39548	32068	134454	41314
14	72684	39247	72120	79355	62832	61425	55896	50151	57353	40198
15	91788	85331	81221	102152	127501	74911	78164	67568	72115	50333
16	2370	3922	2680	5586	4651	4495	3364	4066	1566	4606
17	3727	3319	1759	5441	1416	1266	1612	2985	2506	3597
18	63315	33782	46830	42599	33224	20848	26932	25164	21961	37961
19	19281	18136	9235	13878	8992	5665	14153	12592	8512	12033
20	17934	12403	15419	12930	11309	8564	10574	9112	10420	6188
21	23215	18529	28424	20964	16019	20230	14755	10726	7000	12965
22	304204	105141	122949	154219	110564	90273	101243	88700	49908	121871
23	95874	87210	94253	111027	85104	80831	82620	67488	51651	49917
24	13055	3876	6472	1970	8918	5293	5153	2112	4081	4598
25	8432	6734	9059	7871	6948	6306	6303	5010	6105	6058
26	19643	7115	9993	14075	11377	10948	7874	7407	5399	11527
27	6494	2667	4405	568	4249	3660	2741	3150	481	3278
28	8217	11045	10690	7159	7148	5352	4416	2825	5987	9575

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**JHN_2**	**JHN_3**	**JHN_4**	**JHN_5**	**JHN_6**	**JHN_7**	**JHN_8**	**JHN_9**	**JHN_10**	**MNF_1**
29	105510	93006	116132	132391	88750	91212	87992	78971	68029	39024
30	62102	41663	41588	57105	37110	43980	43438	36451	31884	34466
31	42213	22098	43178	34451	20910	25257	22992	20011	26371	21141
32	24994	10931	11538	16827	11020	10503	9175	8723	5610	8571
33	84211	44977	59631	84909	49557	56104	51267	32450	10463	74790
34	2086	2476	1703	1135	1454	2188	1890	1516	1048	1694
35	45606	31030	35321	36696	26975	34556	20410	22480	15687	32334
36	15471	12721	12114	13018	12582	8980	8933	5640	4005	16052
37	3303	2637	3259	3319	2153	2757	2333	1591	1037	1346
38	6195	3324	2168	1529	1795	1948	1522	625	66	10394
39	26268	11762	15621	17707	14870	14017	11352	9856	8266	23070
40	839	850	1298	350	757	397	558	33	226	408
41	38655	26288	30206	33838	25812	24456	23861	23175	18637	13504
42	9441	9251	6198	5656	2117	6176	6015	8822	7464	8774
43	30835	49378	24177	30142	32455	58040	75877	35461	24506	27416
44	3765	3129	414	1650	1097	964	652	699	404	1688
45	951297	549971	438927	1392771	1419556	1557585	2790655	1385982	250338	834469
46	68398	51040	49066	80765	98374	109733	151906	100685	34465	74064
47	135561	93041	79354	155072	176971	186880	287034	187616	56689	144405
48	246728	154066	136571	370411	423701	425989	735226	424354	89709	263401

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	red non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	MNF_2	MNF_3	MNF_4	MNF_5	MNF_6	MNF_7	MNF_8	MNF_9	MNF_10	**RUBY_1**
1	2533720	2156496	3278842	2372607	3306723	1621257	1621070	2396700	1607848	2195986
2	204277	184509	204864	184893	191868	178741	171816	211546	196871	161649
3	551834	568370	669932	580180	610164	575085	1365772	666839	559781	420306
4	1064310	711682	627627	1198420	993803	1483153	1169755	758805	1176421	1226243
5	29350	30977	443205	6312	23631	1092272	1973	21066	14576	7528
6	1755	4228	3375	555	3825	145	736	2656	3105	1620
7	27138	43643	59949	35238	26562	37138	1008573	51761	6063	59544
8	254	1004	1400	753	1730	0	5025026	2076	1802	3718825
9	5763820	4768831	5308659	5851977	5478091	6147398	1056	6467308	6135219	3977094
10	11674	14666	21438	47368	15772	39243	1145880	22118	35475	883926
11	34646	29933	35487	29324	32757	31735	22420	26831	24415	71726
12	17337	17041	21025	16055	18855	13527	11596	17277	12204	36482
13	3586	3360	2894	47043	1872	30639	22516	1446	36248	1944
14	34289	45764	17888	21221	27408	28728	36628	21126	32981	21386
15	46116	41535	48144	49488	37477	47938	39967	40452	40196	78326
16	2400	1652	3769	2151	3412	1355	1091	638	1711	2169
17	5339	8971	6452	2314	6249	702	873	9792	1155	21192
18	32295	30480	28740	27797	24250	30295	21316	20823	19950	71923
19	11203	7262	8940	8084	9549	7488	9455	6605	7651	22220
20	5330	6083	6096	4878	6331	5882	4003	4510	3953	15750
21	13118	12008	10975	11678	9032	8481	8055	7091	8837	21066
22	63002	54994	64166	55186	41588	33455	33337	37515	26423	110046
23	51305	46607	43980	41706	42017	39407	31816	40033	33785	148143
24	2816	4008	1554	1471	2058	1369	1714	932	1620	2851
25	3087	3244	4657	6209	4356	5721	4161	4607	5279	13887
26	7996	6048	8055	7515	5790	4393	4513	4972	2843	12118
27	3179	2385	2918	1315	363	629	1887	580	1289	2767
28	7680	6128	4361	4664	5090	4908	2918	3628	2850	8468

Supplementary Table 1

	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	black non-aroma	red non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**MNF_2**	**MNF_3**	**MNF_4**	**MNF_5**	**MNF_6**	**MNF_7**	**MNF_8**	**MNF_9**	**MNF_10**	**RUBY_1**
29	36741	40137	42043	38496	41211	36387	31257	30386	31689	79827
30	44015	27715	34871	29042	28518	31129	22351	28106	24536	3146
31	12931	10698	19246	18706	17995	16910	13254	15210	16741	43628
32	6716	4647	7347	5573	4178	3241	3809	3895	3135	9940
33	31825	29335	62178	25654	56300	22716	19812	39814	6944	53582
34	1963	1563	714	1595	1439	574	588	746	686	2932
35	20788	19051	19489	15630	16557	15329	8758	16917	14237	38756
36	8612	9256	9454	6062	7225	6495	4818	5540	3622	16407
37	1546	1261	1247	1378	2179	990	529	328	804	6111
38	5102	2917	1756	1779	1986	778	470	73	526	22145
39	15574	9704	15241	14866	15529	9413	8921	13442	10045	16998
40	216	182	123	120	36	34	0	197	0	4731
41	11192	10332	10856	11000	8894	9448	8434	8701	10907	43949
42	6107	3951	5442	5003	5114	1951	3288	5168	4589	9012
43	13199	16799	19494	16485	20804	13816	12887	13912	15266	15602
44	1919	937	2971	1131	434	479	988	343	1016	4587
45	312766	392824	643599	468013	780767	341550	318020	393865	430240	292998
46	37695	52452	59030	60359	82371	43358	45830	53154	56158	19301
47	65229	96230	100872	108935	148322	83342	81007	96324	107835	31234
48	96687	173828	208882	186383	359040	133593	137305	185457	201362	38754

Supplementary Table 1

	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**RUBY_2**	**RUBY_3**	**RUBY_4**	**RUBY_5**	**RUBY_6**	**RUBY_7**	**RUBY_8**	**RUBY_9**	**RUBY_10**	**UP417_1**	**UP417_2**
1	2076050	3074789	1996961	2434571	2307104	2041732	2894333	2371332	1534293	2506313	3142979
2	202041	194973	242300	215669	211567	251930	224406	193068	189267	275207	259299
3	377217	480223	599973	549551	527630	610925	586823	539773	537057	1048812	852553
4	1042676	714888	1143151	737583	1251174	757764	1166127	991585	889822	2017217	1760746
5	18214	510605	28829	543766	7834	26446	13096	4111	4698	78637	49709
6	828	3532	581	4005	496	188	1008	194	233	926	1850
7	38988	53652	50802	56915	55917	44476	66189	9989	2009	152394	74271
8	5263	1487	924	244	7379	489	868	3842651	4463220	5188230	2999855
9	3385483	2610228	4409725	3937605	4300783	4283490	4374400	4038396	4674485	5387305	3071430
10	10155	11919	18803	25448	733171	24372	6243	581476	751344	965485	4847
11	42292	44114	43060	52504	49112	40770	67890	43151	32768	94314	60001
12	18034	17381	16478	19335	14825	12108	27615	11670	9757	104428	59706
13	26542	1928	3548	35729	1670	4041	1396	9823	9185	44013	1028
14	12109	18505	21631	15230	12793	21287	18227	8413	18484	36296	9285
15	49697	32232	46397	33154	40347	39418	36761	32265	39879	176014	51566
16	1143	1324	1818	1942	1998	828	3492	1148	1261	4616	4336
17	27080	17993	21304	17780	19562	21597	22814	4857	4819	1888	15992
18	43300	31936	24853	50942	30110	22923	45578	25168	26353	69367	24302
19	17666	10876	10211	11024	11774	9673	14324	11960	7252	45433	20560
20	7670	5728	4602	4838	4957	5355	5908	3686	4549	36043	10140
21	4387	4346	4234	4704	4813	7602	2406	5510	5366	23470	8325
22	41335	40346	35776	44851	32275	25190	40696	30060	19179	244550	81280
23	76818	77076	81870	71297	73879	66336	71653	56463	50450	324774	121994
24	7503	4725	2874	3567	2873	1393	2997	1590	1916	7112	10802
25	6978	4933	5109	3973	4028	7015	4633	4757	2140	30198	8992
26	5875	4706	4733	5334	4232	3930	4662	2203	2684	35140	10083
27	2442	1643	2242	2412	3001	1213	605	2020	569	8326	3024
28	8873	6587	2904	2788	5306	5115	4931	4583	5051	15247	4679

Supplementary Table 1

	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
No.	**RUBY_2**	**RUBY_3**	**RUBY_4**	**RUBY_5**	**RUBY_6**	**RUBY_7**	**RUBY_8**	**RUBY_9**	**RUBY_10**	**UP417_1**	**UP417_2**
29	51248	38777	44019	38940	36926	36195	37195	34779	30427	200368	70124
30	1151	1265	1551	1605	584	910	957	61	60	2532	928
31	27494	14707	15238	19826	14448	22115	17894	16895	17328	158847	39714
32	5444	4508	4204	4871	4499	4678	3588	1908	3179	39449	8450
33	46531	79664	42105	57531	35726	39400	47139	40645	23740	99245	86947
34	1901	490	1168	651	733	1051	1655	556	1296	9796	3005
35	18841	10031	11533	12723	10157	14672	9183	12758	13535	104028	42260
36	6483	6363	4534	3977	5318	5395	5452	4300	3554	42055	14338
37	4186	3082	2728	2503	2259	2368	2437	1858	1557	9248	1915
38	16364	5314	2343	8841	6093	4050	4007	819	665	11181	3471
39	11770	8813	8209	8460	9380	8385	8067	7022	7673	95303	18012
40	4038	1487	1043	473	1193	1336	1189	1012	233	10663	868
41	33566	27118	19084	24956	21601	22646	24560	17856	13761	21558	9884
42	6531	4618	5924	4410	3473	3844	6744	2912	4731	21854	4640
43	14865	12536	15106	9634	9582	12217	14217	10333	8466	100895	40001
44	1578	1423	2883	1939	1871	3747	1594	1546	2045	9193	3784
45	280694	369920	287765	207566	134792	260969	324120	167874	102970	1715994	1194346
46	25979	27003	28483	18597	13604	27735	32889	18602	12515	73900	79376
47	45025	57232	48224	31278	27263	47498	54793	34551	19896	132365	170389
48	62697	73664	65993	38660	30868	70039	77926	41384	21873	271588	310708

Supplementary Table 1

	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma		
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Average	Average
No.	UP417_3	UP417_4	UP417_5	UP417_6	UP417_7	UP417_8	UP417_9	UP417_10	white aroma	black non-aroma
1	2682052	2065312	2372714	3647755	2177107	3128140	3030200	2379412	2865296	3644634
2	272158	275515	290302	283485	299361	262775	241718	289836	155971	306922
3	1033569	878403	1742851	984184	984807	834274	823082	1022458	860160	1172016
4	1842996	1933412	1801358	1852317	2055234	1674610	1491599	1705128	1162670	1552671
5	10103	50000	31997	82245	1659909	32232	1189555	42625	276753	295246
6	343	320	1276	563	375	3207	866	348	1289	1710
7	21103	42450	760367	80482	80948	36884	19200	16249	120455	152079
8	3460880	3615497	4246593	951	10019	3506049	3820291	4660019	2343975	1587407
9	3695213	3660078	4463314	3840939	4385877	3674511	4035259	21877	4517917	3398811
10	651599	41892	665814	25915	29081	562892	550387	719748	526673	439825
11	47984	41269	46189	50817	576	58844	43277	1425	60791	112554
12	41208	31183	31013	35820	47577	40490	27166	25570	65890	90997
13	36716	67883	25236	157962	26373	25583	20803	22749	29413	115426
14	44125	19140	40558	23183	20415	18074	20600	8441	70362	72465
15	55832	64956	56244	58733	55864	54462	40906	47392	94115	96247
16	3287	3788	2255	4969	1786	2906	2896	1699	4062	4278
17	4252	980	3010	12416	12749	2604	4744	2117	13016	7718
18	23391	20306	21138	23617	21491	24802	14250	18987	71646	120130
19	18439	14616	18679	14579	16168	18434	12044	9516	24675	41368
20	9019	9482	7929	7939	8278	12101	6036	6155	13933	12247
21	6272	9859	5177	3360	7090	10416	4674	4642	40540	21131
22	84482	54973	53947	60586	50063	73788	50978	39264	154451	191193
23	113620	105068	107998	92983	94325	103057	76816	75225	155467	96676
24	4267	7610	4540	3523	6535	2200	1596	1809	7970	6030
25	6344	5382	6427	4326	8465	9922	4480	4466	12429	9819
26	9154	8269	7744	8117	7382	9278	5936	6381	22154	14129
27	3485	3201	2957	2870	3726	1698	2628	1777	5712	4212
28	7053	7089	4359	1983	2916	3764	3146	3431	13631	7875

Supplementary Table 1

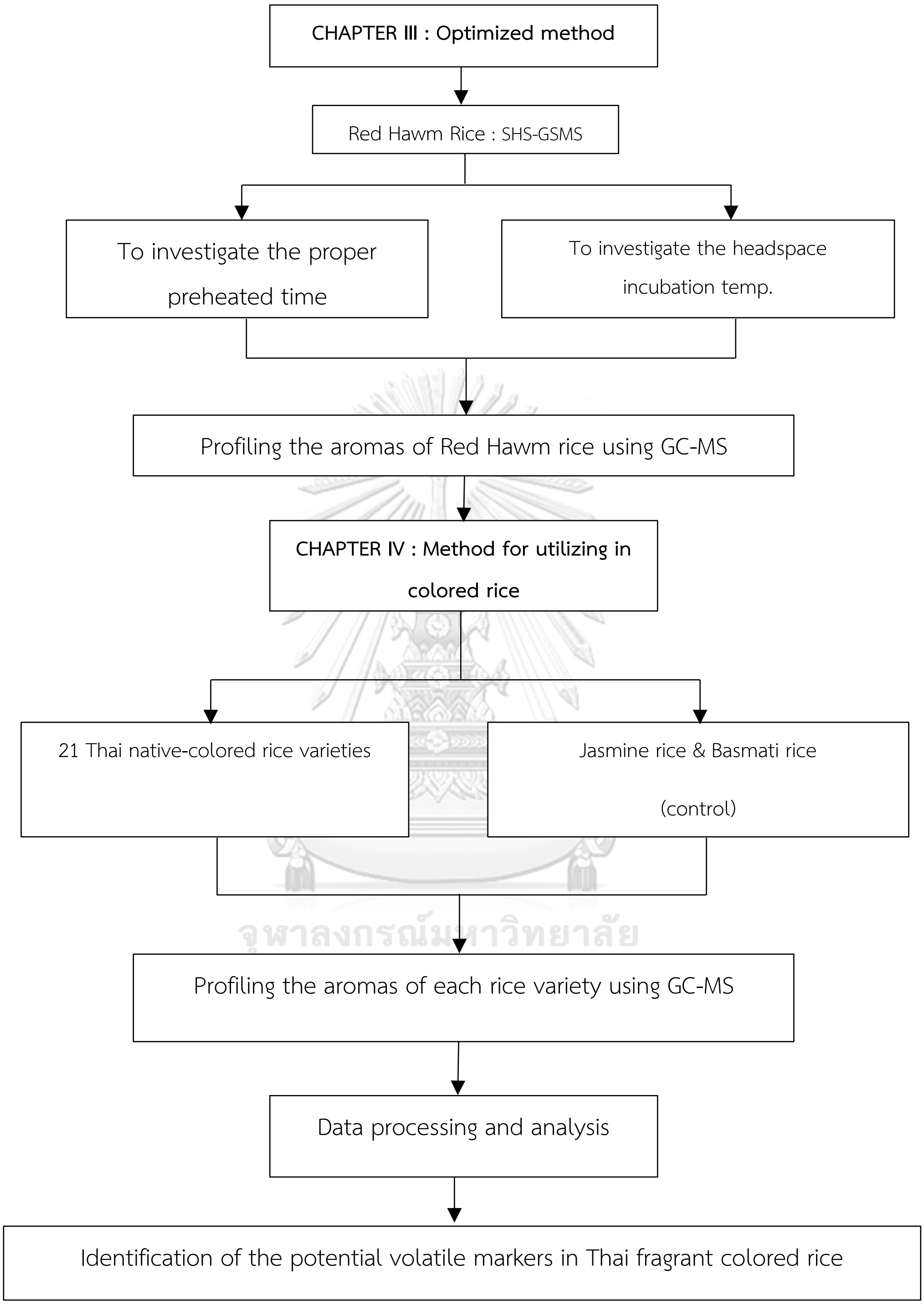
	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma	red non-aroma		
	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Average	Average
No.	**UP417_3**	**UP417_4**	**UP417_5**	**UP417_6**	**UP417_7**	**UP417_8**	**UP417_9**	**UP417_10**	**white aroma**	**black non-aroma**
29	82036	85576	66552	63292	71349	68850	55349	49701	77366	73609
30	150	391	815	304	461	265	250	157	2117	52732
31	34251	52756	27254	27550	35562	45174	19687	20391	36120	40024
32	11435	8741	7896	7898	5778	11187	6712	5998	25251	13998
33	81846	45385	32294	71775	37992	65295	57168	33472	97777	52739
34	1793	2729	2256	1852	1947	3370	1861	1479	4200	1888
35	41709	57162	30700	25458	22047	34856	26344	29251	39240	23935
36	6089	9038	8661	9717	7635	10090	4939	4661	15835	12391
37	2377	2406	2100	2041	3267	3156	2828	1346	3290	2378
38	3895	3182	400	763	1034	380	460	160	26741	9103
39	21111	20036	18732	13924	13435	25757	14931	17933	43098	27768
40	2029	3473	1621	2046	1151	1187	370	334	5665	576
41	13166	12615	7906	8308	8758	9733	7766	7036	59651	28206
42	6019	5468	5385	4650	2504	3247	8418	7270	9805	8677
43	65054	42572	34181	25626	17080	18548	35409	41345	28836	31595
44	2410	3533	3123	4130	1959	3027	250	2649	5418	2808
45	2941132	1128684	512796	777446	951541	729990	1444730	775418	2269966	891528
46	167539	111547	55327	59810	82150	77710	110216	73365	111274	57012
47	335490	224800	108954	119106	146167	132944	218867	146257	212165	108485
48	639687	392226	189273	209765	276370	218016	430330	297969	542438	196453

Supplementary Table 1

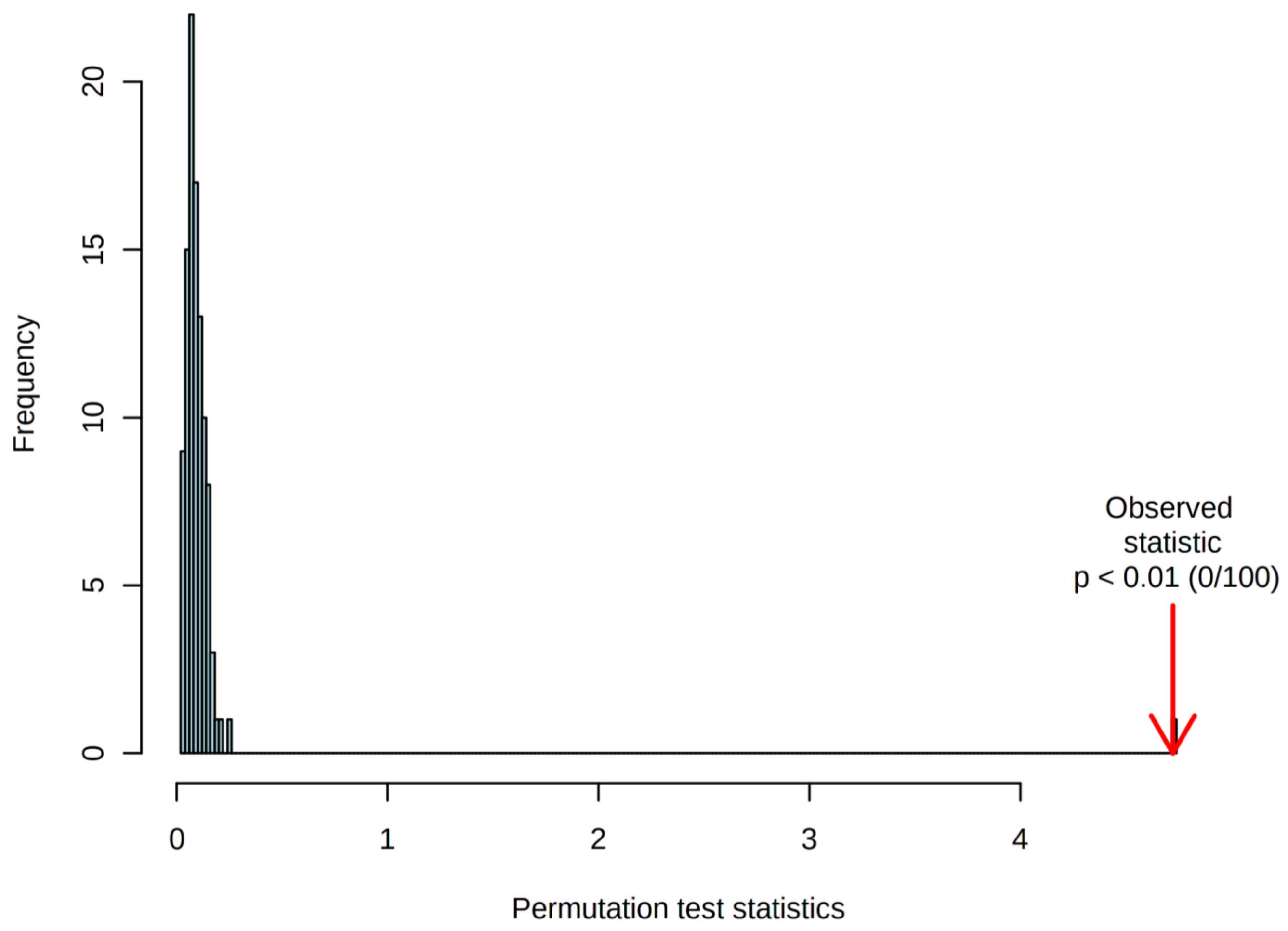
	Average	Average
No.	**black aroma**	**red non-aroma**
1	3007406	2502957
2	239278	241826
3	939391	771723
4	1418445	1402781
5	347389	219607
6	2150	1138
7	141918	86141
8	1833929	2177487
9	3747788	3811375
10	467863	363226
11	49477	46604
12	33871	31392
13	23167	26208
14	61107	20409
15	57246	54522
16	2812	2483
17	7289	11987
18	46746	31737
19	17067	15773
20	8719	8808
21	15820	7386
22	81116	60683
23	67124	99492
24	4746	4114
25	6261	7323
26	8342	7898
27	2524	2630
28	6594	5414

Supplementary Table 1

	Average	Average
No.	**black aroma**	**red non-aroma**
29	60170	62077
30	45073	877
31	23065	33538
32	8332	8018
33	55790	53874
34	1432	2126
35	18366	28300
36	8609	8950
37	1810	2989
38	4724	4778
39	17618	17698
40	398	2024
41	20787	17791
42	5956	6083
43	26815	27163
44	1504	2864
45	789908	730087
46	63789	55782
47	119894	106617
48	245042	187889



Supplementary Figure S1 Diagram of the research framework in "Identification of Aroma Compounds in Thai Colored Rice Varieties"



Supplementary Figure S2 The output of the permutation test

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