Chapter 8

The Deep Purple Color and the Scent are Two Great Qualities of the Black Scented Rice (*Chakhao*) of Manipur

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Additional information is available at the end of the chapter

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Abstract

Specialty rice varieties with unique properties such as unique color, flavor, aroma and nutraceutical qualities are increasingly in demand other than the traditional white rice varieties. Black rice has various nutraceutical properties with high anthocyanin content and the anthocyanin antioxidants are very important in preventing various diseases. Black scented rice of Manipur, which are commonly known as *Chakhao*, are deep purple colored and scented, and are very glutinous, as well. Two *Chakhao* cultivars, *Chakhao Poireiton* and *Chakhao Amubi,* were shown to have high anthocyanin and phenolics content and strong antioxidant activity. The main anthocyanins of *Chakhao Poireiton* are delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside and that of *Chakhao Amubi* are delphinidin 3-galactoside, delphinidin 3-arabinoside and cyanidin 3-galactoside. By GC-MS analysis, a cross mixture of 26 volatile compounds from *Chakhao Poireiton* and 11 volatile compounds from *Chakhao Amubi* were found to be responsible for emanating the aroma. Among the complex mixture of volatile oil components, n-hexadecanoic acid and octadec-9-enoic acid were the most abundant in *Chakhao Poireiton* and 17-pentatriacontene, l3-octadecenal (Z) and hexadecenoic acid eicosyl ester in *Chakhao Amubi*.

Keywords: black scented rice, *Chakhao* rice, Manipur, anthocyanin, nutraceutical properties, flavor (Scent) active compounds

1. Introduction

Rice is the staple food for over half of the world's population. Rice cultivation provides employment for over one billion people directly or indirectly. Besides traditional white or common rice varieties, specialty rice varieties with unique properties such as unique flavor, aroma (unique aromas), color (red, purple), nutrition (glossiness, stickiness and smooth texture), chemical composition, esthetic, waxy (very low amylose content) and superior processing qualities are increasingly in demand. Rice according to the pericarp color can be broadly classified into black (purple), red and white rice. Black rice has high anthocyanin content located in the pericarp layers, which gives it a dark purple color [1, 2]. The demand for various types of specialty rice is increasing in recent years, which are sold for as much as 50% more than traditional rice cultivars [3]. Black rice has been used in various traditional medicines; recently, many researchers have reported that they have several health benefits in various studies, and thus, black rice is being considered as the new superfood by US scientists. In Asian countries, black rice is often consumed after mixing with white rice to enhance flavor, color and nutritional value, which includes high protein, total essential amino acids, vitamin B1 and minerals Fe, Zn, Mn and P, and it is intensely colored because of anthocyanin [4]. One serving of black rice even though contains some calories, but offers a high amount of flavanoid phytonutrients, important fiber, mineral content such as iron and copper, and it is a good source of plant-based protein which is hard to get to plant-based eaters who rely on grains and legumes for protein [5]. Black rice is rich in antioxidant anthocyanin [6, 7]. A spoonful of black rice bran provides the same amount or more anthocyanin than a spoonful of blueberries [8]. Anthocyanin antioxidants are very important in the prevention of cardiovascular disease, protection against cancer, improving brain function, reducing inflammation, etc [9, 10].

Flavor is the primary importance of specialty rice, and superior flavor increases consumer satisfaction and repeatedly purchase [11, 12]. Flavor is composed of taste and aroma, while aroma is conferred by volatile compounds emanating from cooked rice [13]. From cooked rice, a number of compounds have been identified, but only a few make up the characteristic aroma [9, 14, 15]. Based on the aroma, rice cultivars can be separated generally into aromatic and non-aromatic types: Aromatic rice has a relatively diverse range of unique aromas [13] such as jasmine rice, which is characterized as having buttery, corn, dairy, starchy, cooked grain and nutty attributes, and basmati rice, which is characterized as having hay like and earthy attributes [16]. A complex mixture of odor-active compounds comprises the aroma of both aromatic and nonaromatic rice; approximately 300 volatile compounds have been identified from various cultivars of aromatic and nonaromatic rice [17]; and several odor-active compounds in cooked aromatic rice have been determined using odor units [18, 19]. In aromatic rice, 2-acetyl 1-pyrroline (2-AP) is described as having a "popcorn-like" odor by American and "pandan-like" odor by Asian consumers which is synthesized in aerial parts of aromatic rice during growth, and in some nonaromatic types, it is present at a very low negligible concentration [20, 21]. 2-AP is not only the compound responsible for the unique aromas, but their aromas are due to qualitative and quantitative variations in a diverse cross section of odor-active compounds [13]. In cooked rice, lipid-derived odor-active compounds were formed during the degradation of oleic (octanal, heptanal, nonanal, (*E*)-2-nonenal, decanal and 2-heptanone are formed from oleic acid), linoleic (hexanal, pentanol, pentanal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal and 2-pentylfuran are formed from linoleic acid) and linolenic acid [22, 23]. The oxidation of lipid yields rancid odors and also induces various deteriorative reactions with proteins, amino acids and other components [24]. Examples of thermally derived flavor compounds formed in rice during cooking, which

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have seasoning-like and meaty-like aromas, are 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and bis (2-methyl-3-furyl)disulfide, respectively [19]. Thus, the volatile chemistry of rice grain could be due to a single predominant compound or a complex mixture of several compounds associated with a unique flavor and strength of aroma in the diverse set of fragrant rice.

Now, let us have a glance of Manipur, Manipur is a place of exquisite natural beauty and splendor. Manipur is a state of India laying on the northeastern corner of India bordering Myanmar (Burma). The state is rectangular in shape with a precious little valley in the center encircled by mountain ranges on all sides with salubrious climate [25]. The soil cover of Manipur can be divided into two broad types, viz. red ferruginous soil in hill area and alluvium in valley. Rice is the staple food for Manipuris. Agriculture mainly on rice and allied activities are the largest source of livelihood of majority of rural masses and backbone of the state's economy. Rice-based agriculture and allied services provided direct employment to about 70% of the total working population of the state. Rice crop is commonly grown during *kharif* season in Manipur. Rice is largely cultivated by small and marginal farmers. Manipur is endowed with several rice germplasms having special cultural values and unique characters, including colored, aromatic and quality rice landraces.

In this chapter, we reported the volatile oil components responsible for scent of two *Chakhao* cultivars (*Oryza sativa* cv. *Chakhao Poireiton* and *Oryza sativa* cv. *Chakhao Amubi*) of Manipur and reviewed our previous study on anthocyanin content and nutraceutical properties present in these two cultivars.

2. *Chakhao* rice of Manipur

Manipur has a large variety of indigenous rice germplasm which range their adaptation from low-lying lake areas to rainfed uplands of Manipur hills. There is a diverse set of locally adapted aromatic rice, ranging in color from white to red and purple, all of which are very glutinous in nature, also. They are commonly known as *Chakhao*. The literal meaning of *Chakhao* in Manipuri language is delicious rice (*Chak* means rice and *ahoaba* means delicious). *Chakhao* rice of Manipur are poor yielders which are found only in this state of India. Usually, they are grown during *Kharif* season, and they are low yielding, highly lodging, late maturing and also poorly studied as well. Some of the purple color *Chakhao* (black scented rice) cultivars are *Chakhao Poireiton*, *Chakhao Amubi*, *Wairi Chakhao*, *Khurkhul Chakhao*, *Pong Chakhao* and *Chakhao Sempak.* Red color *Chakhao* cultivars are *Chakhao Anganba* and *Langphou Chakhao,* and white color cultivars are *Chakhao Manam Nungshibi* and *Chakhao Angouba*.

The two great qualities of black scented rice of Manipur are their color and scent. These two great qualities make the *Chakhao* a unique specialty rice. They are deep purple in color and fraganced. *Chakhao* are used in community feast and ceremonial purposes as a delicacy, and they are also important in religious rituals. These are one of the high rated dishes served as desserts, flakes, bread, cakes, beverages, chapati and a special snack “Utong Chak” prepared within bamboo sticks [26]. They are sold in the local markets at a high rate. Manipuris *Chakhao* also have potential nutraceutical properties which is used by the traditional medical practitioners.

They have high potential for export to the international markets. However, as they are low yielding, the farmers of Manipur grow *Chakhao* very limitedly.

3. The two great qualities of black scented rice (*Chakhao* rice) of Manipur

3.1. Anthocyanin content and Nutraceutical properties of *Chakhao*

In our previous study [26], we had reported the anthocyanin and phenolics content and antioxidant activity of two black scented rice cultivars of Manipur, *Chakhao Poireiton* and *Chakhao Amubi.* Both cultivars have high anthocyanin, phenolics content and strong antioxidant activity. Using pH differential method, the total monomeric anthocyanin present in the two black scented rice cultivars (*Chakhao Poireiton* and *Chakhao Amubi*) and one non-scented white rice variety (CAUR1) was measured. Acidified methanol extract was used for the measurement, and total monomeric anthocyanin found in *Chakhao Poireiton* and *Chakhao Amubi* was 740 and 692 mg/kg, respectively, which was comparatively higher than non-scented white rice, CAUR1 (134 mg/kg). In our study, we have found out that anthocyanin content in both the *Chakhao* cultivars was higher than white rice variety, and our result is also consistent with the study on other colored rice. Colored rice varieties from Minahasa Regency, North Sulawesi, Indonesia, have higher anthocyanin content from the noncolored rice varieties [27]. Similarly, two colored rice of Thailand were reported to have a comparatively higher anthocyanin content than that of the Thailand non-pigmented rice variety [28]. Eight different pigmented varieties in Thailand were also studied and reported that rice varieties with dark purple color contained a higher amount of anthocyanin [29].

Total phenolics content was estimated for *Chakhao Poireiton* and *Chakhao Amubi* using Folin-Ciocalteu method from acidified methanol extracts, and the results were expressed as gallic acid equivalent. The total phenolics content in *Chakhao Poireiton* was 577 mg/100 g and in *Chakhao Amubi* was 500 mg/100 g of the powdered sample as gallic acid equivalent. The maximum DPPH-free radical-scavenging activity of *Chakhao Poireiton* and *Chakhao Amubi* extracts were 70.28 and 60.84%, respectively, and that of the standard ascorbic acid was 93.73% given by DPPH assay (**Figure 1**) [26]. Though scavenging activity is a little lower than the standard ascorbic acid, the antioxidant activity of *Chakhao Poireiton* and *Chakhao Amubi* extracts was strong enough, thus showing that higher phenolics content and antioxidant capacity are correlated with *Chakhao Poireiton* and *Chakhao Amubi*. Many researchers have reported about the phenolics content and antioxidant activity of other pigmented rice which are consistent with our study on *Chakhao Poireiton* and *Chakhao Amubi* of Manipur. One such study showed that anthocyanin extract of Korean black rice (*Heugjinjubyeo*) exhibited good free radical-scavenging activity [10]. Similarly, total phenol content using Folin-Ciocalteu method and antioxidant activities using thiocyanate method and DPPH-free radical-scavenging assay of different Thai rice white color, red color and black rice cultivars were conducted and reported that colored rice showed higher antioxidant activity from the white rice [30].

In our previous study, we had also reported the different types of anthocyanin present in Manipuris *Chakhao Poireiton* and *Chakhao Amubi*. Further, the acidified methanol extracts of

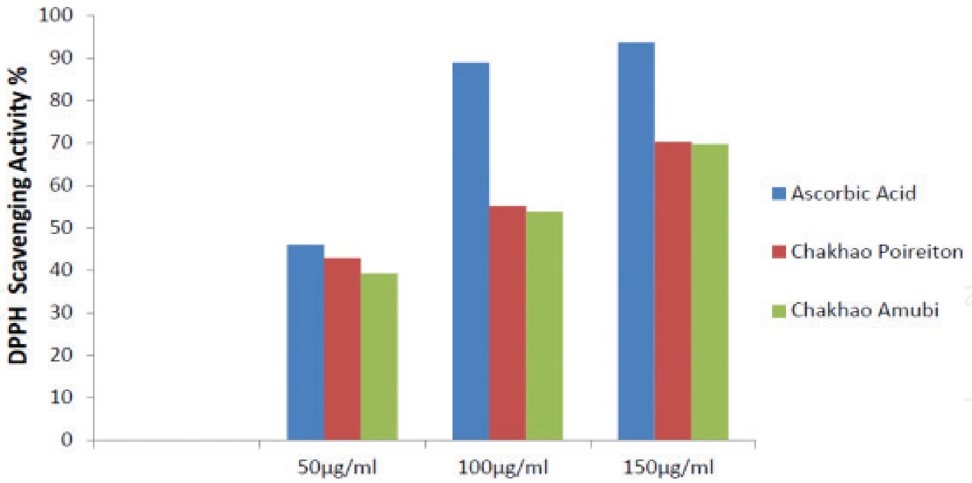

Figure 1. DPPH scavenging activity% of the methanol extracts of *Chakhao Poireiton* and *Chakhao Amubi*.

Chakhao Poireiton and *Chakhao amubi* have been used for HPLC analysis for the identification of anthocyanin components. HPLC analysis identified four main anthocyanins from *Chakhao Poireiton* and three main anthocyanins from *Chakhao Amubi*. The four main anthocyanins of *Chakhao Poireiton* were delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside and that of *Chakhao Amubi* were delphinidin 3-galactoside, delphinidin 3-arabinoside and cyanidin 3-galactoside [26]. Among the identified anthocyanin, delphinidin 3-galactoside was found to be the most predominant in both the cultivars. Similarly, several researchers had also reported about the different types of anthocyanin present in other black rice rather than the Manipuris black scented rice. For example, cyanidin 3-O glucoside and peonidin 3-O glucoside were identified from Korean black rice (*Heugjinjubyeo*) by HPLC analysis [10]. Other researchers also performed and reported the HPLC and LC-MS analyses on black rice extract and identified two major anthocyanins: cyanidin-3-glucoside and peonidin-3-glucoside [31]. From the black rice of Osaka, Japan, by using HPLC method, two anthocyanins have also been identified [32].

In the present scenario, there is an increased interest in the alternative sources of anthocyanin due to a rising demand for economical sources of natural and stable pigments [33]. Anthocyanins have shown to be potent antioxidants which are superior to well-known antioxidants such as butylated hydroxyanisole (BHA), alpha-tocopherol, 6-hydroxy-2,5,7,8-tetramethychromane-2-carboxylic acid (Trolox), catechin and quercetin [34]. Antioxidant capacity is becoming a parameter to characterize food or medicinal plants and their bioactive components. In the human body, dietary antioxidants protect against reactive oxygen species [35]. Anthocyanins may reduce the risk of cardiovascular diseases and cancer with anti-inflammatory, antioxidant and chemoprotective properties [10], and also, if the antioxidants intake is increased, there may have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases [36]. Anthocyanins have been incorporated into the human diet, and due to their diverse physiological abilities to treat conditions such as hypertension, pyrexia, liver disorders, dysentery and diarrhea, urinary problems and the common cold, anthocyanins have been used as traditional herbal medicines [37]. Not only this, the dietary antioxidants can help to fight reactive oxygen species and free radicals and help to decrease the risk of chronic diseases such as coronary heart disease and certain cancers [38]. Our results showed that the black scented rice cultivars (*Chakhao Poireiton* and *Chakhao Amubi*) have better antioxidant properties than noncolored rice varieties; thus, it can be suggested that colored rice varieties could be used as a natural antioxidant source.

3.2. Volatile oil components for scent in *Chakhao*

A complex mixture of volatile compounds comprised the aroma of *Chakhao* rice. GC-MS analysis of the *Chakhao Poireiton* identified 26 volatile compounds (**Table 1**; **Figure 2**). They are in the decreasing order of n-hexadecanoic acid (22.92%), octadec-9-enoic acid (11.66%), 4-beta-H-Pregna (8.78%), 9, 12-octadecadienoic acid (Z.Z) (8.11%), benzene methyl (5.61%), 2-furancarboxaldehyde 5-methyl (3.92%), g-hexadecenoic acid octadecyl ester, (Z) (3.88%), 9-hexadecenoic acid, eicosyl ester, (Z) (3.75%), 2-furancarboxaldehyde (3.41%), pentadecane (2.90%), I 7-pentatriacontene z (2.59%), stigmast 5-EN-3-OL. (3.beta.24S)-(2.00%), tetradecanoic acid (1.91%), stigmast-5-EN.3-OL, oleat (1.68%), 9-octadecenoic acid (Z), 9-octadecenyl ester, (Z) (1.67%), dodecane (1.59%), furan, 2,2'-methylenebis[5-methyl (1.59%), 9-hexadecenoic acid, 9-octadecenyl ester, (Z.Z) (1.58%), furan 2-(2-furanylmethyl)-5-methyl (1.51%), benzene butyl (1.44%), benzofuran 4, 7-dimethyl (1.20%), benzofuran, 2-methyl-3 (0.78%), undecane (0.71%), butanenitrile, 3-methyl (0.25%).

Peak	R. Time	Name	Area	Area%
1.	3.720	Butanenitrile 3-methyl	104,009	0.25
2.	4.540	Benzene methyl	2,295,838	5.61
3.	6.873	2-Furancarboxaldehyde	1,393,622	3.41
4.	11.768	2-Furancarboxaldehyde 5-methyl	1,603,643	3.92
5.	15.012	Benzene butyl	588,613	1.44
6.	16.549	Undecane	289,797	0.71
7.	16.765	Benzofuran 2-methyl-3	l8,639	0.78
8.	18.310	Benzene, pentyl	31,571	1.30
9.	18.943	Furan 2-(2-furanylmethyl)-5-methyl	619,648	1.51
10.	19.660	Dodecane	649,180	1.59
11.	20.133	Benzofuran 4, 7-dimethyl	491,294	1.20
12.	21.742	Furan, 2,2'-methylenebis[5-methyl]	649,017	1.59
13.	27.782	Pentadecane	1,188,621	2.90
14.	33.804	Tetradecanoic acid	781,382	1.91
15.	37.940	n-Hexadecanoic acid	9,379,913	22.92
16.	41.112	9, 12-Octadecadienoic acid (Z.Z)	3,3l7,895	8.11
17.	41.236	Octadec-9-enoic acid	4,771,521	11.66
18.	41.317	Oleic acid	l,336,655	3.27

Peak	R. Time	Name	Area	Area%
19.	48.343	g-Hexadecenoic acid octadecyl ester. (Z)	1,588,616	3.88
20.	48.818	9-Hexadecenoic acid, eicosyl ester, (Z)	1,535,517	3.75
21.	49.042	I 7-Pentatriacontene z	1,059,294	2.59
22.	51.890	9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)	684,629	1.67
23.	51.966	9-Hexadecenoic acid. 9-octadecenyl ester. (Z.Z)	647,022	1.58
24.	53.000	4-Beta-H-Pregna	3,591,642	8.78
25.	53.419	Stigmast-5-En.3-oL. Oleat	687,915	1.68
26.	56.337	Stigmast 5-En-3-oL. (3.Beta.24S)	818,755	2.00
			40,924,254	100.00

Table 1. Volatile oil profiling using GC-MS of *Chakhao Poireiton*.

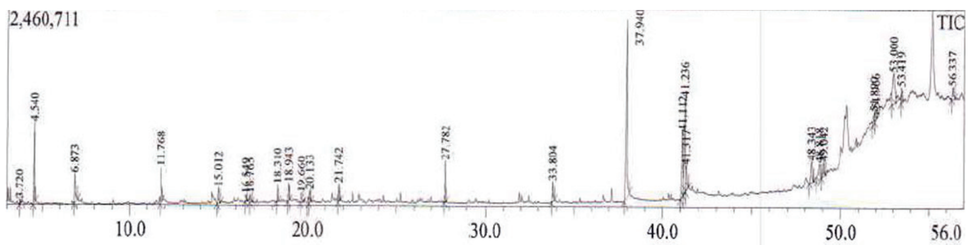

Figure 2. GC-MS chromatogram of *Chakhao Poireiton.*

GC-MS analysis of the *Chakhao Amubi* identified 11 volatile compounds (**Table 2**; **Figure 3**). The decreasing order of volatile compounds comprising the aroma of *Chakhao Amubi* analyzed by GC-MS is as follows: 17-pentatriacontene (40.60%), l3-octadecenal (Z) (12.03%), 9-hexadecenoic acid eicosyl ester-(Z) (11.98%), tetracosamethyl-cyclododecasiloxane (10.27%), Z-9-pentadecenol (9.79%), 9-octadecenoic acid (Z)-tetradecyl ester (0. 4.29%), toluene (3.38%), l-(+)-ascorbic acid 2,6-dihexadecanoate (2.21%), Z.Z-3-13-Octadecedien-I-o I (1.96%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) (1.93%), cyclononasiloxane octadecamethyl (1.56%). All the compounds together contributed to the unique aroma of their respective cultivars and their aroma increases during cooking, which may be due to the interaction of the compounds with the protein and lipids.

Among the complex mixture of volatile oil components, n-hexadecanoic acid and octadec-9-enoic acid were the most abundant compounds emanating the scent in *Chakhao Poireiton,* and in *Chakhao Amubi*, 17-pentatriacontene, l3-octadecenal (Z) and hexadecenoic acid eicosyl ester were the most abundant compounds. Each variety has a unique fragrance resulting from a number of volatile compounds which may vary from well-characterized popcorn-like aroma/2-AP-associated aroma although little is known about their relationships with aroma/flavor [39]. 2-AP has been found as one of the odor-active compounds which give aroma to the black rice [4]; however, in the present study of *Chakhao Poireiton* and *Chakhao Amubi*, no 2-AP has been detected. Similarly, 2-AP was not detected in black rice, instead reported 94 volatile compounds of which nonanal, butylated hydroxytoluene, 1-hexanol, naphthalene and 1-octen-3-ol were the main volatile compounds [28]. Earlier, concentration of 2-AP has been used as an indicator of aroma in the selection of aromatic lines due to its significant importance in aromatic rice; however, it does not hold good for all the aromatic rice. And also, the overall aroma during cooking may also be associated with dissociation of the starch-lipid complex and enhancement of the formation of a cross section of lipid-derived volatiles compounds.

Peak	R. Time	Name	Area	Area%
1.	4.550	Toluene	748,852	3.38
2.	37.880	l-(+)-ascorbic acid 2,6-dihexadecanoate	490,0l1	2.21
3.	48.618	Cyclononasiloxane octadecamethyl	346,471	1.56
4.	49.428	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)	427,185	1.93
5.	49.635	Z.Z-3-13-octadecedien-I-o I	434,892	1.96
6.	50.685	9-octadecenoic acid (Z)-tetradecyl ester	950,550	4.29
7.	50.866	l3-octadecenal, (Z)	2,667,664	12.03
8.	51.096	9-hexadecenoic acid. eicosyl ester (Z)	2,655,953	11.98
9.	51.241	Tetracosamethyl-cyclododecasiloxane	2,277,003	10.27
10.	51.682	Z-9-pentadecenol	2,169,969	9.79
11.	55.156	17-pentatriacontene	9,001,555	40.60
			22,170,105	100.00

Table 2. Volatile oil profiling of *Chakhao Amubi*.

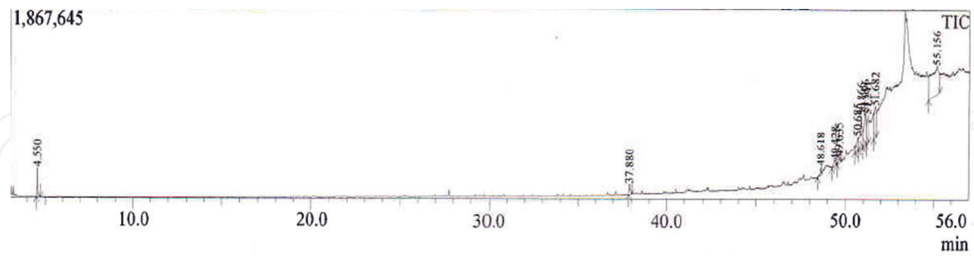

Figure 3. GC-MS chromatogram of *Chakhao Amubi.*

During the rice-breeding program, evaluation of rice is done in many ways according to consumer desires and references. Some of the traits are easy to assess, while some are very difficult. Flavor assessment is one among the difficult traits. Assessment of flavor requires time, cost and traditional sensory panels which are not accurate and also limit the number of progeny. Thus, the study of volatile oil components of different *Chakhao* rice would replace the sensory analysis and reduce time and cost as well. The potential of using the scent chemistry would make a rapid progeny selection at the very initial and accurate screening of a large number of progeny for premium new cultivars with the desired traits increasing consumer satisfactions.

4. Conclusion

Nowadays, people are seeing forward more to the supplementation of natural antioxidant in the diet; thus, the consumption of pigmented rice will be a great thinking for the improvement in human health. Rice varieties with higher anthocyanin pigment have stronger scavenging activity than white rice varieties, and thus, these pigmented rice varieties are reducing agents and possess strong radical-scavenging activity. *Chakhao* rice extracts could be a potential source of antioxidative phytochemicals and useful ingredient for nutraceuticals or functional food products rather than the toxic synthetic colorants. The supplementation of *Chakhao* in the diet would have a great impact on human health. More publicity on the relationship between antioxidants and disease risk mechanisms would increase consumption of the anthocyanin-containing rice. Establishment of antioxidant capacity in rice crop will result in the betterment of whole as rice crop constitutes the main food for populations in different countries.

The approach of identification of individual rice aroma would make possible the selection of multiple flavor types and the development of superior new cultivars for a wide cross section of flavors without using sensory tests. The separation and characterization of these compounds may be of potential use in rice-breeding programs focusing on flavor. A better understanding of the flavor (taste and aroma) of *Chakhao* will increase consumer preference and will help selection of better *Chakhao* for breeding with great grain quality. The anthocyanin and volatile oil extract from Manipuri *Chakhao* rice have great future potential which can be used in the industries as a natural food coloring and odor-active substances in beverages and also in pharmaceutical industries.

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