

## Review Article

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# Extraction, characterization, quantification, and application of volatile aromatic compounds from Asian rice cultivars

<https://doi.org/10.1515/revac-2021-0137>

received December 31, 2020; accepted May 30, 2021

**Abstract:** Rice is the main staple food after wheat for more than half of the world's population in Asia. Apart from carbohydrate source, rice is gaining significant interest in terms of functional foods owing to the presence of aromatic compounds that impart health benefits by lowering glycemic index and rich availability of dietary fibers. The demand for aromatic rice especially basmati rice is expanding in local and global markets as aroma is considered as the best quality and desirable trait among consumers. There are more than 500 volatile aromatic compounds (VACs) vouched for excellent aroma and flavor in cooked aromatic rice due to the presence of aromatic hydrocarbons, aldehydes, phenols, alcohols, ketones, and esters. The predominant VAC contributing to aroma is 2 acetyl-1-pyrroline, which is commonly found in aerial parts of the

crop and deposits during seed maturation. So far, literature has been focused on reporting about aromatic compounds in rice but its extraction, characterization, and quantification using analytical techniques are limited. Hence, in the present review, extraction, characterization, and application of aromatic compound have been elucidated. These VACs can give a new way to food processing and beverage industry as bioflavor and bioaroma compounds that enhance value addition of beverages, food, and fermented products such as gluten-free rice breads. Furthermore, owing to their nutritional values these VACs can be used in biofortification that ultimately addresses the food nutrition security.

**Keywords:** VACs, 2-AP, biofortification, *Oryza sativa*, nutraceuticals, bioflavor

## 1 Introduction

Rice is the main staple food commodity after wheat and maize in many parts of the Asian countries, which is more than 90% global rice consumption rate [1]. Being an important energy and nutrient source in the form of carbohydrate, rice is additionally known for its excellent sweet aroma. Among the various rice types, aromatic rice received wider popularity in Asia and maximum acceptance in the East Asia, European countries, and USA [2–4]. Aromatic rice cultivars have secured the prime position in Indian and global market [5]. Its characteristics such as pleasant, sweet aroma and superior grain quality (chemical and physical properties) are the reasons for consumer preference, higher market value, and export revenues [6–8]. The standard market price can improve the socioeconomic condition of farmers in developing countries such as India and Pakistan, which are involved in growing quality aromatic rice. There are a number of locally adapted small to medium size quality aromatic rice cultivars in Asian countries with mild to strong flavor and fragrance [9–12]

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due to the presence of volatile aromatic compounds (VACs). These VACs were synthesized by different biochemical and metabolic pathways [5,13,14].

In the literature, more than 500 aromatic compounds have been reported and identified till date [15] in Asian aromatic rice such as hydrocarbons, aldehydes, ketones, alcohols, heterocyclic compounds, phenols, esters, and other miscellaneous [5,16]. The principal compound for its sweet fragrance is 2-acetyl-1-pyrroline (2-AP) heterocyclic chemical compound [17–19], which has close association with aromatic rice particularly Basmati and Jasmine rice varieties from India and Thailand [20–23]. The 2-AP produces unique “nutty-popcorn” aroma [5] and has shown the maximum flavor dilution factor among cultivated Asian rice varieties after quantification [24,25]. Previous studies suggested that there is a difference in the occurrence and quantity of VACs contents concerning rice cultivars [12,26].

Different methods are available for extraction, characterization, and quantification of VACs. Traditionally purge and trap [26], steam distillation (SD) using the Likens and Nickerson apparatus, solvent extraction (SE) method, SD coupled with SE, and simultaneous distillation extraction (SDE) method [18,21,26–28] were used for the extraction of VACs from aromatic rice. Moreover, the main drawback of these methods is non-automate and time-consuming processes starting from sample preparation to final step for analysis as it depends on various organic solvents that affect the aroma quantity and quality. Liyanaarachchi et al. [12] identified different groups of VACs by performing SD and SE extraction protocol and suggested that SE is a more efficient method for the extraction of aromatic compounds because it requires relatively lesser sample size and time.

To overcome the issues faced by conventional methods, recently, analytical techniques such as solid-phase micro-extraction (SPME) with the advantage of gas chromatography (GC) coupled with mass spectrometry (GC-MS) [29], flame ionization detector (GC-FID), nitrogen phosphorus detection (GC-NPD) [30,31], olfactory (GC-O), pulsed flame photometric detector (GC-PFPD) [32], headspace solid-phase micro extraction (HS-SPME) coupled with GC-MS [33–35], and nuclear magnetic resonance (NMR) [36] were adopted for rapid and efficient identification, extraction, characterization, and quantification of VACs [24]. Turbo Matrix Headspace Trap coupled with GC-NPD and GC-MS analytical techniques is used for the quantification of VACs from aromatic rice with the main focus on 2-AP [27]. A simple extraction protocol for aromatic compound is the GC-MS approach [37]. Investigation on VACs had gained much attention of different groups of researchers [25,38,39], but very few studies have focused on extraction, characterization, quantification [15], and

application of aromatic compounds. In the present review, we briefly discuss the aforementioned analytical techniques for better understanding of aromatic compounds.

In the global market, prime role is played by aromatic rice such as basmati and brown rice. This aromatic rice has lesser glycemic index compared to white plain rice, which means slow release of energy that maintains the blood sugar level stable, thereby it can be utilized in diabetic diet [40]. The studies on aromatic rice such as Kalanamak (black rice) and brown rice show nutraceutical effects on human health in the form of richer source of dietary fibers, phenolics, antioxidant property, proteins, vitamins, and minerals such as Fe and Zn [41–43]. The ayurvedic medicinal property of aromatic red rice includes higher polyphenols, anthocyanins, and antioxidant property [44]. This aromatic rice can be further improved to develop biofortified rice rich in Fe and Zn to achieve food security and zero hunger [45]. The byproduct of rice can be processed by value addition in terms of rice cake and rice bread (gluten free) are preferred more by northern India, and fermented products such as idli and dosa are preferred more by southern India, and Khanom jeen a type of noodles has gained more attention in Korea [5].

The VACs can be preferentially used by beverage industry to add aroma in the beverages such as rice wine, which is preferred by the major part of China as traditional alcohol drink. In germinated brown rice (GBR), higher quantity of vitamins and gamma aminobutyric acid (GABA) is present [46]. This GABA helps in the reduction of hypertension as well as neurotransmitter inhibitor in the central nervous system [47]. So, the research can dig into more nutraceutical potential to develop GABA aromatic rice [48]. The consumption of GABA rice for a longer period of time can be useful in reduction of hypertension, preventing high blood pressure and cardiovascular diseases [49]. The medicinal and nutrigenomic importance of brown rice is due to the presence of phytochemicals, bioactive compounds, and antioxidants that function as anti-diabetic, anti-cholesterol, and cardio-vascular remission agents [40]. By looking into the importance of GBR in nutritional and health benefits, the quality of GBR has been improved using autoclave facility to maintain taste, aroma, grain texture, and physiological ingredients [50]. There are reports on genetic and molecular markers linked to 2-AP and other volatile compounds related to BADH2 gene. This information can be harnessed to develop biofortified rice. The truthful studies are needed to know the enzymatic biochemical synthesis pathway in the field condition (flowering and grain maturity) [51], post harvesting, and during storage to develop the better quality of biofortified aromatic rice [5,52,53] and utilization of these aromatic compounds to improve the value addition of product in food processing industry.

## 2 VACs in Asiatic rice cultivars and their aromatic properties

Conventionally, rice varieties are classified into two groups based on aroma as aromatic and non-aromatic [54]. Across the globe, rice consumers are known to have a strong affinity for aromatic varieties with huge demand in the current market for their specific aroma properties, besides their appearance and taste. Both aromatic and non-aromatic rice varieties possess various typical volatile compounds released from the grain, often termed as VACs [55] that impart aroma to the rice. Even though majority of VACs identified in both types of rice are similar, their relative proportion varies significantly between aromatic and non-aromatic cultivars [56]. Although constant efforts were made to investigate the key compounds responsible for aroma in rice, neither a single compound nor a group of compounds were identified so far, to be responsible for the complete rice aroma.

Aroma is a very complex sensation that could be described as typically pleasant smell arising from plant parts (such as leaves, stem, root, fruits, and grains) or cooking of plant's parts [31]. It is a mixture of volatile compounds with low molecular weight (<300) and high vapor pressure. These compounds can freely cross cellular membranes and can be released into the surrounding environment owing to their peculiar physical properties [57]. The complete group of volatile chemicals generated from plants comprises thousands of inorganic and organic compounds that originate from major pathways of secondary metabolic activities [58]. There are three significant pathways involved in the biosynthesis of main aroma components in plants that include shikimic acid pathway, degradation of lipids for the formation of short-chain alcohols and aldehydes, and terpenoid pathway [59]. These aroma molecules perform a wide range of activities in plants that could act as semio-chemicals (messengers for communication), pheromones; activation of defense responses [60]; and involved in plant–animal interactions [61,62] and plant reproduction [63].

With respect to rice, so far researchers identified more than 500 volatile compounds [15] to potentially contribute to the perception of rice aroma, depending on their concentrations and sensory thresholds. This delineates the fact that diversity in the chemical composition of different VACs could be responsible for imparting varietal differences in rice aroma. Nevertheless, among the identified huge number of volatile chemicals, only a small number have been reported to be responsible for the aroma in cooked rice [64]. Initially, 2AP was identified to

be the key constituent of aroma in cooked rice [17], which was supported by the findings of previous studies [65,66]. Although the compound is present in all types of rice cultivars, fragrant cultivars had this particular VAC in significantly higher concentrations [18]. Later many researchers reported that besides 2-AP other volatile compounds may also contribute to varietal differences in aroma [67–69]. They include a series of compounds such as aldehydes, alcohols, ketones, phenols, hydrocarbons, organic acids, pyrazines, pyridines, esters, and other compounds [70–73].

## 3 VACs in different variants of rice

### 3.1 Raw and cooked rice

Volatile compounds produced in different types of rice, viz., raw, cooked, scented, specialty types, and the key contributors of aroma, demonstrated tremendous variation between different research groups, due to differences in method of isolation and the type of rice analyzed. Raw rice is considered to have mild or weak aroma in comparison to cooked rice and major contributors for aroma in raw brown rice include hydrocarbons [67]. In raw rice, 73 compounds comprising alcohols, aldehydes, alkyl aromatics, furans, ketones, terpenes, and naphthalenes were identified [74]. Besides these, 2-AP had been found as the major aroma contributor of raw rice [75,76]. There were (>100) volatiles extracted from rice grain samples of aromatic and non-aromatic varieties using a dynamic HS-SPME system coupled to a two-dimensional gas chromatography (GC × GC) [77]. Few compounds such as 1,3-octadiene, 1-octen-3-yl acetate, isomenthol, estragole, and *trans*-anethole were noticed in rice samples for the first time and the study reported eight key volatile compounds, i.e., pentanal, hexanal, 2-pentyl-furan, 2,4-nonadienal, pyridine, 1-octen-3-ol, and (*E*)-2-octenal, to be accountable for the dissimilarity between aromatic and non-aromatic rice varieties.

Since aroma of cooked rice is influenced by multitude of factors, VACs of cooked rice necessarily differ from the VACs of raw or uncooked rice [78–80]. Raw rice preserved for more than 1 year often produce stale flavor due to the accumulation of VACs such as hexanal, aldehydes, and various alcohols [81–85], whereas during cooking it produces off-flavors due to the free fatty acid (FA) synthesis and oxidation of lipids generating several carbonyl compounds [81]. Aisaka [86] reported C346 carbonyls in stored rice to be tenfold higher than that of fresh rice. Besides this, it was reported that stored rice

after cooking generated only 45% of volatiles in comparison to cooked fresh rice that had 3–5-fold higher volatile compounds. Attempts made to regulate the stale flavor of stored rice through addition of amino acids L-lysine hydrochloride and L-cysteine during cooking could effectively inactivate the activity of carbonyl groups resulting in reduction of off-flavor [86,87].

Yajima et al. [127] identified 100 volatile compounds consisting of hydrocarbons and alcohols (13 each), aldehydes (16), ketones and acids (14 each), esters (8), pyrazines (6), phenols (5), pyridines (3) along with eight other compounds, and revealed 92 compounds among them to be responsible for flavor of cooked rice. Buttery et al. [26] identified nine compounds to be the key contributors for aroma in cooked rice viz. 2-AP, (*E,E*)-2,4-decadienal, (*E*)-2-decenal, (*E*)-2-nonenal, decanal, nonanal, octanal, 4-vinylguaiacol, and 4-vinylphenol based on odor threshold values. Besides these compounds a study reported aldehydes, phenols, nitrogen (N<sub>2</sub>), and sulfur (S)-based VACs to be major contributors for the flavor of brown rice during cooking [25]. Fukuda et al. [68] characterized rice varieties differing for amylose content and identified the functional relationship between amylose and volatile emission in rice. Glutinous varieties (without amylose) exhibited a distinct flavor due to high concentration of unsaturated aldehydes and these volatiles were termed as glutinous-rich volatiles [68]. However, among these glutinous volatiles, indole displayed negative correlation with amylose content. Other volatiles that differed in the cooked rice with varying amylose content comprised ketones, alcohols, heterocyclic volatiles, FAs, fatty esters, and phenolic volatiles. Volatile profile of GBR varieties belonging to different rice ecotypes viz., *indica* and *japonica* revealed significant differences not only in the relative abundance of VACs (aldehydes and alkanes) but also some of the volatiles were specific to the ecotypes. Among the identified 35 VACs, 2-pentyl-furan, hexanal, and pentanal were in higher proportion as compared to other volatile chemicals. In addition to this, the study reported slight differences between aroma profile of polished and brown rice [88]. These research findings highlight that aroma of cooked rice relies on pre- and post-harvest conditions that include drying, milling, and storage apart from genetic factors. Concurrent to this, it was reported from many studies that concentration of principal aromatic volatile compound 2-AP is also highly influenced by milling, wherein higher content is observed in cooked rice as compared to brown rice but the compound is reported to notably degrade during storage [75,88]. In the similar manner, Deng et al. [89] reported significant improvement in the

key aroma components (aldehydes, alcohols, and ketones) of cooked *Japonica* rice (Wuchang) and Jasmine rice (Complete Wheel) through high hydrostatic-pressure (HHP) processing and suggested HHP processing as an alternative for improving flavor of cooked rice.

In addition to this, it was reported that VACs released after cooking are different from those released in the field at flowering time implying the synthesis of VACs to be dependent on various developmental stages of plants [79,90]. Rice plant growth consists of different phases comprising vegetative, reproductive, grain filling, and maturity, wherein synthesis and availability of various metabolites including volatile compounds are highly variable. Hinge et al. [79] screened accumulation pattern of 14 volatiles including 2-AP at various developmental stages in scented and non-scented rice varieties and reported accumulation of maximum volatiles during seedling stage that decreased gradually during reproductive and maturity stages. Among the 14 volatiles identified, 10 were accumulated in high concentrations in scented as compared to non-scented varieties. The study also reported accumulation of 2-AP to be highest in mature grains followed by booting stage. This clearly epitomizes the involvement of multitude of factors in accumulation of various volatiles during synthesis, accumulation, and degradation processes [91,92].

Among the several VACs reported, 2-AP is considered as one of the more pronounced odorants in cooked aromatic rice [32,69,93], non-aromatic rice [25,69,80,94], and black rice [95]. 2-AP is known to have popcorn or butter-like odor [39] or pandan-like odor describing the plant that has enormous quantity of this compound [96]. This volatile has lowest odor thresholds and contain 1-pyrroline ring, wherein the hydrogen at position 2 is substituted by an acetyl group containing a methyl ketone group. The pyrroline ring makes the compound highly unstable and volatile [17,19]. Even though the chemical is found in some non-scented cultivars, its concentration is found to be negligible or below threshold level (0.0015 mg/kg) and cannot be perceived easily [18,21,97]. In aromatic rice genotypes, 2-AP can be detected in all plant parts except in the roots [39,94].

Other most commonly reported VACs of cooked rice apart from 2-AP include hexanal, octanal, indole, (*E*)-2-nonenal, 4-vinyl-2-methoxyphenol, and (*E,E*)-2,4-nonadienal. Among these, (*E*)-2-nonenal is considered to produce a fatty, cucumber, beany, tallow, and woody-like aroma [71,98]. (*E,E*)-2,4-decadienal possess a waxy-like and fatty aroma [6,76,99], while octanal has citrus-like flavor and (*E,E*)-2,4-nonadienal produces nutty, fatty flavor [95]. On the other side, higher concentration of hexanal is reported to generate rancid and oxidative off-flavor in cooked rice [28,85].

### 3.2 Scented rice

Rice cultivars that possess potential aroma compared to traditional cultivars are popularly known as aromatic rice with other names such as scented, pecan, and popcorn rice. Many studies conducted on profiling of VACs in several scented rice varieties summarized 2-AP to be a principal aroma constituent contributing to aroma [21,30,39,69,71,100–105]. Nevertheless, extensive studies conducted by Yajima *et al.* [106] and Mahatheeranont *et al.* [67] on aroma profiling of cooked scented varieties of rice revealed 114 and more than 140 compounds, respectively, to be responsible for the full rice aroma. Furthermore, profiling of aroma components of traditional rice (Koshihikari) and scented rice (Kaorimi) revealed higher concentrations of 1-hexanal, 1-hexanol, 4-vinylphenol, and lower amount of indole groups in the former than in the scented rice [106]. Supporting this, recently a study reported N-heterocyclic class of compounds (2-AP, 2-acetyl-1-pyrrole, and indole) as the major distinguishing volatiles between scented and non-scented rice varieties [79]. Similarly, Bryant and McClung [107] reported huge diversity in the volatile composition of aromatic and non-aromatic rice cultivars besides 2-AP, using SPME fibers in conjunction with gas chromatography/mass spectrometer (GC-MS). Nevertheless, Tsuzuki *et al.* [108] reported negligible qualitative variation in sulfur-containing volatile compounds between scented (Shiroi-kichi) and ordinary (Koganenishiki) rice varieties after cooking. Similarly, Grimm *et al.* [109] and Widjaja *et al.* [21] reported VACs such as (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, and (*E,E*)-2,4-decadienal from both fragrant and non-fragrant rice varieties of cooked glutinous or waxy rice. Supporting this, Yajima *et al.* [106] identified a compound – pyrrolidine for the first time, to be responsible for aroma of any type of cooked rice. Similarly, Sansenya *et al.* [110] documented aroma compounds to be more abundant in fragrant than in non-fragrant rice varieties. These studies support the fact that not all, but majority VACs noted so far from cooked aromatic and non-aromatic rice varieties are the same, except for their relative proportion.

### 3.3 Speciality rice

Red and black rice are speciality types of rice with reddish brown and dark purple pericarp, respectively, due to the accumulation of anthocyanin pigments. They possess high nutritional profile as compared to white rice and contain distinct aroma [111]. In a study conducted to

analyze the volatiles of red and black rice, 129 volatile chemicals were extracted from the bran through hydro-distillation. In red rice bran, compounds such as myristic acid, nonanal, (*E*)-beta-ocimene, and 6,10,14-trimethyl-2-pentadecanone were reported, whereas myristic acid, nonanal, caproic acid, pentadecanal, and pelargonic acid were identified as key compounds of black rice bran. Besides, guaiacol present in higher concentrations was found to be responsible for aroma characteristic of black rice. However, both the rice types reported traces or negligible amounts of 2-AP [112]. Yang *et al.* [91] identified 35 VACs, wherein higher concentrations of hexanal, 2-pentylfuran, nonanal, and 2-AP were reported in cooked white rice and black rice. Major volatiles contrasting between these rice types comprised 2-AP, *p*-xylene, indole, and guaiacol. Based on odor thresholds and olfactometry studies, it was reported that 2-AP (popcorn like) and guaiacol (smoky, black rice-like) were the principal odorants for imparting peculiar fragrance to black rice. In a detailed study conducted to analyze the chemistry of six distinct rice flavor groups including both scented (basmati, jasmine, two Korean japonica cultivars, black rice) and non-scented rice variety, 25 volatiles were reported to be major odorants based on odor intensity [95]. Further based on odor threshold and odor active values (OAVs), 13 volatiles viz., 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, 1-octen-3-ol, (*E*)-2-octenal, (*E,E*)-2,4-nonadienal, 2-heptanone, (*E,E*)-2,4-decadienal, decanal, and guaiacol were identified to play a decisive role for imparting aroma differences between different rice flavor groups. The effects of various degrees of milling on the volatile profile of raw and cooked black rice were studied using SPME and GC-MS. Among 101 volatile compounds reported, 44 were absent in raw rice, whereas 20 compounds were specific to cooked black rice, and products of FA oxidation were identified in both raw and cooked black rice. Furthermore, it was identified that partially milled black rice retained 80% guaiacol preserving the characteristic smoky flavor and highly preferred for consumption than completely milled black rice [113].

### 3.4 Types of aroma in rice

Rice aromas were classified into five distinct groups as green, fruity/floral, roasted, nutty, and bitter based on the chemical composition of different VACs [5]. It has been highlighted from many studies that no single chemical was ought to be completely responsible for aroma in cooked rice, rather a combination of various volatiles

in fixed proportions is essential for the generation of specific aroma. Thus, aldehydes, ketones, and certain alcohols were found to be accountable for green or woody-type aroma, whereas volatiles belonging to heptanone, ketone groups, and 6-methyl-5-hepten-2-one were essential for the generation of fruity and floral aroma. Similarly, nutty aroma is produced through benzaldehyde and 2-pentylfuran, while bitter aroma is due to the presence of benzaldehyde and pyridines [16].

### 3.5 Synthesis of aroma compounds in rice

As envisaged earlier although a large group of compounds have been recognized from different aromatic and non-aromatic varieties of rice, determining the relative role of each volatile compound responsible for the perception of aroma of rice is a difficult task and remains unfulfilled [95]. Many compounds in cooked rice have been estimated and quantified using odor units and aroma extract dilution analysis [25,26]. These compounds can be divided into distinct classes based on their origin as Maillard reaction products, lipid degradation products, and thermally induced products. These gateways of synthesis are often reported to play a critical role in the formation of either pleasant or unpleasant flavors in cooked and processed foods [114].

#### 3.5.1 Acetyl 1-pyrroline

Till date, 2-AP has been considered as the chief volatile responsible for rice aroma, and initially, L-proline was identified to be the precursor of 2-AP in rice [115]. But later contradictions regarding the origin of 2-AP have raised, and polyamine degradation was identified to be the prominent pathway for 2-AP synthesis in rice. In the polyamine degradation pathway, the polyamines are converted to  $\gamma$ -aminobutyraldehyde (GAB-ald) by obstructing the formation of GABA due to the inactive *badh2* enzyme (coded by *osbadh2*) leading to the accumulation of GAB-ald. Subsequently, the accumulated GAB-ald reacts with methylglyoxal in a non-enzymatic manner and produces 2-AP [116]. Since GABA and methylglyoxal are critical for stress tolerance and their biosynthesis is strictly restricted in scented rice cultivars, the accumulation of 2-AP often results in the sacrifice of stress tolerance [117,118]. Conversely, the polyamines are converted into GAB-ald, which instantly gets converted to GABA by the activity of functional *BADH2* enzyme, ultimately inhibiting 2-AP biosynthesis in

non-aromatic rice [119]. Alternate pathway for biosynthesis of 2-AP includes non-enzymatic reaction between methylglyoxal and P5C, an immediate precursor of proline [120]. The synthesis of 2-AP can take place both through enzymatic (gene dependent) and non-enzymatic (gene independent) pathways. However, enzymatic pathways of 2-AP synthesis also intricate with glycolysis and polyamine degradation, while non-enzymatic pathway produce 2-AP directly.

#### 3.5.2 Maillard reaction products

The Maillard reaction in food produces a wide range of sensory-active compounds (including color, taste, and aroma). It involves a chemical reaction between the primary amino group of an amino acid, peptide, or related compound with the carbonyl group of a reducing sugar. The resulting key aroma compounds although produced in very minute concentrations of 1  $\mu\text{g}/\text{kg}$  to 1  $\text{mg}/\text{kg}$  contribute significantly to the flavor because of their low odor-perception thresholds. The reaction tends to occur during storage, and the rate at which reaction progresses is highly temperature dependent. 2-Phenylethanol and phenylacetic acid are Strecker degradation products of the amino acid L-phenylalanine [121,122] that contribute a rose-like odor [21,25] in scented and non-scented rice. 2-Aminoacetophenone is a degradation product of tryptophan [123] considered to be responsible for producing naphthalene or floor polish odor in brown rice [124]. Strecker degradation is considered a corollary to the Maillard reaction that involves generation of aldehydes or ketones through oxidative decarboxylation of  $\alpha$ -amino acids by an oxidation reagent [125,126]. Besides these, a diverse range of products including formation of nitrogen-containing heterocyclic compounds and sulfur-containing heterocyclic compounds are also produced through this reaction [25,127,128].

#### 3.5.3 Lipid degradation products

Degradation of lipids can occur by both oxidation and thermal induction processes. During cooking, oxidation of unsaturated lipid acyl chains acts as a major channel for volatile production. Lipid oxidation products besides producing rancid odors are also involved in promoting various deteriorative reactions by reacting with amino acids, proteins, and other components [129]. In cooked rice, breakdown of principal unsaturated FAs, oleic, linoleic, and linolenic acids often yields volatile compounds [130]. Hexanal, pentanol, pentanal, (*E*)-2-octenal, (*E,E*)-2,4-decadienal, and

2-pentylfuran are formed from degradation of linoleic acid [95,131], whereas octanal, heptanal, nonanal, (*E*)-2-nonenal, decanal, and 2-heptanone are the breakdown products of oleic acid. Vanillin, another volatile compound produced through oxidation of lipids in cooked brown rice cultivars, produces a pleasant flavor and contributes to the aroma enhancing consumer preference [25,132]. In contrast, hexanal contributes to consumer rejection due to its rancid odor generated during cooking [28]. Lam and Proctor [14] reported significant accumulation of this compound in partially milled rice as compared to completely milled, fresh rice. Furthermore, the concentrations of volatiles such as (*E*)-2-nonenal (rancid), octanal (fatty), and hexanal (green) producing distinct off-flavors have been reported to significantly escalate with duration of storage.

#### 3.5.4 Thermally induced products

Although Maillard reaction is the major pathway for synthesis of majority of the thermally induced volatile compounds, some volatiles such as furanones with pleasant, sweet aroma cannot be synthesized through this chemical reaction [133]. Furanones such as 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and bis-(2-methyl-3-furyl)-disulfide, the products of thermally derived flavor compounds, are known to impart seasoning-like and meaty-like aroma, respectively, to cooked rice [25]. Besides furanones, other volatiles produced in cooked rice through a combination of thermal and enzymatic processes by decarboxylation of ferulic acid include 2-methoxy-4-vinylphenol, 4-vinylguaiaicol, and 4-vinylphenol that produce undesirable pharmaceutical odor [134]. Among these, 4-vinylguaiaicol is a guaiaicol derivative in rice that has an unpleasant, spicy, nutty, and clove-like odor [14,91,135], while guaiaicol was reported to be a unique odorant in black rice [95,111,112]. The off-flavor imparted by 4-vinylguaiaicol could be attributed to the undesirable changes in guaiaicol caused by the migratory loss of aroma-active compound and breakdown of volatiles due to lipid oxidation and thermal degradation processes [21].

## 4 Classification of general aromatic compounds present in Asian aromatic rice cultivars

The VACs of aromatic rice have been explored by many researchers using traditional and modern analytical

techniques [25,39,136,137]. It is not a single compound but a complex of more than 500 VACs [16] responsible for pleasant, sweet fragrance in aromatic rice, which is considered a desirable trait by consumers and consequently makes it more preferable than other rice cultivars. The aroma produced by most of the VACs have nutty popcorn-like flavor mainly due to the contribution of 2-AP at a larger concentration as compared to other volatile compounds [16,138]. Studies have been conducted to distinguish aromatic cultivars based on 2-AP concentration via biochemical and molecular test [139] but very limited research has been conducted on differential fragrance pattern of another volatile compound in different aromatic rice. Bryant *et al.* [71] identified the genetic variability in VACs in different aromatic and non-aromatic rice cultivars in freshly harvested rice, during storage and post-storage using SPME/GC-MS. They also suggested that there are some unique volatile compounds restricted to special aromatic cultivars only. The VACs were used as a key marker to distinguish the aromatic and non-aromatic rice cultivars by following HS-SPME coupled with GC × GC-TOFMS [73]. The differential classification of VACs in Asian aromatic rice cultivars was briefly reported by different group of researchers, which is explained in Table 1.

## 5 Extraction of aromatic compounds from Asian aromatic rice cultivars

Extraction of volatile compounds is a challenging task as compared to non-volatile compounds. Extraction of VACs is a prerequisite for their accurate quantification. There are many extraction methods available. Ideal method is one which can minimize the chemical modification of VACs during extraction procedure. A comprehensive review on extraction and quantification method for VACs is available [16]. These methods have been briefly described as follows:

1. Direct extraction: this procedure separates the compounds on the basis of their relative solubility either in liquid phase or solid phase. SE is the most widely used method for extracting compound of interest from plant products. The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; and (4) the extracted solutes are

Table 1: Classification of general aromatic compounds present in Asian aromatic rice cultivars

Sl. No.	Rice cultivars	Grain type	Cultivated area and cultivar type	VACs	Solvents used in traditional methods	Extraction and quantification methods	References
1.	Mushk Budji, Pusa Sugandh-3, Ambemohar/Gobindobhog, Basmati 370, Basmati-386, Amritsari Basmati, Haryana Basmati-1 (HB-1), Sabarmati, Improved Sabarmati, Joha, Tulaipanji, Sona Masuri, Kalanamak, Pusa Basmati-1 (PB-1), Dubraj, Sharbati, Bora saul, Samba/Gobindobhog, Vishnubhog, Vishnuparag, Royal, and Kamavatya	Small, medium, and long slender	India temperate and <i>Japonica</i> type	Alcohol, aldehyde, ketones, pyridines, heterocyclic organic compound, pyridine, and toluene	Fresh distilled diethyl ether	HS-SPME/GC-FID/GC-O	[10,17,39,70,92,96,136]
2.	Nang Thom Cho Dao, Tam Xoan, Nep Hoa Vang, Tam den, Tam tra, Lua Ngu, Thai Binh, Nam Dinh, Ha Tay, Hai Phong, Lam Thao, Nep Hoa Vang, Nep Rong, Nep Bac, Long An, Tam Canh, and Khao Dawk Mali 105	Medium bold to long slender type	Vietnam <i>Indica</i> and <i>Japonica</i> type cultivars	Aldehyde, ketones, alcohol, hydrocarbon, esters, phenols, acids, aromatic compounds, and nitrogen-containing compound	KOH, dichloromethane	SPME, GC-MS	[67,175–177]
3.	Paw hsan hmwe, Mee Done Taung, Mee Done Hmwe, and Nga kywe Taung Pyan	Medium	Myanmar, <i>Indica</i> and <i>Japonica</i> type cultivar	2-AP	KOH	GC-MS	[177,178]
4.	Riceberry, KDML 105, RD 6, RD 15 Hawm Supanburi, Hawm Klong Luang, Red Cargo rice, Jasmine, Golden Elephant, Khao jao 15, Khao Bpraa Jeen, and Khao jao Hom Nin	Small, medium, and long	Thailand, north, and north eastern parts of country/ <i>Indica</i> type	Aldehyde, ketone, toluene, esters, carboxylic acids, aromatic hydrocarbons, and furans	—	GC-FID, GC-O, HS-GC-MS	[110,134,179]
5.	Yamada Nishiki, Kabashiko, Jakou, Nioi Mochi, Kamari, Jakou Mochi, Towanishiki, sasanishiki, and Narukosan Koutou	Small	Japan/ <i>Indica</i> and Japanese type cultivars	Pheno, alcohol, aldehydes, and heterocyclic compound	—	SPME-GC/REMPI-TOFMS	[135,180–182]
6.	Basmati 370, Basmati Pak, Basmati 185, Basmati 385, Super Basmati, Basmati-2000, and Shaheen Basmati	Long slender	Pakistan/ <i>Indica</i> and <i>Japonica</i> type	Aldehyde, alcohols, acids, heterocyclic organic compound, ketones, 2-AP, and vanillin	Dichloromethane	HS-SPME, GC/O, GC-MS	[25,135,183]

(continued)

Table 1: (continued)

Sl. No.	Rice cultivars	Grain type	Cultivated area and cultivar type	VACs	Solvents used in traditional methods	Extraction and quantification methods	References
7.	Chingura, Sakarkhora, Radhuni Pagal, Kaljira, Kataribhog, Modhumala, Tulsi, Mohonbhog, Rajbhog, Badshahbhog, Kataribhog, and Khaskani	Small and medium	Bangladesh/ <i>Indica</i> and <i>Japonica</i> type	Aldehyde, alcohol, ketones, acids, heterocyclic organic compound, vanillin, and terpenoids	Dichloromethane	GC/O, GC-MS	[25,184]
8.	Tarom, Sadri, Hassan Saraie, Domsiah, Binam, Hassani, Salari, Mirza Anbar-boo, Domsiah, Mosa Tarom, Mehri, Firoz, Neda, and Nemat	Short, medium and long slender	Iran/ <i>Indica</i> and <i>Japonica</i> type	Toluene, aldehyde, aliphatic hydrocarbons, sulfur compound, carboxylic acids, and furans	—	Cold-fiber SPME-GC-TOF-MS	[55,185]
9.	Pandan wangi, Rojolele, Bengawan Tunggal, Sintanur, Batang Gadis, Pandanwangi, Kurik Kusut, Mentik wangi, Pare Pulu Mandoti, Situ Bagendit, Radah Putih, Mentik Susu, Sintanur, Batang Gadis, AnakDaro, Mandoti, and CichMerah	Medium	Indonesia/ <i>Indica</i> and <i>Japonica</i> type	Aldehyde, furan, pyrrolone, toluene, alcohol, acids, heterocyclic organic compound, ketone, and aromatic compounds	Dichloromethane and KOH	HS-SPME	[77,139,186]
10.	Suyunuo Wuxiangjing, Zhengxian 8 (ZX), Nanjing 38 (NJ), and Yannuo 12 (YN)	Long and medium grained	China/ <i>Indica</i> , <i>Japonica</i> type	Aldehyde, alcohol, heterocyclic organic compounds, ketones, phenol, acid, and esters	SD	SPME/GC-MS, GC-MS	[151,187]

- collected [140]. Selection of solvent is a crucial step, and it depends upon solubility of the desired compound in solvent.
2. Distillation: distillation utilizes boiling point differences for separation of compound which involves purification of compound from liquid mixture. Hydrodistillation and SD are commonly used methods for the extraction of volatile compounds.
  3. Simultaneous SD extraction: it is used for the extraction of aromatic/volatile compounds which merges vapor distillation and SE. This method is quick and gives concentrated substance even with small solvent volumes.
    - (a) SE: in this process, a compound is transferred from one solvent to another due to differences in solubility between these two solvents.
    - (b) Headspace (HS) extraction: in this type of extraction, a volatile material is extracted from a heavier raw sample. An HS sample is usually extracted utilizing a vial containing the sample, the solvent, a matrix modifier, and the HS. Volatile components from the complex sample can be extracted and isolated in the HS of a vial. Once the sample is transferred followed by sealing of vial, volatile compounds diffuse into the gas phase until the HS acquires an equilibrium state. The sample is then collected from top of the vial, i.e., an HS.
      - i. Dynamic HS extraction: it is comprised of purging the HS with a large volume of inert gas which eventually removes the volatile compounds. Purge and trap is a known method of dynamic HS extraction.
      - ii. Static HS extraction: it is typically used for the determination of volatile and semi-volatile analytes in liquid mixtures.
      - iii. Multiple HS extraction: it extracts a sample and calculates the amount of desired compound in relation to a known standard.
    - (c) SD continuous extraction: in this multilayer distillation system, a steam is directed through the raw plant material. The mixture of steam and volatile compounds is collected followed by condensation to produce a liquid having two separate layers of oil and water.
  4. Simultaneous SD and SE: simultaneous micro SD/SE is an efficient method of extracting semi-volatile, flavor, and fragrance compounds for subsequent separation by GC.
  5. SE followed by direct injection: this approach is based on a simple SE followed by direct injection in GC which may be coupled to tandem mass spectrometry (MS).
  6. Solid-phase extraction: in these techniques, compounds dissolved in a liquid mixture are separated from other compounds on the basis of their physical and chemical properties. SPME is a modern technique that consists in direct extraction of the analytes with the use of a small-diameter fused silica fiber coated with polymeric stationary phase. It is a solventless extraction procedure which is especially suitable for trace analysis. It is used for the analysis of volatile compounds due to low cost, simplicity, solvent-free extraction, and speed.
  7. HS-SPME: it combines the advantage of HS extraction and solid-phase extraction. It is a simple, rapid, solvent-free, and cost-effective extraction mode, which can be easily hyphenated with GC-MS for the analysis of volatile organic compounds (VOCs) [141].
  8. Supercritical fluid extraction (SFE): it is a separation and extraction process of volatile compound by the use of supercritical fluids as the solvent. This solvent is less toxic in comparison to the organic solvents. The carbon dioxide (CO<sub>2</sub>), alone or in modified form, is an extensively used extraction solvent.
- ## 6 Characterization of aromatic compounds from Asian aromatic rice cultivars
- Plants undergo diverse stages of growth and development, simultaneously secrete different levels of hormones, metabolites (primary and secondary), and various chemical compounds such as non-volatile organic compounds (nVOCs) and VOCs of lipophilic liquids having low molecular weight and high vapor pressure. Physical properties of VOC compounds permit easy movement across the cellular membranes as well as into the surrounding environment [57]. In the past, more than 1,700 VOCs have been characterized from angiosperm and gymnosperm families covering 90 different plants [142]. Biosynthesis of VOCs relies on the presence of carbon, nitrogen, and sulfur as well as reaction energy governed by primary metabolism.
- Broadly based on the biosynthetic origin, VACs are categorized into several chemical classes of alcohols, acids, esters, aldehydes, ketones, lactones, phenols, sulfides, furans, terpenoids, phenylpropanoids (benzenoids), FA derivatives, and amino acid derivatives [143]. The profiling of volatile compounds of rice has been reported by various researchers around the world, some of them have also reported that the corresponding aromatic compounds have pleasure odor through GC, mass spectroscopy (MS), and other advanced instrumentation techniques [16,25,76,143,144]. Consequently, around 500 different VACs have been reported

in rice around the world and among them 2-AP, 2-acetylpyrrole,  $\alpha$ -pyrrolidone, and pyridine have been reported for enhancing consumer acceptability of rice, while hexanal, acetic acid, and pentanoic acid gained from lipid oxidation are responsible for the reduced acceptability of rice [28,75,145]. 2-AP is discovered as the most significant flavoring constituent of cooked rice, and its presence cannot be ignored from detection in the large number of rice varieties [5,146]. Chemically, 2-AP holds N-heterocyclic ring of five-carbon in its structure, which is named as 1-(3,4-dihydro-2H-pyrrol-5-yl) ethenone according to IUPAC. A cyclic imine and a ketone with pyrroline are substituted in the structure of 2AP (Table 2). VOCs from the Indian Basmati rice comprised 13 hydrocarbons, 14 acids, 13 alcohols, 16 aldehydes, 14 ketones, 8 esters, and 5 phenols. Furthermore, 2-AP has been reported as a core aromatic compound, which is found in all aerial plant parts of Indian cultivar of Basmati rice [147,148]. Recently, Hinge *et al.* [79] have also reported that besides 2-AP several volatile organic compounds such as hexanal, nonanal, octanal, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, heptanal,

pentanal, (*E*)-2-octenal, 4-vinylphenol, 4-vinylguaiacol, 1-octen-3-ol, decanal, guaiacol, indole, and vanillin are also contributing in the aromaticity of Basmati rice cultivars.

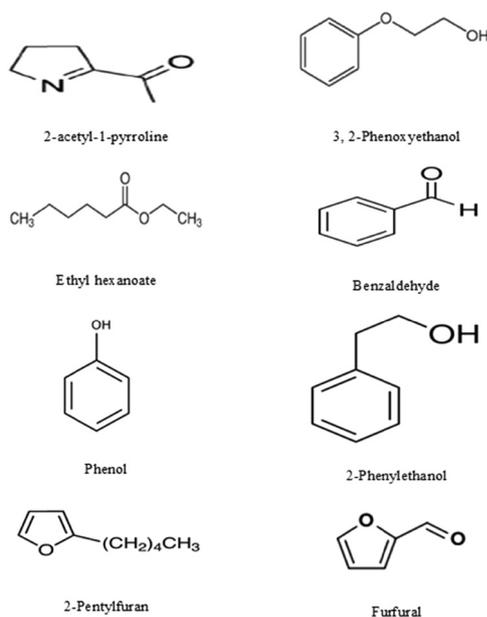
Comparative characterization of aroma volatiles was performed at vegetative and mature stages in Indian Basmati (Basmati-370, scented), non-Basmati rice (Ambemohar-157, scented), and IR-64 (non-scented) cultivars [149]. The researcher's group has reported the presence of 26 volatile compounds in three rice cultivars at vegetative and mature stages. The aromatic compound 2-AP was found to be the core contributor for aromaticity at vegetative and mature stages of AM-157 and BA-370 cultivars. Further with inclusion of 2-AP, 1-octanol, 1-octen-3-ol, (*E*)-3-octen-2-one, and aliphatic aldehydes octanal, (*E*)-2-nonenal, nonanal, heptanal, hexanal, decanal, and (*E*)-2-octenal were prime aromatic compounds present in matured seeds of scented rice cultivars, while during vegetative phase 1-octanol, (*E*)-3-octen-2-one, heptanal, octanal, nonanal, hexanal, 1-octen-3-ol, (*E*)-2-nonenal, (*E*)-2-octenal, phenylacetaldehyde, and pentanal aromatic compound were detected.

**Table 2:** Chemical characterization of VOCs of Asiatic aromatic rice

Sl. No.	Chemical group	VOCs	References
1.	Alcohols	Pentanol, ( <i>Z</i> )-3-hexen-1-ol, 1-hexanol, 1-octen-3-ol, 1-hexanol, 2-ethyl-hexanol, 1-octanol, linalool, 3, 4-dimethylcyclohexanol, 2 nonen-1-ol, carveol, 3,7-dimethyl-1-octanol, 2-hexyl-1-octanol, 2-hexadecanol, 2-methylpropanol, 2-pentanol, 1-butanol, 3-methylbutanol, 2-hexanol, 1-pentanol, and 1-octen-3-ol	[77,79,95,149]
2.	Esters	Ethyl hexanoate, ethyl heptanoate, ethyl octanoate, methylsalicylate, 5 acetic acid, 1,7,7-trimethyl-bicyclo(2,2,1)hept-2-yl, ester methyl 2-aminobenzoate, ethyl laurate, ethyl benzoate, and geranyl acetate	[25,149]
3.	Aldehyde	Benzaldehyde, 2 phenylacetaldehyde, vanillin, pentanal, hexanal, ( <i>E</i> )-2-hexenal, heptanal, ( <i>Z</i> )-2-heptenal octanal, ( <i>E,E</i> )-2,4-octadienal ( <i>E</i> )-2-octenal, nonanal, ( <i>E,Z</i> )-2,6-nonadienal, ( <i>E</i> )-2-onenal, oecanal, ( <i>E,E</i> )-2,4-nonadienal, $\beta$ -cyclocitral, (2,6,6-trimethyl-1-cyclohexen-1-yl) acetaldehyde, and ( <i>E,E</i> )-2,4-decadienal	[26,77,149]
4.	Phenols	Phenola, 2 2-methoxyphenol, 3 2-phenoxyethanol, and 2-methoxy-4-vinylphenol	[149]
5.	Ketones	2-Heptanone, 6-methyl-2-heptanone, 6-methyl-5-hepten-2-one, ( <i>E</i> )-3-octen-2-one, 2,2,6 trimethylcyclohexanone, 2-nonanone, ( <i>E</i> )-5-ethyl-6-methyl-3-hepten-2-one, 4-cyclopentylidene-2-butanone, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, 2 undecanone, 6,10-dimethyl-2-undecanone, and $\beta$ -ionone	[16,79,161]
6.	Terpenes	Pinene, camphene, 3-carene, $\alpha$ -limonene, azulene, $\beta$ -elemene, isolongifolene, longifolene, $\beta$ -caryophyllene, aromadendrene, and valencene	[79,149]
7.	Carboxylic acid	Benzoic acid, hexanoic, octanoic, nonanoic, decanoic, myristic, pentadecanoic, stearic, and tridecanoic	
8.	Aliphatic hydrocarbons	Nonane, 4-methyldecane, dodecane, tetradecane, pentadecane, heptadecane, nonadecane, allylcyclohexane, ( <i>E</i> )-5-methyl-4-decene, ( <i>Z</i> )-3-undecene hydrocarbon, ( <i>Z</i> )-3-dodecene, 7-tetradecene, 1-tetradecene, 1,1-diethoxy-2-methylpropane, 1,1-diethoxy-2-methylbutane, 1,1-diethoxy-3-methylbutane, 1,1-diethoxypropane, 1,1-diethoxyhexane, 1,1-diethoxynonane, and 1,1-diethoxy-2-phenylethane	[16,67,79,149]
9.	Aromatic hydrocarbons	<i>p</i> -Xylene, toluene, 1-isopropyl-2-methylbenzene, 1-isopropyl-4 methylbenzene, acetophenone, guaiacol, <i>p</i> -cresol, 4-vinylguaiacol, 4-vinylphenol, benzyl alcohol, 2-phenylethanol, and furfural	[79,149]
10.	Furans	1,2-Pentylfurana	[149]

The VACs presence in Indian Basmati rice and characterized VACs belong to chemical groups as alkane, alkene, ketone, aromatic hydrocarbon, terpenes, alcohols, aliphatic aldehydes, aromatic aldehydes, *N* heterocyclic, ester, phenol-containing compounds, carboxylic acid, and furan [148–151] (Figure 1). The characterization of VACs compound (2-AP) from 208 paddy varieties of Indian origin having aromatic property reported varying intensity of 2-AP ranging from 0.05 to 4.49 ppm [145]. The highest level of 2-AP in tested rice genotypes was 4.49 ppm in variety RD 1214 Dubraj. Some of the important aromatic compounds present in Asian paddy are presented in Table 2.

Black rice is the most preferable rice in Asian countries in Korea, as it is often blended with non-aromatic white rice prior to cooking for increasing aromaticity, flavor, color, and nutritional value. Thirty-five volatile compounds from Korean black rice (Geumjeong-ssal) among the different classes of chemicals responsible for aromaticity are broadly classified into aromatic, nitrogen-containing, alcohol, aldehyde, ketone, and terpenoid groups [95]. The black rice comparable to white rice has high degree of aromatic and nitrogen-containing compounds than the white non-aromatic rice. In black rice, 2-AP (9.7%) is the least abundant aromatic compound of total volatiles and hexanal (25.3%) is the most abundant with nonanal (14.8%) and 2-pentylfuran (10.4%) [91]. Around 140 volatile compounds of the Khao Dawk Mali 105 brown rice were extracted by capillary GC-MS [67].



**Figure 1:** Structural formula of aromatic compounds present in Asian paddy.

Among the extracted compounds, 70 volatiles were identified as aromatic compounds, including 2-AP, a key aroma compound of brown rice. The aromatic compounds are present in Asian brown rice of the three varieties Malagkit Sungsong (IMS), Basmati 370 (B 370), and Khaskhani (KK) [25]. A total of 41 aromatic compounds including 2-AP were identified and of them 11 are reported for the first time as rice-derived compounds such as 2-aminoacetophenone and bis-(2-methyl-3-furyl) disulfide are in prominently higher concentration. Nasi Pandan wangi from Indonesia and Indian Basmati rice hold the highest proportion of aromatic compounds viz. 2-AP; at the same time, hexanal and 2-pentylfuran were found to be the most prominent volatile compounds for Jasmine and Mentik Wangi [77]. The different levels of accumulation of volatile compounds in aromatic rice depend on the environmental factors [152].

## 7 Quantification of aromatic compounds from Asian aromatic rice cultivars

GC and MS have revolutionized the field of analytical measurements from complex mixture. GC is an analytical technique used for the separation of chemical components from sample mixture followed by detection and quantification. This is especially used for compounds that can be vaporized without decomposition. A vaporized sample is injected into GC column, and components will be resolved due to flow of inert gas utilized as medium. MS measures the mass-to-charge ratio of ions represented as mass spectrum. This technique can be applied to pure samples as well as complex mixtures. Combination of GC and MS for simultaneous separation and quantification of volatile compounds has been widely used to study rice aroma. Modifications of GC-MS are utilized for accurate quantification of volatile compounds from sample mixture. Some are narrated as follows.

1. GC-flame ionization detector (GC-FID): a flame ionization detector (FID) measures analytes in a gas stream. It is frequently coupled with GC for the detection of analytes.
2. GC-olfactometry (GC-O): here, compounds resolved from the GC are analyzed by both FID detector and human olfactory system separately. This combination makes it more appropriate technique for analysis of aromatic volatile compound such as 2-AP.
3. GC-NPD: nitrogen-phosphorus detectors (NPDs) for GC are specific to nitrogen- or phosphorus-containing

compounds. NPD is also known as a thermionic-specific detector which uses thermal energy to ionize an analyte.

4. Tandem GC-MS: GC followed by MS. Tandem MS uses two or more mass analyzers coupled together to increase resolution for analysis of chemical samples.
5. GC-time-of-flight-MS: the time-of-flight (TOF) analyzer uses an electric field to accelerate the ions and then measures the time they take to reach the detector. TOF MS deals with increase in time taken by analytes to travel during MS, which eventually leads to the higher resolution.
6. GC-MS with SIM: selected ion monitoring (SIM) allows the mass spectrometer to detect specific compounds with very high sensitivity. The instrument is pre-adjusted to measure the masses of selected compounds.
7. SHs-GC-FID and SHs-GC-NPD: static HS GC-FID and static HS GC-NPD. In case of static HS gas chromatography, the sample is placed in a sealed container and after an appropriate incubation time the gas in the HS of the container is sampled and analyzed by GC.
8. Capillary GC-MS: capillary GC is a high-resolution analytical method. A selective stationary phase and an efficient column preparation method are the two main factors for GC column separation with high resolution.

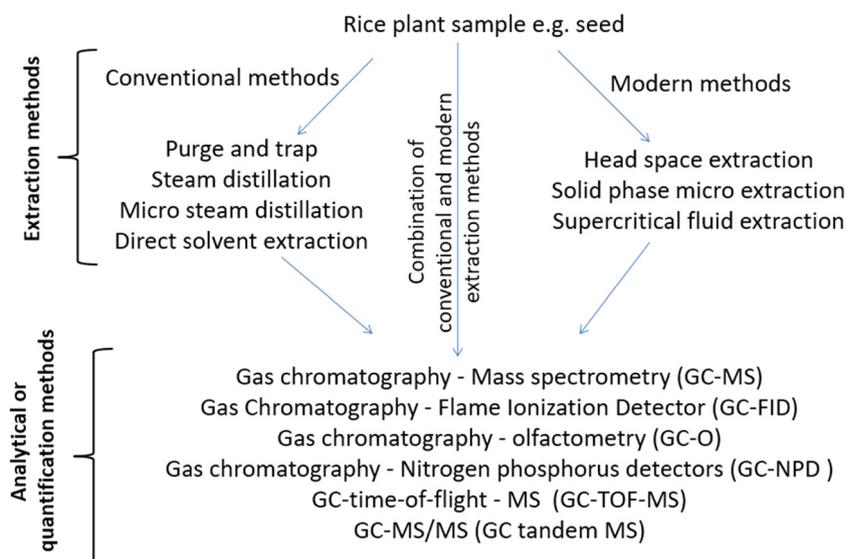
Purge and trap method, SDE, SE followed by direct injection, SPME, HS analysis, and SFE are most commonly utilized for extraction of 2-AP, while GC with nitrogen-phosphorus detector (NPD), flame ionization

detector (FID), MS, or olfactometry as a detector are most widely used techniques for quantification of 2-AP [31]. The extraction and quantification of VACs are briefly explained in Figure 2.

## 8 Applications

### 8.1 Nutraceuticals

Nutraceuticals can be defined as a part of food that allegedly provide benefit to human health by either decreasing or preventing the incidence of disease [153]. Specialty rice varieties with unique properties such as color, flavor, and aroma are documented to possess nutraceutical values and maintain huge market demand than the traditional white rice varieties. Pigmented along with aromatic rice varieties that are enriched with pleasant taste and odor are also associated with innumerable health benefits [154]. Brown rice is the dehusked whole grain rice containing bran and germ. Due to the presence of bran and germ, brown rice retains a significant amount of dietary fiber, vitamins, and minerals that are present in negligible or trace amounts in the milled white rice. It contains low calories and also serves as a good source of magnesium, phosphorus, selenium, manganese, and vitamins and majority of these minerals are reported to be deposited in the bran itself [155]. Manganese and selenium present in brown rice play an important role against



**Figure 2:** Methods for extraction and quantification of aroma in rice.

free radicals and act as anti-cancerous agents. Brown aromatic basmati rice contains 20% more fiber than other brown rice varieties, which prevents the formation of cancerous cells in the body [156]. Brown rice contains naturally occurring bran oil, which helps in reducing LDL forms of cholesterol [110].

Similarly, colored rice varieties (black and red) could be either semi-polished or unpolished. They inherit their color from anthocyanin pigments that are known to have antioxidant properties, free radical scavenging activity, along with other health benefits. Besides this, they possess other pigmented compounds such as cyanidin-3-*O*- $\beta$ -*D*-glucopyranoside in enormous quantity [157] that is associated with diverse functional properties including protection against cytotoxicity [158] and anti-neurodegenerative activity [100]. Red-colored varieties of rice are reported to be iron and zinc rich, whereas black rice varieties are high in protein, fat, and crude fiber. Reports suggest intake of black rice to be highly beneficial for the elimination of reactive oxygen species, lowering of cholesterol levels due to the presence of vitamin E, phytic acid, and  $\gamma$ -oryzanol [159,160]. In addition to this, colored rice varieties also possess phytochemical compounds in large amounts including flavonoids and wall-bound phenolics that are necessary for breaking digestive enzymes, promoting digestion [161]. Consumption of red rice rich in proanthocyanidins reduces the risk against type 2 diabetes [162] and anthocyanins present in black rice possess hypoglycemic effect [163]. In Asian countries, white rice is often mixed with black rice to enrich flavor, color, and nutritional content [95]. Asem et al. [164] analyzed the anthocyanin, phenolics, and antioxidant activity of two black scented rice cultivars Chakhao Poireiton and Chakhao Amubi and reported high amounts of all these nutraceutical compounds than white rice.

Although rice contains higher levels of complex carbohydrates and is considered as a food with high glycemic index, several traditional varieties have been identified to have a low glycemic index [165] and basmati rice is one among them [166]. Furthermore, it is widely known that phytate present in cereals severely interfere with dietary iron (Fe) and restrict its absorption into human body. Basmati rice is known to produce a metallothionein-like protein, rich in cystine that helps in iron absorption and the corresponding gene has been used in the biofortification programs for the development of Fe-rich rice varieties [167]. In addition to basmati, several traditional scented varieties of rice have been reported to possess higher levels of Fe and zinc (Zn) and could be used to develop micronutrient-rich cultivars through biofortification programs [167]. These VACs can give a new way for

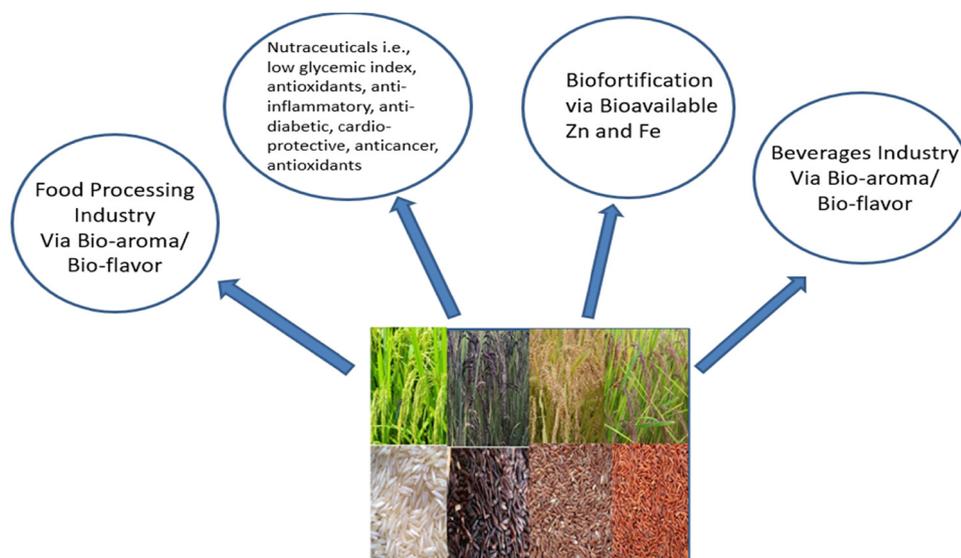
the development of biofortified aromatic rice cultivars, which can return higher exchange in global market.

## 8.2 Value addition to beverage industry

In the last decade, cereal-based beverages undoubtedly gained popularity with acknowledged beneficial effects on human health and predominant sensory traits. The health and functional properties of these beverages could be mainly attributed to the bioactive phytochemicals present in them that include phenolic compounds, carotenoids, tocopherols, dietary fibers, phytosterols,  $\gamma$ -oryzanol, and phytic acid [168]. Among various cereal-based beverages, roasted cereal grain tea is a caffeine-free beverage typically prepared by boiling the roasted whole grains or powder or by brewing in hot water. The aroma that arises from volatile compounds is a key feature of roasted cereal grain tea and a principal indicator of the tea quality. The major volatile compounds reported in these beverages comprise alcohols, alkanes, aldehydes, esters, and pyrazines. Of these, alcohols often contribute to sweet, floral, and fruity odors, with positive impact on the aroma of tea [169,170]. Among various cereal-based teas available, rice tea has been a conventional beverage for many people across the globe and prepared using different variants of rice viz., brown rice, white rice, and black rice. Few studies reported a total of 37 volatiles in white rice tea, pre-dominated with aldehydes, alkanes, and furans and 49 volatile compounds in the beverage prepared from black rice including alkanes, aldehydes, alkenes, and pyrazines [171,172]. Typically, rice wine is prepared from various kinds of rice or even rice brans among which, red rice wine made from unpolished aromatic red rice was reported to have premium quality, with a sour taste and wine-like fruit aroma [173]. Since, red rice is characterized by high nutritional value and immense health properties, the wine prepared from red rice through fermentation is documented to possess high biological value with essential sensory properties [174]. Thus, VACs have the potential to be used as bioflavor and bioaroma compounds to enhance value addition of beverages (Figure 3).

## 9 Conclusion and future perspective

Aromatic Asian rice cultivars are predominantly judged by superior and standard grain quality, aromaticity,



**Figure 3:** Application of different aromatic Asian rice cultivars.

cooking quality, and appearance, which make it worth of revenue generation in global market. The characteristic features of aromatic rice are due to the presence of number of VACs. These aromatic compounds were present in diverse germplasm of rice in Asian countries and classified into different chemical groups. There were several conventional methods used for extraction, characterization, and quantification of VACs, which were cost and time consuming. To overcome this, an advancement has been taken place to develop cost-effective analytical techniques. Till date, more than 500 VACs have been identified in aromatic rice cultivars and derived products. Owing to the presence of number of VACs, aromatic rice has significant nutraceutical values, which is gaining noteworthy interest due to the secretion of scented compounds and also show nutraceutical effects on human health in the form of richer source of dietary fibers, phenolics, antioxidant property, proteins, vitamins, and minerals such as Fe and Zn. Furthermore, these VACs can be specifically used by beverage industry as bio-aroma or bio-flavor to increase the value addition of beverages such as rice wine, rice tea, and other alcohol drinks. Adoption of these health-based crops could ensure nutritional and food security along with growth of food processing sector.

**Acknowledgments:** The authors show their gratitude to Director of the ICAR – Indian Institute of Seed Science, Mau, Uttar Pradesh for providing all the facilities and support required in this program.

**Funding information:** Authors state no funding involved.

**Author contributions:** Vinita Ramtekey: writing – original draft; Susmita Cherukuri: writing – review and editing, Kaushalkumar Gunvantray Modha and Ashutosh Kumar: writing – review; Udaya Bhaskar Kethineni, Govind Pal, Arvind Nath Singh, and Sanjay Kumar: writing – supervision, editing, and visualization.

**Conflict of interest:** Authors state no conflict of interest.

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