RICE FLAVOR CHEMISTRY

by

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ABSTRACT

Rice is the most important food crop consumed by man in the world. Because of its importance, rice breeders have been focused to develop rice quality. Rice flavor quality is a critical breeding objective in that the quality has a significant impact on consumer preference. Using a dynamic headspace system with Tenax trap, GC-MS, GC-olfactometry (GC-O), and multivariate analysis, the aroma chemistry of various rice flavor types (aromatic rices, colored rices, waxy rices) was analyzed and characterized. First, in black rice with a unique flavor, a total of 31 odor-active compounds were determined. Among them, 2-acetyl-1-pyrroline (2-AP), guaiacol, indole, and *p*-xylene largely influenced the difference the aroma in cooked black rice and white rice. The former two compounds were key aroma compounds responsible for the unique character of black rice due to lower odor threshold and unique odor description. Second, a total of 29 major odor-active compounds from six distinctly different rice flavor types (basmati, jasmine, two Korean japonica cultivars, black rice and a non-aromatic rice) when cooked, were determined based on their odor activity value. Among them, 13 odor-active compounds, including 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 could separate and characterize the individual rice flavor types. Third, premium-quality, black-pigmented, and waxy rice types were discriminated based on six odor-active compounds [(*E*)-2-hexenal, heptanal, (*E*)-2-octenal, guaiacol, (*E*)-2-nonenal, and decanal] using canonical discriminant analysis (CDA). Two discriminant equations obtained by CDA provided a predictive model for discriminating the three types of specialty rice. Fourth, volatile compounds emanating from Ilpumbyeo (traditional white rice), Heugjinjubyeo (black pigmented), and Jeogjinjubyeo (red pigmented) of cooked rice milled to different degrees (0, 6 and 8 % by weight) were compared to ascertain their site of origin. Removal of the bran qualitatively and quantitatively affected the volatile compounds formed with certain volatiles increasing, indicating the endosperm as their primary site of origin and a second group decreasing or eliminating, indicating the rice bran as the primary site of origin. These information from four experiments may be of potential use in rice breeding programs focusing on flavor quality.

INDEX WORDS: Flavor chemistry; Rice flavor; Odor-active compound; Volatile compounds; Specialty rice; Aromatic rice; Colored rice; Black pigmented rice; *Oryza sativa*; Pigment; Milling; Rice bran; Starch lipid; Dynamic headspace; Tenax trap; 2-Acetyl-1-pyrroline; Guaiacol; GC-O; Principal component analysis (PCA); Canonical discriminant analysis (CDA); Odor activity value (OAV)

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CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the leading food crop in the world. It is grown in at least 115 countries with an estimated production of 630 million tons in 2005 (FAO, 2007). Approximately 95% of the production is in Asia (e.g., Bangladesh, Burma, China, India, Indonesia, Japan, Korea, Thailand and Vietnam) and it is consumed by over one-half of the world's population. Rice is typically consumed as a staple and represents the principal source of carbohydrate and protein for human. It is also grown and consumed in America, Europe and Africa, where its popularity is progressively increasing (Bhattacharjee et al., 2002). In the United States (~1.5% of the world's production), it is grown primarily in Arkansas, California, Louisiana, Mississippi and Texas and is consumed widely, though seldom as a primary staple (Suwansri et al., 2002).

There are many types of rice grown around the world and they exhibit a diverse array of properties. Rice quality attributes include appearance, color, texture, chemical composition, flavor and degree of gelatinization with cooking. Rice quality, which encompasses cultural differences as well as physicochemical attributes, significantly affects consumer preference. Japanese consumers prefer rice with low amylose and protein contents, which gives a sticky cooked product (Isono et al., 1994). Indian consumers consider aroma, taste and grain length the most critical quality traits, while Asian consumers in the United States consider appearance, flavor and stickness the most important acceptance factors (Bergman et al., 2000; Suwansri et al., 2002).

Flavor, a primary sensory quality attribute for consumers, is composed of taste and odor (aroma). Taste is perceived by chemoreceptors within taste buds on the surface of tongue, while odor is assessed by chemoreceptors in the olfactory epithelium. Rice flavor studies to date have largely focused upon identification of the volatiles emanating from cooked rice and to a much lesser extent, the relative importance of individual compounds to the overall aroma. A number of volatile compounds have been detected and identified with GC-MS. For example, 64 volatile compounds were identified as emanating from a California long-grain rice cultivar (e.g., seven alcohols, fifteen aldehydes, nine ketones, four ester, eight acids, ten aromatics, ten nitrogen compounds, etc.) (Buttery et al., 1988). At present, a relatively small number of volatiles are thought to be critical components of the aroma (i.e., odor-active compounds); however, research to date has dealt with only a small number of lines and flavor-types. Superior flavor increases consumer satisfaction, overall acceptability and the probability of repeated purchase (Bergman et al., 2000), hence selection for flavor in rice breeding programs is critical.

Most countries cultivate rice from the *Oryza* genus, which has around twenty different species. *O. sativa* and *O. glaberrima* are the primary species and are grown in Asia and Africa, respectively. *O. sativa* has three important ecogeographic races: *japonica*, *indica* and *javanica.* Depending on differences in production conditions, ethnicity, and flavor preference, each country has different cultivar preferences. *Japonica* and *indica* have distinct differences in grain appearance, color, flavor and chemical composition. For example, *japonica* has medium to short grains (also called round grains) while *indica* grains are long, thin and flat. In addition, many *indica* cultivars are aromatic, whereas *japonica* cultivars, preferred in China, Japan and Korea, are typically non-aromatic (Chaudhary and Tan, 2001).

In rice breeding, primary selection criteria include yield, quality, and agronomic traits such as insect resistance and herbicide tolerance. A diverse cross-section of aromatic (scented) cultivars has been developed with distinct flavors that are preferred in some Southeast Asian countries. The leading aromatic rice cultivars are: Basmati 370 (India), Goolarah and YRF 7 (Australia), Hieri (Japan), Della (America) and Kha Dawk Mali 105 (or KDML 105), also known as "Jasmine rice" (Thailand) (Chaudhary, 2003). While some progress has been made in our understanding of rice flavor chemistry, flavor remains one of the most difficult traits to select in breeding programs. Current flavor selection procedures are highly subjective and not conducive to screening large number of lines.

Rice flavor selection decisions are currently made initially by chewing individual grains or sniffing the aroma of leaf tissue or grains after either heating in water or reacting with KOH or I_2-KI (Sood and Sidiq, 1978; Hien et al., 2006). The technique is not quantitative and only allows classifying progeny as scented, moderately scented and non-scented. Decisions are made primarily based on the amount of a single volatile compound [2-acetyl-1-pyrroline (2-AP)], even though a number of aroma compounds are known to be important. Likewise, sensory tests such as chewing or sniffing are highly subjective with individuals varying widely in their ability to determine the flavor. Even a well-trained assessor's ability declines with successive samples and with injuries incurred with chewing the grains. Sample size and time requirements can also limit the utility of sensory tests. Chemical methods using caustic substances, such as KOH or I_2-KI, can cause damage to the olfactory system after sniffing numerous samples. Therefore, current sensory and chemical tests are not adequate due to numerical, accuracy and other limitations.

The use of molecular markers associated with 2-AP can indicate the presence of the gene but do not indicate the level of expression (Garland et al., 2000; Cordeiro et al., 2002). Although molecular markers have certain advantages (e.g., small sample size and detection in the early stages of cultivar development), few markers have been identified for other flavor attributes. An ideal flavor selection method for breeding programs should be a rapid and accurate method that is amenable to screen large numbers of progeny. Accurate selection is dependent upon knowing the chemistry of preference of the targeted population of consumers.

An analytical method for flavor assessment facilitates selection of lines with superior flavor attributes. This allows selection of rice lines identified with preferred flavor attributes for a targeted population, using a principal component reference standard after characterization of the chemistry of preference of the population. Once superior cultivars/lines have been developed, sensory panels within the population make a final assessment. This method is rapid, accurate and conducive to screening large numbers of lines. Wang and Kays (2003) used a principal component reference standard as an efficient marker for selecting sweetpotatoes for flavor. The principal component reference standard was developed using multivariate analysis of the 17 most important aroma-active compounds and the relative sweetness of individual sugars multiplied times their respective concentrations. An analytical method for flavor assessment allows accurate classification of clones for flavor, the simultaneous selection of multiple flavor types, and the development of superior new cultivars for a wide cross-section of flavor without using sensory tests.

In rice breeding programs such a method would facilitate selection for flavor, increase the rate of flavor improvement, and allow selecting specific or unique flavor types.

The following research is designed to: 1) identify volatile compounds in a black rice cultivar and characterize the key odor-active compounds; 2) compare odor-active compounds in six distinctly different cooked rice flavor types; 3) characterize and discriminate specialty rices (premium-quality, black-pigmented, and waxy rice) based on odor-active compounds, and 4) investigate the origin of volatile compounds in rice grains.

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CHAPTER 2

LITERATURE REVIEW

Due to the importance of rice flavor, the identification and quantification of rice volatiles has been a major interest and a number of volatile compounds have been reported (Grosch and Schieberle, 1997; Maga, 1984; Tsugita, 1986). Several odor-active compounds contributing to the flavor of cooked rice have been identified (Buttery et al., 1988; Jezussek et al., 2002; Widjaja et al., 1996). The following review focuses on rice flavor chemistry, factors affecting flavor, and breeding for improved flavor.

A. RICE FLAVOR CHEMISTRY

1. Identification of Rice Flavor

The first steps in characterizing aroma are the identification and quantification of the volatile compounds emanating from cooked rice. A diverse cross-section of volatiles has been identified thus far (e.g., >320), however, there has been tremendous variation in the compounds identified between studies (Maga, 1984; Tsugita, 1986). The variation appears to be largely due to differences in method of isolation and the type of rice analyzed. This is especially so in studies using steam distillation for collection in that volatile compounds with relatively high molecular weights and low vapor pressures were also identified.

Several different analytical methods have been used for the isolation of rice aroma compounds: solvent extraction (Bergman et al., 2000), distillation methods [modified Likens-Nickerson simultaneous distillation-extraction (Widjaja et al., 1996), and steam distillation (Tava and Bocchi, 1999)], and headspace methods [solid-phase microextraction (SPME) (Grimm et al., 2001), and Tenax trapping (Buttery et al., 1988)]. Solvent extraction is one of the simplest methods, however, recoveries of volatile compounds were low and variable depending on the solvent used and method of concentrating. The method has another limitation in that it extracts lipids with the aroma constituents and requires their separation. Simultaneous distillation-extraction (SDE) is a rapid isolation method that gives good recoveries of volatiles with the exception of many polar and very water soluble compounds which are poorly absorbed. Heat-induced formation of artifacts and the decomposition of labile components are also drawbacks of this method (Reineccius, 2006).

Headspace methods are classified into static and dynamic (Kolb, 1999). The static headspace method with solid phase micro-extraction is widely used in environmental, petrochemical, botanical, forensic and clinical analyses due to its simplicity and speed. It does not require organic solvents for either sample preparation or cleanup, reducing the possibility of forming artifacts. Its major disadvantage is that it is largely limited to non-polar or semi-polar volatiles. In addition, the fiber is fragile and sensitive. The sensitivity of the fiber can result in contamination during manual injection, resulting in poor reproducibility. Tenax trapping is popular with the dynamic headspace method. It absorbs volatile compounds from the headspace that are subsequently desorbed thermally or using a solvent prior to analysis. Tenax is widely used due to its ability to adsorb and desorb a wide-range of organic volatiles. It traps just the volatile compounds, is easy to prepare, and is quite sensitive (i.e., ppb range).

2. Odor-Active Compounds Affecting Rice Flavor

Among the more than 320 volatile compounds identified in cooked rice, only a small number have been reported as important to the aroma (Table 2.1). These have been characterized using odor units, charm analysis or aroma extract dilution analysis (AEDA) using gas chromatography-olfactometry (GC-O) as reviewed by Acree (1997). An odor unit or odor activity value (OAV) is obtained by dividing the concentration of the individual compound by its odor threshold, the lowest detectable concentration. Compounds with high odor units contribute more to the aroma and are important in flavor differences among foods (Reineccius, 2006). However, flavor can not be explained by just odor units since the aroma is made up of a complex mixture of compounds. Charm analysis and aroma extract dilution analysis involve smelling a serial dilution of the aroma sample and recording the presence or absence of individual odorants (Blank, 1997). Charm analysis measures the dilution value over the entire time the compound elutes from the column while AEDA simply determines the maximum dilution value detected. With AEDA the data is expressed as a flavor dilution factor (FD factor) that is the ratio of concentration of the odorant in the initial extract to its concentration in the most diluted extract in which the odor was detected by GC-O. Such a dilution analysis is relatively simple and multiple assessments of each odorant assure relatively precise results. It does, however, require using a solvent to extract and dilute the sample and can be time-consuming since only one panelist can smell the sample at a time (Van Ruth, 2001). In addition to odor units and AEDA, two other GC-O methods are utilized: Osme and nasal impact frequency (NIF), also referred to as surface of nasal impact frequency (SNIF). The Osme method assesses the importance of an odorant to the overall aroma based on relative sensory intensity assessed by sniffing the non-diluted aroma isolate. Several panelists evaluate the odor intensity and duration of each odorant (Grosch, 2001).

The NIF method considers odorants detected by the greatest number of panelists the most important. This method, however, requires eight to ten untrained panelists (Reineccius, 2006).

Several odor-active compounds in cooked rice have been determined using odor units (Buttery et al., 1988) and AEDA (Jezussek et al., 2002) and can be divided into groups based upon their origin (e.g., Maillard reaction, lipid degradation, thermally induced products) (Table 2.1). In cooked and processed foods, these avenues of synthesis often play a significant role in the formation of either pleasant or unpleasant flavors (Whitfield, 1992).

a. 2-Acetyl-1-Pyrroline

2-Acetyl-1-pyrroline (2-AP), reported to be the most important aroma compound in scented rice, has a popcorn or butter-like odor (Buttery et al., 1983). Asians describe it as a "pandan-like" odor after the pandan plant that has significant quantities of the compound (Paule and Powers, 1989). In breads and certain cereal products, 2-AP is formed through the reaction of glucose and proline via the Maillard reaction. 1-Pyrroline and 2-oxopropanal were identified as important intermediates in the generation of 2-AP (Hofmann and Schieberle, 1998). However, 2-AP in scented rice is synthesized in its aerial parts from L-proline and L-ornithine enzymatically during growth and not during cooking (Yoshihashi et al., 2002). Its formation in plants varies with genetic factors and production conditions (e.g., location, cultivation method, harvest time, temperature) (Hien et al., 2006; Itani et al., 2004; Yoshihashi et al., 2004). To obtain higher concentrations of 2-AP in aromatic rice, it is recommended that the crop be grown in a cool climate with relatively low levels of nitrogen fertilization and harvested earlier than ordinary cultivars (Itani et al., 2004). Drought stress is also know to increase the concentration of 2-AP through an increased accumulation of proline (Yang and Kao, 1999). After harvest, there is a decline in concentration with increasing storage duration that is acerbated by higher temperatures (Yoshihashi et al., 2005).

Interest in 2-AP is due to its importance in the flavor of scented rice. Although it is found in some non-scented cultivars, its concentration is thought to be negligible (Buttery et al., 1986; Grosch and Schieberle, 1997). Several methods have been developed for the quantification of 2-AP which can be detected in as little as 0.3 and 0.5 g of uncooked scented rice with solvent extraction (Bergman et al., 2000) and indirect steam distillation (Mahatheeranont et al., 2001), respectively. Tanchotikul and others (1991) also developed a rapid method for quantification of 2-AP in 1 g samples that utilizes chromatographic separation, and selected ion monitoring mass spectrometry. Automated static headspace gas chromatography can also be used to quantify 2-AP rapidly with high recovery and sensitivity (Sriseadka et al., 2006). Stable isotope dilution allows correction for losses due to 2-AP's relatively highly volatility (i.e., a vapor pressure of 0.793Torr at 25°C) (Yoshihashi, 2002). 2-AP is extracted from the grain using a solvent that contains a stable isotope-labeled internal standard allowing quantification. Stable isotope dilution assay uses an isotopomer of the analyte as the internal standard. The flavor compounds to be quantified are synthesized and labeled with a stable isotope and are added to the sample before measurement. Knowing the initial concentration of the labeled compounds, flavor compounds in the sample can be identified and accurately quantified by GC-MS. This approach is especially important for liable or reactive compounds, however, it is time-consuming (e.g., synthesis of the isotope-labeled standards) and expensive (Werkhoff et al., 2002).

Synthesis of 2-AP in rice plants is thought to be controlled by a single recessive gene (*fgr*) on chromosome 8, the expression of which can be readily altered effecting the final concentration within the tissue (Cordeiro et al., 2002). Genes encoding betaine aldehyde dehydrogenase (BADH2), eukaryotic-type carbonic anhydrase, and 3-methylcrotonyl-CoAcarboxylase are linked *fgr* gene and are thought to be involved in the synthesis of 2-AP (Bradbury et al., 2005b; Chen et al., 2006).

b. Maillard Reaction Products

The Maillard reaction involves a chemical reaction between the carbonyl group of the open-chain form of a reducing sugar and the primary amino group of an amino acid, peptide, or similar compound. It occurs during storage, however, the rate of which is greatly facilitated by heat. The Maillard reaction contributes significantly to flavor formation in foods resulting in a diverse range of distinctive odors (BeMiller and Whistler, 1996). Several Maillard reaction products in cooked rice are thought to be important in the flavor (Table 2.1). 2-Phenylethanol and phenylacetic acid are Strecker degradation products of the amino acid L-phenylalanine (Etschmann et al., 2005; Hofmann and Schieberle, 2000) and 2-aminoacetophenone is a Strecker degradation product of tryptophan (Christoph et al., 1999). Strecker degradation is considered a part of the overall Maillrad reaction (BeMiller and Whistler, 1996). 2-Aminoacetophenone is thought to be an off-odor in brown rice in that it has a naphthalene or floor polish odor (Rapp et al., 1993). 2-Phenylethanol and phenylacetic acid in scented (i.e., Jasmine, Basmati, Goolarah and YRF9) and non-scented rice (Pelde) contribute a rose-like odor (Jezussek et al., 2002; Widjaja et al., 1996).

c. Volatile Lipid Degradation Products

Both oxidative and thermally induced degradation of lipids result in the formation of volatiles. For example, the oxidation of unsaturated lipid acyl chains is a major route for volatile synthesis during cooking. Lipid oxidation products can not only yield rancid odors but also induce various deteriorative reactions with proteins, amino acids and other components (Nawar, 1996). Cooked rice has odor-active compounds formed during the degradation of oleic, linoleic and linolenic acid, the principal unsaturated fatty acids present (Zhou et al., 2002a) (Table 2.1). Octanal, heptanal, nonanal, (*E*)-2-nonenal, decanal and 2-heptanone are formed from oleic acid, whereas hexanal, pentanol, pentanal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal and 2-pentylfuran are formed from linoleic acid (Monsoor and Proctor, 2004). Vanillin was found in cooked brown rice cultivars and contributed to the aroma (Jezussek et al., 2002). The presence of vanillin, formed via the β-oxidation pathway, was preferred by many consumers because of its positive impact on flavor quality (Schwab, 2000). In contrast, hexanal contributes to consumer rejection due to its rancid odor (Bergman et al., 2000). Hexanal formation is greater in partially milled than in fully milled rice (Lam and Proctor, 2003). During storage (*E*)-2-nonenal (rancid), octanal (fatty), and hexanal (green) significantly increase with their odors contributing to off-flavors formation with age (Lam and Proctor, 2003). Aldehydes such as octanal, nonanal, (*E*)-2-nonenal, decanal, (*E*)-2-decanal, and (*E*,*E*)-2,4-decadienal are common components of other foods even though they have low odor thresholds (Buttery et al., 1988).

d. Thermally Induced Volatiles

Although many thermally induced volatile compounds are formed via the Maillard reaction, some are not. Furanones, which have a pleasant sweet aroma, are important aroma compounds in many fruits (Sanz et al., 1997). The furanones are also detected in some thermally processed foods. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone and *bis*-(2-methyl-3-furyl)-disulfide, formed in cooked rice, are good examples of thermally derived flavor compounds that have seasoning-like and meaty-like aromas, respectively (Jezussek et al., 2002). During cooking sweetpotato also synthesizes a complex mixture of thermally induced volatile compounds that involve both enzymatic and thermal reactions (Kays and Wang, 2000). Thermal and enzymatic processes produce 2-methoxy-4-vinylphenol, 4-vinylguaiacol and 4-vinylphenol in cooked rice through the decarboxylation of ferulic acid conferring undesirable pharmaceutical odors (Coghe et al., 2004).

3. Volatile Chemistry Associated with Rice Products

a. Rice Cake

Rice cakes made of glutenous rice are popular in Korea, Japan, China and Vietnam due in part to their reduced caloric value and convenience and are often consumed with various flavorings. Kurasawa and others (1969) extracted hydrogen sulfide, ammonia and several carbonyl compounds from rice cakes. Of the carbonyl compounds, phenylhydrazones were quantitatively the most abundant followed by acetaldehyde, isobutyraldehyde, and propionaldehyde. Buttery and others (1999) subsequently identified 65 volatile compounds using high flow dynamic headspace isolation with Tenax trapping. 3-Methylbutanal, dimethyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, 4-vinylguaiacol, hexanal, (*E*,*E*)-2,4-decadienal, 2-methylbutanal, 2-acetyl-1-pyrroline, 1-octen-3-ol, and 1-octen-3-one were important contributors to the aroma based on their odor thresholds and concentrations.

b. Rice Bran

Over 170 volatile compounds have been identified in rice bran of which 4-vinylguaiacol and 4-vinylphenol contribute to its characteristic odor (Fujimaki et al., 1977; Tsugita et al., 1978). Lactones and 2-acetylthiazole derived from thermal reactions also play a critical role in rice bran aroma (Tsugita et al., 1978). Rice bran oil extracted from the pericarp, seed coat, and aleurone layer is popular in Japan, India, Korea, China, and Indonesia for cooking and salads due to its stability at elevated temperatures and nutritional components such as antioxidants and micronutrients (e.g., vitamin E complex, γ-oryzanol, phytosterols, polyphenols, squalene) (Ghosh, 2007). During deodorization of the bran oil, phytosterols and tocotrienols are removed due to their high volatility, giving it a milder flavor. Oryzanol, however, is not sufficiently volatile and is not removed during deodorization (Van Hoed et al., 2006).

c. Soong-neung

Soong-neung is made by boiling in water the roasted, slightly burned rice in the bottom of the rice cooker and served as a beverage after meals in Korea. Volatile compounds in soong-neung were mainly pyrazines and carbonyls of which 2-methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine, acetaldehyde, propanal, isobutyraldehyde and isovaleraldehyde are important. For example, 2,3-dimethylpyrazine and 2,5-dimethylpyrazine contribute nut-like and roasted cereal-like odors that are characteristic of soong-neung (Cheigh et al., 1975). The compounds were produced between 125 to 155 °C (Nam et al., 1973).

d. Rice Wine

Rice wine is made from uncooked fermented rice. Rice wine, made with various red cultivars, is produced by fermentation and has a fruity aroma and a characteristic sour taste (Teramoto et al., 1994). Alcohol and ester compounds in red rice are quantitatively increased in the presence of *Monascus* species, a group of filamentous fungi, during fermentation. 3-Methyl-1-butanol, ethanol, ethyl acetate, 2-methyl-1-propanol, ethyl butanoate, and 3-methylbutyl acetate are responsible for the overall flavor of the product (Chung et al., 2004). In Shaoxing yellow rice wine, ethyl acetate, methanol, *n*-propanol, isobutanol, β-phenyl ethanol and acetylene have been identified (Yin, 2006). Sake (Japanese rice wine) aged for a number of years is popular due to its unique aroma with “caramel”, “burnt”, and “heavy” notes and mellow taste. 3-

Hydroxy-4,5-dimethyl-2(5H)-furanone was reported to confer the burnt odor; 3-methylbutanal, methional, and dimethyltrisulfide also contributed to the aroma (Isogai et al., 2005; Takahashi et al., 1976).

B. FACTORS AFFECTING RICE FLAVOR

1. Chemical Composition

The primary chemical components of rice are lipids, starch and proteins, which are known to vary with production environment, soil, and cultivar. For example, the lipid content ranges from 1 % to 4 %, protein from 6 % to 8 % and starch from 60 % to 80 % among cultivars (Kennedy and Burlingame, 2003; Mano et al., 1999). Rice composition also varies with storage duration and degree of milling. The lipid content decreases significantly during storage due to lipase action, which increases the concentration of free fatty acids. Changes in starch and protein during storage tend to be minimal. Normal milling to produce white rice removes the pericarp, seed coat, testa, aleurone layer and embryo significantly reducing the concentrations of proteins and lipids which are concentrated in the sub-aleurone layer (Zhou et al., 2002a).

a. Lipid Derived Volatiles in Rice.

Rice lipids are generally classed as starch lipids which are associated with the starch granules and non-starch lipids, from other cellular components. Non-starch lipids are primarily located near the surface (i.e., bran); and as a consequence, are significantly reduced during milling. The free fatty acids in rice are mainly palmitic, stearic, oleic and linoleic acid (Zhou et al., 2002a).

Volatile formation from lipids results from lipolysis, lipid oxidation and decomposition. Lipase produces free fatty acids that may undergo oxidation. In addition to enzymatic action, heat during cooking also results in lipolysis. Hydroperoxides formed with lipid oxidation readily decompose yielding a variety of products with varying molecular weights and odor thresholds. Decomposition products include aldehydes, ketones, alcohols, furanones, acids, lactones and hydrocarbons, many of which impact flavor (Nawar, 1996). During storage, the activity of lipase and lipoxygenase increases, resulting in an enhanced production of the volatile compounds, particularly hexanal which contributes to off-flavor (Zhou et al., 2002b). The 2-alkanone is produced from saturated fatty acids in substantially larger quantities during thermal oxidation.

Lipids are also important to the flavor of foods because they increase the binding of lipophilic flavor compounds. Lipids, therefore, can moderate both flavor release and perception (Reineccius, 2006).

b. Protein Derived Volatiles in Rice.

The protein content of brown rice is between 6.6 to 7.3 %, however, after milling it declines to 6.2 – 6.9 %, indicating that a significant portion is interior to the aleurone layer of the grain. Much of rice protein is present in protein bodies that are stable even during heating. The primary protein in rice is glutenin, which is insoluble in water. Albumin and globulin are also present, are water soluble, and are prevalent in the bran (Zhou et al., 2002a). The concentration of volatile sulfur compounds (e.g., hydrogen sulfide, dimethyl sulfide) formed from protein is decreased by protein oxidation (Zhou et al, 2002b).

Volatile sulfur compounds are not major contributors to the aroma for several reasons. Carbonyl compounds formed by lipid oxidation can react with sulfhydryl groups on cysteine or methionine decreasing the formation of volatile sulfur compounds. Likewise, protein oxidation reduces the level of volatile sulfur compounds (e.g., hydrogen sulfide, methylmercaptan, dimethyl sulfide, dimethyl disulfide and sulfur dioxide) formed during cooking (Zhou, 2002b).

The Maillard reaction is the primary route for aroma formation in foods. A diverse range of products are formed such as nitrogen-containing heterocyclic compounds (pyrazine, methoxypyrazine, pyrrole, pyridine, pyrroline, pyrrolidine, pyrrolizine and piperine), oxygen-containing heterocyclic compounds (maltol, furaneol, cyclotene, oxazole and oxazoline), and sulfur-containing heterocyclic compounds (thiazole and thiophenes) (Reineccius, 2006).

Chemical interaction between flavor compounds and proteins involves reversible, weak, hydrophobic interactions, stronger ionic effects and irreversible covalent bonds. With flavor compounds that exhibit only hydrophobic interactions, binding increases with increasing carbon chain length. In addition, the extent of flavor binding depends upon the type and amount of protein (Reineccius, 2006).

c. Carbohydrate Derived Volatiles in Rice.

Rice is classified as waxy or non-waxy based on the amylose content of the grain. Waxy rice has very low levels of amylose (e.g., 0 to 5 %). Amylose content is a key indicator for predicting the behaviour of rice during cooking and processing in that it influences the texture, water absorption ability and hardness (Zhou, 2002a).

Waxy rice has a higher free fatty acid content than non-waxy rice. Therefore, the increased concentration of free fatty acids in waxy rice may lead to enhance the formation of volatile carbonyl compounds through lipid oxidation (Zhou et al., 2002a).

Polysaccharide may also influence flavor release through vapor pressure reduction due to chemical binding (e.g., ionic, hydrophobic, covalent, hydrogen bonding, and Van der Waals forces) or by influencing mass transfer rate due to enhanced resistance. For example, free amylose forms a helical structure that has hydrophobic areas that will contain certain aroma compounds (Reineccius, 2006).

2. Effect of Storage Conditions on Rice Flavor

Freshness is an important quality attribute for rice and aging leads to a number of physicochemical changes (e.g., pasting properties, chemical composition, texture, color and flavor). Postharvest changes in rice flavor can be partly controlled with storage conditions. Modified atmosphere storage conditions, for example, greatly help to maintain rice flavor through the reduction of oxidation. Optimum storage conditions are moisture content below 15 %, temperature below 15 °C, 70 % RH, and a gas atmosphere of 2-7 % O_2 and 3-5 % $CO_{2.}$

a. Storage Duration

A number of studies have reported that rancidity increases during storage concurrently with increasing levels of hexanal, a lipid oxidative product. In addition to hexanal, octanal (fatty) and 2-nonenal (rancid) also increase during storage and contribute to the decline in flavor quality (Lam and Proctor, 2003). Aging of jasmine rice results in a decreased concentration of 2-AP and increases the off-flavor compounds hexanal and 2-pentylfuran (Wongpornchai et al., 2004). As storage duration increases, the activities of lipase and lipoxygenase increases, leading to progressively increasing the amounts of hexanal (Suzuki et al., 1999).

b. Storage Temperature

Storage temperature affects change in both desirable and undesirable compounds. The concentration of 2-AP varies with temperature. 2-AP concentration in rice stored at 30 °C is dramatically decreased over that 5 and 25 °C due to its highly volatility and lipophilic properties. Higher extraction temperatures (e.g., 75 °C) result in lower recovery of 2-AP than at 40 °C (Yoshihashi et al., 2005). High storage temperature (35 °C) increases lipase and lipoxygenase activity and the concentration of hexanal, pentanal and pentanol (Suzuki et al., 1999).

c. Degree of Milling

White rice is produced through the abrasive removal of the brown surface layer from the individual grains. The pericarp includes the seed coat, testa, and aleurone layer. Milling reduces the concentration of lipids, protein, fiber, reducing and total sugars, ash and a number of minor components such as vitamins, and free amino and fatty acids. At the same time, milling improves the sensory quality of stored rice, in part through a reduction of oxidation products (Piggott et al., 1991; Zhou et al., 2002a; Zhou et al., 2002b). The reduction in hexanal levels between brown and milled rice indicates that the compound is mainly found in the bran. 2-AP concentration, in contrast, did not decline with milling indicating an endosperm origin (Bergman et al., 2000). Yoshihashi and others (2005) reported that the concentration in the bran was higher than that in milled rice. 2-AP in the bran is lost faster than from the endosperm where it is thought to be bound to starch, even after extended storage.

3. Genetic Factors

The chemical composition and characteristic aroma varies widely among cultivars. Waxy rice has lowerer levels of amylose (0-5 %) than non-waxy rice, altering cooking and processing requirements, and the edibility of the product (Chaudhary, 2003). Black and red pigmented rice have anthocyanin pigments (cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin-diglucoside, and cyanidin 3-rutinoside) (Abdel-aal et al., 2006), the presence of which impacts the flavor. Red rice contains up to 50 times higher tannin content than the common brown rice which increases its astringency (Goffman and Bergman, 2004). In addition, there is variation of 2-AP content among aromatic cultivars ranged from Makagkit (760 μg/kg), Goolarah (691), YRF 9 (670), Basmati 370 (610), IR 841-76-1 (560), Jasmine (156), to Della (76) (Grosch and Schieberle, 1997).

4. Environmental Factors

Production conditions such as temperature, location, harvest time and soil type influence the aroma of the harvested product (Rohilla et al., 2000). Day/night temperatures of 25/15 ºC during ripening resulted in better aroma of basmati rice (Bhattacharjee et al., 2002; Juliano, 1972) while early transplanting diminishes the aroma (Ali et al., 1991). 2-AP concentration in Khao Dawk Mali 105 differed due to production location ranging from 87 to 532 ppb (Yoshihashi et al., 2004). The 2-AP concentrations in Hieri, Miyakaori, and Sari Queen (Japanese aromatic cultivars) were higher in brown rice harvested early and ripened at a low temperature (Itani et al., 2004). The basmati and jasmine types had stronger aromas when harvested at the beginning of winter (Lorieux et al., 1996). High altitude and low soil moisture also increase 2-AP concentration in aromatic rice (Nakamura, 1998; Yang and Kao, 1999), the latter through a increased proline concentration, a substrate in 2-AP biosynthesis. For superior quality Itani and others (2004) recommended cultivation at a cool temperature and high altitude, the application of low levels of nitrogen, early harvesting, and drying at a low air temperature. Singh and Singh (1997) also indicated environmental factors affecting aroma formation in aromatic rice, such as cool weather during flowering and grain development; fertile soil, direct sowing, production on lighter soils and upland conditions, low soil moisture during grain filling, and manual dehulling.

C. BREEDING FOR IMPROVING RICE FLAVOR

Additional information on the inheritance of aroma in rice would facilitate the development of new cultivars with superior flavor. Dong and others (2001) and Mahalingam and Nadarajan (2005) crossed aromatic/non-aromatic and aromatic/aromatic cultivars. F_1 plants from the former were non-aromatic and had non-aromatic to aromatic segregation ratios in the F_2 population of 3:1 indicating that a single recessive gene controls “aroma” in aromatic rice. Aroma in this case refers to the presence of 2-AP. However, some lines displayed segregation ratios of 9:7 (non-aromatic to aromatic) indicating two recessive genes are involved in the aroma.

Several methods have been advocated for selecting rice with superior flavor (i.e., 2-AP concentration). These include a sensory test where the grains are chewed or the leaf tissue or grain is sniffed after reacting the tissue with solution of KOH or I_2-KI (Sood and Sidiq, 1978; Hien et al., 2006). The technique is convenient and relatively simple; however, it is highly subjective and not very quantitative. It only allows classifying progeny as scented, moderately scented and non-scented. Recently, molecular markers associated with 2-AP were utilized for making selection decisions in rice breeding programs. It allows determining the strength of the aroma in aromatic rice using only a small sample and detection in the early stages of cultivar development (Bradbury et al., 2005a; Chen et al., 2006; Jin et al., 2003). Several rapid methods for analytically measuring 2-AP concentration have been developed (Grimm et al., 2001; Sriseadka et al., 2006). The primary flaw in the proceeding methods is the assumption that 2-AP concentration is indicative of the overall aroma. Since 2-AP is either not present or found in very low concentrations in non-aromatic types, the methods are not applicable. To begin to address this deficiency, Laguerre and others (2007) analyzed the volatile compounds in aromatic rice using SPME and mass spectrometry and applied to multivariate analysis to discriminate aromatic rice. This allowed discriminating between aromatic and non-aromatic rice cultivars.

Rice aroma is far more complex than these methods can currently address. A better understanding of rice aroma chemistry with the identification of the critical odorants and determination of their contribution to the overall aroma is needed as well as an understanding of the variation in aroma among the diverse range of flavor types currently available. Hopefully a better understanding of rice aroma chemistry will eventually allow making rapid, accurate selection decisions using analytical techniques that are amenable to assessing large numbers of progeny. Such methods will also allow characterizing the chemistry of preference of targeted consumer populations, facilitating the development of superior new cultivars.

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Table 2.1. Odor-active compounds from cooked rice.

Volatile compounds	Mol. wt	Odor description
2- Acetyl-1-pyrroline [a,b,c]	111.14	Popcorn-like
Lipid degradation products		
Hexanal [a]	100.16	Green
Octanal [a]	128.21	Citrus-like
Nonanal [a]	142.24	Floral, fruity
Decanal [a]	156.27	Soapy
(*E*)-2-Nonenal [a]	140.22	Fatty, tallowy
(*E*,*E*)-2,4-Decadienal [a,c]	152.23	Fatty
4,5-Epoxy-(*E*)-dec-2-enal [c]	168.23	Metallic
2-Pentylfuran [b]	138.21	Beany
Vanillin [c]	152.15	Vanilla-like
Maillard reaction products		
2-Phenylethanol [b]	122.16	Rose-like
Phenylacetic acid [c]	136.15	Rose-like
2-Aminoacetophenone [c]	135.16	Naphthalene, floor polish
Thermally induced products		
3—Hydroxy-4,5-dimethyl-2(5H)-furanone [c]	128.13	Seasoning-like
Bis-(2-methyl-3-furyl)-disulfide [c]	226.32	Meaty
4-Vinylguaiacol [a,c]	150.17	Phenolic, medicinal, spicy
4-Vinylphenol [a]	120.15	Phenolic, medicinal

[a] Buttery et al. (1988), [b] Widjaja et al. (1996), [c] Jezussek et al. (2002).

CHAPTER 3

VOLATILE FLAVOR CHEMISTRY OF COOKED BLACK RICE USING DYNAMIC HEADSPACE, MULTIVARIATE ANALYSIS AND GC-O[1]

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[1] Yang, T.S., K.S. Lee, O.Y. Jeong, K.J. Kim, and S.J. Kays. To be submitted to *Journal of Agricultural and Food Chemistry.*

ABSTRACT

Black rice (*Oryza sativa* L.), an aromatic specialty rice popular in Asia, has a unique flavor, the volatile chemistry of which has not been reported. The objectives of this research were to study volatile profiles of cooked black rice and characterize the odor-active compounds. Fifty-eight volatile compounds were identified by GC-MS using a dynamic headspace system with Tenax trapping. Aromatics and aldehydes were quantitatively in the greatest abundance, accounting for 72.1 % of total relative concentration of volatiles. The concentration of 2-acetyl-1-pyrroline (2-AP) was high, exceeded only by hexanal, nonanal, and 2-pentylfuran. A total of 31 odor-active compounds determined by GC-O, was applied to principal component analysis (PCA) demonstrating significant differences between a black and a traditional white rice cultivar in terms of aroma, explaining 95.2 % of the total variation. 2-AP, guaiacol, indole, and *p*-xylene largely influenced the difference between the aroma in cooked black and white rice. 2-AP and guaiacol were major contributors to the unique character of black rice based on odor thresholds, relative concentrations, and olfactometry.

KEYWORDS: Flavor chemistry; odor-active compound; *Oryza sativa*; dynamic headspace; Tenax trap; 2-acetyl-1-pyrroline; guaiacol; GC-O; principal component analysis

INTRODUCTION

Quantitatively rice is the single most important food crop worldwide. It is consumed as a staple by over one-half of the world's population with approximately 95% of production being in Asia (*1*). Most countries cultivate rice from the *Oryza* genus, which has more than twenty different species. *O. sativa* L. and *O. glaberrima* Steudel are the most widely grown and are cultivated primarily in Asia and Africa, respectively. In addition to traditional white or common rice, a diverse cross-section of specialty rice types have been developed that have unique flavor, nutritional, textural, esthetic or other properties that often garner higher prices in the market place (*2*). While there have been a number of studies on rice flavor chemistry, many of the uniquely flavored specialty rice types such as black rice have not been characterized.

Black rice is intensely colored due to anthocyanins found in the surface cells of the grain. It has a number of nutritional advantages over common rice such as higher protein, total essential amino acids, vitamin B_1 (*3*) and minerals (Fe, Zn, Mn and P) (*4*), the latter of which varies with cultivar and production location (*5*).

Black rice is popular in Asian countries where it is often mixed with white rice prior to cooking to enhance the flavor, color and nutritional value. Interest in black rice is indicated by the number of accessions held in germplasm collections, e.g., China – 359, Sri Lanka – 50, Indonesia – 42, India – 30, the Philippines – 25 and Bangladesh – 24. Black rice is currently available in the United States and the European Union and it is anticipated that demand will progressively increase in the future (*2*).

Cyanidin 3-glucoside and peonidin 3-glucoside are the primary anthocyanins in black rice (*6*). They are thought to exhibit antioxidant properties beneficial to health (e.g., reduction of reactive oxygen species, anti-inflammatory properties, reduction of serum cholesterol) (*7-8*). In addition, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester and 4-carbomethoxy-6-hydroxy-2-quinolone in black rice displayed antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay (*9-10*).

Black rice has a relatively intense flavor that is distinctly different from other types of aromatic rice. Flavor is considered the single most critical quality trait in rice affecting consumer preference (*11*). Over 300 volatiles compounds have been identified from various cultivars of aromatic and non-aromatic rice (*12*). The volatiles identified vary with the degree of milling (*13*), isolation technique (e.g., Tenax trapping, simultaneous distillation-extraction, solvents, steam distillation, and solid phase microextraction) (*14-18*), cooking method (*14, 19*) and storage duration (*20*). Among the volatiles identified, there are a relatively small number of odor active compounds. For example, 2-acetyl-1-pyrroline (2-AP) has a very low odor threshold and is considered to be critical to the flavor of aromatic rice (*14*). While aromatic types assessed to date contain 2-AP, they have very different aromas indicating that other compounds contribute to their respective flavors. In that the aroma chemistry of black rice has yet to be characterized, the objectives were to identify volatile compounds in a cooked black rice cultivar and characterize the key odor active compounds versus a typical non-aromatic white rice cultivar.

MATERIALS AND METHODS

Materials. To investigate volatile compounds of cooked black rice, a dehulled black rice cultivar (Geomjeong-ssal Brand Premium Korean black rice, 1.84 kg package, Korean Farm Inc., Santa Fe Springs, CA) was purchased locally in GA. The black rice was not milled since the pigmentation is present in the surface cells. A milled common white rice (Jungilpum Brand Premium Korean non-aromatic rice, 9.07 kg, AOFW CO., Doraville, GA) was also purchased in a local supermarket to characterize the key odor active compounds that distinguish black rice from a typical non-aromatic white rice cultivar. Samples were sealed in glass and held at -20°C until analysis.

Chemicals. Analytical standards utilized were: benzaldehyde, decane, decanal, (*E*)-2-decenal, dodecane, guaiacol, heptanal, heptane, (*Z*)-linaloloxide, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, undecane (Sigma-Aldrich Inc., St. Louis, MO); (*E*,*E*)-2,4-decadienal, (*E*)-2-hexenal, 1-hexanol, *d*-limonene, 2-nonanone, 2-methylnaphthalene, hexanal, (*E*)-2-octenal, 1-octen-3-ol (Aldrich Chem. Co., Milwaukee, WI); octanal, *p*-xylene (Fluka Chem. Co., Milwaukee, WI); naphthalene, toluene (J. T. Baker Inc., Phillipsburg, NJ); 2-ethyl-1,4-dimethylbenzene, indole, (*E*)-2-nonenal, 2-pentylfuran, 4-vinylguaicol (TCI America, Portland, OR); 4-methyloctane (Pfaltz & Bauer Inc., Waterbury, CT); 3-octen-2-one (Alfa Aesar, Ward Hill, MA).

Sample Preparation. Since black rice is generally cooked with white rice, 5 ratios of black to white rice ranging from 0 to 100% were tested. Due to differences in water absorption between cultivars, the amount of water added to a given amount of rice (mL/g) was established for the same cooking time and good texture after cooking by preliminary tests and varied as indicated in **Table 3.1**.

Isolation of Volatiles Using Tenax Trap. **Figure 3.1** illustrates the isolation procedure using a dynamic headspace system with Tenax trap. Rice samples (60 g) were added to distilled water and cooked for 25 min at 100 ℃ in a specially constructed 1 L glass beaker with entry and exit ports. The entry and exit ports were wrapped with aluminum foil to minimize diffusive losses during cooking. Immediately after cooking the beaker containing the cooked rice was placed in a hot water bath (70 °C) and purified air was passed through the beaker (150 mL/min for 60 min) exiting into a Tenax trap where the volatiles were collected. The 10 cm long, 6 mm o.d., 4 mm i.d. stainless-steel sorbent trap (Scientific Instrument Services, Inc., Ringoes, NJ) held 150 mg 60/80 mesh Tenax-TA® (Alltech Assoc. Inc. Deerfield, IL) contained with silated glass wool. The Tenax trap was preconditioned at 280 °C for 2 hr with purified He at 20 mL/min. A 50 mL glass flask was placed between the exit port and the trap to collect any condensation. Using a vacuum sampling pump (Aircheck Sampler, Model 224-44XR, Eighty-Four, PA), the rice volatiles were swept from the exit port of the beaker into the Tenax trap using air purified by passing it through an activated charcoal filter (Alltech Assoc. Inc. Deerfield, IL) [Pyrex glass tube (10 cm × 1 cm i.d.) with 7 cm of 2.5 g of charcoal].

Short Path Thermal Desorption. The volatile samples were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min onto the gas chromatograph column (GC/MS, Model 6890N/5973, Agilent, Palo Alto, CA) using an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, NJ). Analytes were collected on the first 4 cm of the GC column using a CO_2 cooled cryofocus trap (-40 °C) (SIS 2” Cryo-Trap, Scientific Instrument Services, NJ). After desorption, the cryofocus trap was rapidly heated up to 200 °C and the GC separation program started.

GC/MS Analysis. Identification and quantification of the volatile compounds were performed using a GC/MS equipped with a 30 m length, 0.25 mm i.d., 0.25 µm film thickness, fused silica capillary column (HP-5MS, Agilent, Palo Alto, CA). Injection port temperature was 225 °C with a split ratio of 5:1. Helium was used as the carrier gas at a flow rate of 1.0 °C/min. The column temperature was held at 40 °C for 1 min and then programmed at 1.5 °C/min to 65 °C which was held for 1 min, increasing at 2 °C/min to 120 °C, held for 1 min, and increased at 15 °C/min to 280 °C and held for 5 min. MS conditions were: ion source 230 °C; electron energy 70 eV; multiplier voltage 1247 V; GC/MS interface zone 280 °C; and a scan range of 35 to 350 mass units.

Identification and Quantification of Volatile Compounds. The volatiles were identified based on comparison of their mass spectra and relative abundances with NIST 02 and Wiley 7 spectral libraries. The identity of a compound was confirmed by comparison of the Kovats’ retention index (RI) and mass spectra with authentic standards. Kovats’ RIs are determined using a non-polar HP-5MS column and a series of *n*-hydrocarbons (C_7-C_{15}) and compared with those reported previously in the literature and listed at http://webbook.nist.gov/. The levels of the volatile compounds were expressed as δ-carvone equivalents (assuming all of the response factors were 1). The concentrations are considered relative data since recovery after extraction and calibration factors related to the standard were not determined. The internal standard, δ-carvone, was introduced by placing 5 mL in a sealed 1 L Erlenmyer flask. After 24 hrs, 10 mL of air saturated with δ-carvone was removed and injected into the glass beaker containing cooked rice at the beginning of volatile collection. The concentration of the internal standard in the trapped sample was determined *via* direct GC injection of a range of concentrations in hexane. The results were expressed as the average of three replicates of five ratios of black to white rice.

Gas Chromatography-Olfactometry (GC-O). Black rice was cooked (100 g rice/100 mL distilled water) for 30 min and the volatiles trapped, desorbed and chromatographed as previously described. The desorbed odorants were assessed using an Olfactory Detector Outlet (ODO II, SGE Intl., Austin, TX) attached to the Agilent 6890N GC (30 m length, 0.25 mm i.d., 0.25 µm film thickness, fused silica capillary column). The temperature program was the same as described above. The split ratio was 0.5:1 and helium was used as the carrier gas at a flow rate of 2.0 mL/min. An aroma extract of the sample was characterized by describing the aroma of the individual components and assessing their intensity by 3 trained assessors. The 3 assessors were trained by describing 15 materials with different flavor: popcorn-like (popcorn), starchy (rice starch), woody (toothpicks), cooked grain (cream of wheat), corn (cream style corn), nutty (roasted peanut), floral (Jasmine scent), dairy (2% milk), hay (hay), barn (white pepper), buttery (butter), green (alfalfa), rancid (vegetable oil), waxy (candle) and earthy (mushroom) odors. The trained assessors were familiar with GC-O from preliminary tests and previous research. Odorants perceived by all 3 assessors were classified as odor active compounds. The respective odor intensities were scored from 1 to 5: 1 = very weak; 2 = weak; 3 = intermediate; 4 = strong; 5 = very strong.

Data Analysis. Analysis of variance and principal component analysis (PCA) (*21*) were carried out by using the SAS system for Windows v8 to assess differences in odor-active compounds of between black and white rice.

RESULTS AND DISCUSSION

Black Rice Flavor Chemistry. Fifty-eight volatile compounds emanating from cooked black rice, collected using a dynamic headspace system with a Tenax trap (**Figure 3.1**), were identified and quantified by GC-MS (**Table 3.2**). There were 23 aromatic, 10 hydrocarbon, 4 nitrogen-containing, 6 alcohol, 10 aldehyde, 3 ketone and 2 terpenoid compounds, the relative proportions of which are displayed in **Figure 3.2**. The relative proportion of the main classes of volatiles in 100 % black rice was significantly different from that in 100 % white rice. The relative proportion of aromatic, hydrocarbon and nitrogen-containing compounds in black rice was significantly greater than in white rice, while white rice had higher relative proportions of alcohols, aldehydes and ketones. Aldehydes (38.1 %) and aromatics (34.0 %) quantitatively represented the highest percentage of the total volatiles emanating from black rice. Aldehydes, identified in the decreasing order of their relative proportion, were hexanal, nonanal, octanal, heptanal, (*E*)-2-octenal, decanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, (*E*)-2-hexenal, and (*E*)-2-decenal. All aldehydes were detected through GC-O and were considered odor-active compounds in cooked black rice (**Table 3.3**). The aldehydes, products derived predominantly *via* lipid oxidation, had low odor thresholds and were considered to be important contributors to the overall aroma of black rice. Aromatics in black rice included 18 benzene, 2 phenol, 1 furan and 2 naphthalene derivatives. Among them, 2-pentylfuran and 4-vinylguaiacol were reported as odor-active compounds in California long-grain rice and brown rice cultivars, respectively (*14, 22*). 1-

Ethyl-2-methylbenzene, 1-ethyl-4-methylbenzene, 1,3,5-trimethylbenzene, 2-ethyl-1,4-dimethylbenzene, and 1,2-dimethoxybenzene found in black rice have been reported as volatile components of rice bran (*23*), derivatives of which (e.g., 1-ethyl-3-methylbenzene, 1,2,3-trimethylbenzene and 2-ethyl-1,3-dimethylbenzene) also emanated from black rice. Since black rice, unlike milled white rice, contains bran, the presence of such volatiles from this portion of the grain is not surprising. As rice is progressively milled to remove the bran, the chemical composition of the remaining portion of the grain changes. Therefore, the degree of milling can influence the volatile profile obtained (*13*). In black rice the surface bran layer produces several benzene derivatives that are not present in milled rice and appear to significantly contribute to the volatile profile obtained. While hydrocarbons (11.8 %), N containing compounds (8.3 %), aliphatic alcohols (5.4 %), aliphatic/alicyclic ketones (1.6 %) and terpenoids (0.8 %) were quantitatively substantially lower in abundance, several represented significant contributors to the aroma. For example, 2-acetyl-1-pyrroline (2-AP) is considered the single most important volatile in aromatic rice (*14*). In black rice it represented 7.9 % of total volatiles and had the fourth highest relative concentration, following hexanal (20.6 %), nonanal (12.1 %), and 2-pentylfuran (8.5 %); its concentration increased dramatically with increasing proportion of black rice in the mixture and was not present in 100 % white rice sample (**Figure 3.3A**). 2-AP has a very low odor threshold in water (0.1 ppb) (**Table 3.3**) and confers a popcorn-like odor (*23*) that is considered a critical component in the overall aroma of black rice. 2-AP is often reported as a key aroma compound in many food products (e.g., aromatic rice, bread flouer, green tea, popcorn) (*24-27*), so its presence in black rice is not surprising. Although hexanal, nonanal, and 2-pentylfuran were abundant in black rice, their relative concentrations were 5.3, 1.5, and 1.9 times lower than in white rice. They were not considered significant contributors to the unique character of the aroma of black rice in that their concentrations increased as the percentage of white rice increased (**Table 3.2**). The relative proportion of aldehydes in cooked white rice was 56.0 %, followed by alcohols (17.5 %), aromatics (14.8 %), ketones (8.7 %), hydrocarbons (1.6 %), terpenoids (1.2 %), and nitrogen-containing compound (0.3 %) (**Figure 3.2**). Hexanal (36.4 %) was the most abundant compound followed by benzaldehyde (6.9 %), nonanal (6.0%), and 2-pentylfuran (5.3%) (**Table 3.2**).

Odor-Active Compounds of Black Rice Using GC-O. Thirty-one compounds emanating from black rice were characterized as odor-active based upon GC-O (e.g., 12 aromatics, 10 aldehydes, 3 alcohols, 2 ketones, 3 nitrogen-containing compounds and 1 hydrocarbon) (**Table 3.3**). Of these, 16 compounds were of intermediate intensity (≥ 3) while 2-AP, (*E*)-2-nonenal, nonanal, hexanal and 3-octen-2-one were classified as strong (≥ 4) (**Table 3.3**).

The 12 detected aromatic compounds included 7 benzenes, 2 phenols, 1 furan and 2 naphthalenes. The benzene derivatives had low odor intensities except for *p*-xylene apparently due to their high odor thresholds (**Table 3.3**). The phenolic compounds, guaiacol and 4-vinylguaiacol, were greater than intermediate in intensity and had low odor thresholds. Guaiacol, in particular, was not detected in the 0 and 5 % black rice samples, but was found in the 20, 50 and 100 % samples (**Figure 3.3A**). Guaiacol was described as having a “smoky” or “black rice-like” aroma by assessors. Based on its characteristic odor and low odor threshold, it was a principal contributor to the unique aroma in cooked black rice. Guaiacol has previously been reported as a key aroma compound responsible for the smoked odor in smoked salmon (*28*).

Many of the aldehydes were found to be equal too or greater than intermediate in intensity with the exception of (*E*)-2-hexenal, decanal, and (*E*)-2-decenal. The aldehydes are thought to be mainly produced via lipid oxidation and decomposition. Lipid oxidation in rice occurs by 1) oxidative reaction of unsaturated fatty acids mediated via enzymatic, thermal or light reactions and 2) thermal mediated oxidative reactions of saturated fatty acids (*29*). Lipase and lipoxygenase activity increases with storage duration in rice, enhancing the production of lipid oxidative products (*30).* Several studies have reported increased rancidity during rice storage concurrent with elevated levels of hexanal (green tomato odor), octanal (citrus odor) and 2-nonenal (cucumber odor) (*31*). The concentrations of hexanal, octanal, and (*E*)-2-nonenal (18.9 %, 26.2 % and 16.1 %, respectively) in black rice were significantly lower than in white rice (**Figure 3.3B**).

2-AP (popcorn odor), indole (sour odor), and 2-methylpyridine (ash odor), are nitrogen-containing compounds believed to be derived from the bran (*24*). The protein content of black rice is higher than in brown rice (*3*) and may serve as a nitrogen source in the biosynthesis of these volatiles. 2-AP which was not present in the white rice sample, was readily detected when only 5 % black rice was present (**Figure 3.3A**) and contributed significantly to the unique aroma.

Several odor-active, lipid derived alcohol and ketone compounds, were found: 1-pentanol (plastic odor), 1-heptanol (green odor), 1-octen-3-ol (mushroom odor), 3-octen-2-one (rose odor) and 2-nonanone (fruity odor). The concentrations of 1-pentanol, 1-octen-3-ol, 3-octen-2-one, and 2-nonanone which were found to be greater than intermediate in intensity, were lower than in white rice (**Table 3.2**) and did not appear to be significant contributors to the distinctive different aroma of black rice.

Comparison of Odor-Active Compounds in Black and White Rice Using Multivariate Analysis. Since black rice is generally prepared as a mixture with white rice, the volatiles emanating from various ratios (**Table 3.1**) were compared. Principal component analysis (PCA) was used with odor-active compounds of cooked black rice to establish differences among the five rice samples and to determine which volatiles contributed most to the differences. **Figure 3.4** is a PCA biplot of the five ratios of black to white rice based upon the concentration of the odor-active compounds. The first principal component (PC 1) explained approximately 87.3 % of the total variation. As the percentage of black rice increased, the plotted position based on the PC 1, moved sequentially toward the 100 % black rice sample. The second principal component (PC 2) explained 7.9 % of the total variation indicating the difference between pure rice samples and mixed rice samples; collectively PC 1 and 2 accounted for over 95.2 % of the variation. The level of precision of the analytical method is indicated by the precise separation of the individual samples, and the distinct separation of samples containing only 5 % black rice (95 % white) from those of 100 % white (**Figure 3.4A**). The compounds with negative value on the PC 1 were in decreasing order of their contribution (**Figure 3.4B**): indole (29), guaiacol (21), *p*-xylene (7), 4-methyloctane (6), 2-ethyl-1,3-dimethylbenzene (20), 2-AP (10), 2-methylpyrimidine (4), 1,3,5-trimethylbenzene (14), toluene (1), *o*-xylene (8), 2-ethyl-1,4-dimethylbenzene (19), 4-vinylguaiacol (30), and naphthalene (25) (numbers correspond to **Table 3.2**). They indicate the main odor-active compounds are derived exclusively or predominately from black rice. Indole, guaiacol, *p*-xylene, and 2-AP were greater than intermediate in intensity. In contrast, the compounds with a positive value on the PC 1 were in decreasing order of their contribution, (*E*)-2-decenal (27), 1-heptanol (12), heptanal (9), 1-octen-3-ol (13), 2-nonanone (22), octanal (16), benzaldehyde (11), (*E*)-2-hexenal (5), 1-pentanol (2), 2-pentylfuran (15), and hexanal (3). They are indicative of a white rice origin. Therefore, the main odor-active compounds with negative and positive values characterized the different aromas between cooked black rice and white rice.

2-AP, guaiacol, indole, and *p*-xylene are responsible for the unique aroma in cooked black rice (**Figure 3.4B**).

A broad cross-section of volatiles, of which aromatics and aldehydes predominate, emanate from cooked black rice. Of these, 31 were considered odor active; 22 were also found in white rice, however, 9 were unique to black rice (2-AP, guaiacol, toluene, *o*-xylene, 2-ethyl-1,4-dimethylbenzene, 2-ethyl-1,3-dimethylbenzene, 4-vinylguaiacol, 4-methyloctane, and 2-methylpyridine). Critical odorants were 2-AP and guaiacol which appear to be major contributors to the characteristic aroma of black rice due to their high intensity and the unique description, respectively. The uniqueness of the aroma and the level of precision of the analytical technique described allowed detecting even a small number of black rice grains in white rice.

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Table 3.1. The Ratio of Black to White Rice, the Amount of Water and Cooking Time for Each Sample

ratio of black to white rice	black rice (g)	white rice (g)	amount of water (mL)	cooking time (min)
1:0	60.0	0.0	60.0	25
1:1	30.0	30.0	75.0	25
1:4	12.0	48.0	84.0	25
1:19	3.0	57.0	88.5	25
0:1	0.0	60.0	90.0	25

Table 3.2. Concentration of Volatiles Identified in Various Ratios of Cooked Black and White Rice[a]

RI[b]	compound	concentration (ng/100g)					method of identification[c]
		black100%	black 50%	black 20%	black 5%	white100%	
	aromatics						
760	toluene	47.7 ± 4.7	3.6 ± 0.9	ND[d]	ND	ND	MS, RI, STD
851	ethylbenzene	37.8 ± 0.5	28.8 ± 2.2	22.3 ± 1.8	15.4 ± 1.4	16.5 ± 1.5	MS, RI
859	*p*-xylene	60.3 ± 0.5	45.2 ± 3.3	30.1 ± 0.9	18.4 ± 0.3	23.5 ± 0.3	MS, RI, STD
886	styrene	5.1 ± 0.1	5.8 ± 0.5	4.9 ± 0.0	5.9 ± 0.2	7.5 ± 0.1	MS, RI
887	*o*-xylene	66.3 ± 2.4	ND	ND	ND	ND	MS, RI
952	benzaldehyde	64.1 ± 3.8	135.5 ± 5.8	299.8 ± 10.3	355.3 ± 15.3	442.3 ± 8.6	MS, RI, STD
958	1-ethyl-3-methylbenzene	14.0 ± 0.1	7.2 ± 0.6	5.3 ± 0.4	ND	ND	MS, RI
968	1-ethyl-4-methylbenzene	5.9 ± 0.8	8.0 ± 0.5	ND	ND	ND	MS, RI
985	1-ethyl-2-methylbenzene	7.6 ± 0.2	6.6 ± 0.1	ND	ND	ND	MS, RI
988	1,3,5-trimethylbenzene	53.6 ± 0.9	68.4 ± 6.2	ND	ND	ND	MS, RI
992	2-pentylfuran	180.8 ± 8.4	216.6 ± 11.0	273.3 ± 5.2	311.8 ± 4.8	341.6 ± 9.2	MS, RI, STD
1005	1,3-dichlorobenzene	4.3 ± 0.2	ND	ND	ND	ND	MS, RI
1015	1,2,3-trimethylbenzene	14.8 ± 0.4	13.2 ± 0.9	8.9 ± 0.4	ND	ND	MS, RI
1043	benzenacetaldehyde	13.4 ± 0.5	20.0 ± 2.1	ND	ND	ND	MS, RI
1058	1-methyl-3-propylbenzene	6.7 ± 0.0	ND	ND	ND	ND	MS, RI
1065	1-ethyl-3,5-dimethylbenzene	9.9 ± 0.3	8.3 ± 0.0	ND	ND	ND	MS, RI
1072	2-ethyl-1,4-dimethylbenzene	30.6 ± 1.5	ND	ND	ND	ND	MS, RI, STD
1079	2-ethyl-1,3-dimethylbenzene	15.8 ± 0.5	6.7 ± 0.6	ND	ND	ND	MS, RI
1086	guaiacol	68.9 ± 2.8	47.7 ± 1.7	9.1 ± 0.3	ND	ND	MS, RI, STD
1148	1,2-dimethoxybenzene	6.0 ± 0.3	3.1 ± 0.1	ND	ND	ND	MS, RI
1172	naphthalene	21.7 ± 0.4	22.0 ± 0.4	27.3 ± 2.2	11.7 ± 0.2	13.7 ± 0.2	MS, RI, STD
1281	2-methylnaphthalene	14.7 ± 0.2	14.8 ± 1.5	17.5 ± 0.4	26.9 ± 0.4	31.4 ± 0.2	MS, RI, STD
1311	4-vinylguaiacol	16.8 ± 0.5	20.3 ± 2.4	12.5 ± 0.3	9.3 ± 1.6	ND	MS, RI, STD
	aliphatic/alicyclic hydrocarbons						
820	2,4-dimethylheptane	6.2 ± 0.3	ND	ND	ND	ND	MS, RI
827	2,4-dimethyl-1-heptene	9.6 ± 0.3	5.3 ± 0.4	ND	ND	ND	MS, RI
852	4-methyloctane	11.7 ± 0.3	9.5 ± 0.2	ND	ND	ND	MS, RI, STD
900	nonane	8.5 ± 0.5	10.8 ± 0.0	6.6 ± 0.0	6.1 ± 0.1	4.5 ± 0.1	MS, RI, STD
1000	decane	7.6 ± 0.2	9.8 ± 0.6	ND	ND	ND	MS, RI, STD
1026	3-ethyl-2-methyl-1,3-hexadiene	9.7 ± 0.9	23.4 ± 1.7	40.4 ± 1.6	45.6 ± 1.6	53.7 ± 0.3	MS, RI
1100	undecane	9.1 ± 1.1	6.1 ± 0.7	7.5 ± 0.0	13.6 ± 0.3	14.0 ± 0.3	MS, RI, STD
1200	dodecane	46.2 ± 0.5	31.6 ± 1.3	17.1 ± 1.0	8.2 ± 0.2	5.7 ± 0.2	MS, RI, STD
1300	tridecane	90.7 ± 1.8	61.8 ± 3.1	32.6 ± 1.9	15.2 ± 0.3	8.4 ± 0.3	MS, RI, STD
1400	tetradecane	58.2 ± 1.7	49.3 ± 2.4	36.9 ± 3.0	21.6 ± 0.5	20.8 ± 0.8	MS, RI, STD
	N containing compounds						
816	2-methylpyridine	2.9 ± 0.7	2.4 ± 0.0	ND	ND	ND	MS, RI
918	2-acetyl-1-pyrroline	169.3 ± 6.3	130.8 ± 13.1	98.4 ± 9.1	29.7 ± 1.5	ND	MS, RI
1213	benzothiazole	10.0 ± 0.5	8.1 ± 0.3	8.7 ± 0.3	19.3 ± 0.9	12.2 ± 3.3	MS, RI
1289	indole	41.9 ± 0.2	31.3 ± 1.9	18.9 ± 1.0	18.2 ± 1.5	12.4 ± 0.2	MS, RI, STD
	aliphatic alcohols						
771	3-methyl-1-butanol	5.5 ± 0.2	26.1 ± 1.9	85.1 ± 15.3	256.9 ± 13.9	304.5 ± 7.4	MS, RI
772	(*S*)-2-methyl-1-butanol	9.0 ± 1.3	41.2 ± 11.6	51.5 ± 4.0	58.6 ± 0.3	61.1 ± 0.8	MS, RI
787	1-pentanol	21.4 ± 1.6	105.7 ± 11.1	264.0 ± 0.5	243.0 ± 8.2	293.5 ± 4.4	MS, RI, STD
870	1-hexanol	20.3 ± 1.3	83.9 ± 9.7	222.0 ± 4.6	226.2 ± 11.4	267.8 ± 1.7	MS, RI, STD
969	1-heptanol	4.6 ± 0.2	21.0 ± 2.3	47.6 ± 4.1	57.1 ± 3.5	66.5 ± 0.7	MS, RI, STD
984	1-octen-3-ol	57.1 ± 4.2	96.0 ± 1.5	153.8 ± 5.9	165.7 ± 6.1	197.3 ± 2.7	MS, RI, STD
	aliphatic aldehydes						
803	hexanal	440.2 ± 13.8	837.5 ± 75.5	1507.3 ± 63.3	1790.0 ± 42.7	2327.9 ± 27.0	MS, RI, STD
857	(*E*)-2-hexenal	7.5 ± 0.6	14.5 ± 1.5	25.6 ± 3.4	28.6 ± 1.7	36.7 ± 0.6	MS, RI, STD
903	heptanal	41.0 ± 1.3	73.9 ± 7.7	117.3 ± 4.6	129.8 ± 3.1	153.0 ± 1.0	MS, RI, STD
1005	octanal	46.6 ± 1.5	78.1 ± 8.9	133.0 ± 4.7	151.9 ± 3.7	177.7 ± 4.6	MS, RI, STD
1058	(*E*)-2-octenal	35.0 ± 2.1	54.4 ± 4.5	134.0 ± 2.2	165.8 ± 6.5	214.0 ± 0.3	MS, RI, STD
1106	nonanal	258.3 ± 0.2	328.5 ± 6.5	384.5 ± 7.3	362.2 ± 11.4	381.5 ± 5.9	MS, RI, STD
1160	(*E*)-2-nonenal	16.1 ± 1.0	27.8 ± 2.3	60.1 ± 3.3	81.3 ± 3.7	100.2 ± 1.1	MS, RI, STD
1206	decanal	34.5 ± 1.1	48.7 ± 2.7	68.3 ± 0.6	68.0 ± 5.4	53.9 ± 6.0	MS, RI, STD
1262	(*E*)-2-decenal	6.0 ± 0.5	24.8 ± 1.3	46.0 ± 1.5	55.3 ± 4.4	63.8 ± 4.1	MS, RI, STD
1315	(*E*,*E*)-2,4-decadienal	15.2 ± 1.7	46.9 ± 0.5	65.7 ± 1.6	70.3 ± 3.8	72.4 ± 4.2	MS, RI, STD
	aliphatic/alicyclic ketones						
1036	3-octen-2-one	8.7 ± 1.0	28.9 ± 1.7	57.7 ± 3.0	55.0 ± 0.7	57.9 ± 0.6	MS, RI, STD
1093	2-nonanone	6.8 ± 0.8	13.1 ± 1.3	26.9 ± 1.3	27.9 ± 1.3	31.3 ± 0.5	MS, RI, STD
1449	(*E*)-geranylacetone	17.8 ± 1.4	36.1 ± 0.4	59.9 ± 0.6	60.1 ± 2.3	69.7 ± 1.5	MS, RI
	terpenoids						
1024	*d*-limonene	7.9 ± 0.7	7.9 ± 0.3	9.1 ± 0.5	52.6 ± 1.4	74.8 ± 0.4	MS, RI, STD
1069	(*Z*)-linaloloxide	7.9 ± 0.2	17.8 ± 0.4	12.0 ± 0.1	ND	ND	MS, RI, STD

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[a] Values expressed as δ-carvone equivalent (ng/100g) and given as average ± standard deviation (n=3). [b] Retention index based on a series of *n*-hydrocarbons. [c] Method of identification: MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI, by comparison of RI with those from the literature; STD, by comparison of retention time, and spectrum of an identified compound with those of an authentic compound. [c] ND = not detected.

Table 3.3. Odor Intensity, Odor Description, and Odor Threshold of Odor-Active Compounds in Cooked Black Rice

no.	RI[a]	odorant	identification[b]	intensity[c]	odor description[d]	reported odor threshold (ppb)
1	760	toluene	MS, RI, STD	2.4	caramel, paint	1000 [e]
2	787	1-pentanol	MS, RI, STD	3.4	plastic	4000 [f]
3	803	hexanal	MS, RI, STD	4.0	green tomato, green	5 [f]
4	816	2-methylpyridine	MS, RI	1.0	ash	
5	857	(*E*)-2-hexenal	MS, RI, STD	1.0	green, apple	17 [f]
6	852	4-methyloctane	MS, RI, STD	3.2	nutty	
7	859	*p*-xylene	MS, RI, STD	3.6	medicinal	530 [e]
8	887	*o*-xylene	MS, RI	2.3	peanut	1800 [e]
9	903	heptanal	MS, RI, STD	3.9	floral	3 [f]
10	918	2-acetyl-1-pyrroline	MS, RI	4.4	popcorn	0.1 [f]
11	952	benzaldehyde	MS, RI, STD	2.2	almond	350 [f]
12	969	1-heptanol	MS, RI, STD	0.6	green	3 [g]
13	984	1-octen-3-ol	MS, RI, STD	3.7	mushroom	1 [f]
14	988	1,3,5-trimethylbenzene	MS, RI	1.5	hospital	
15	992	2-pentylfuran	MS, RI, STD	2.5	floral, fruit	6 [f]
16	1005	octanal	MS, RI, STD	3.8	citrus	3 [f]
17	1036	3-octen-2-one	MS, RI, STD	4.0	rose	
18	1058	(*E*)-2-octenal	MS, RI, STD	2.9	nutty, cooked flour	3 [f]
19	1072	2-ethyl-1,4-dimethylbenzene	MS, RI, STD	2.4	paint thinner	
20	1079	2-ethyl-1,3-dimethylbenzene	MS, RI	2.7	varnish	
21	1086	guaiacol	MS, RI, STD	3.2	smoky, black rice-like	3 [f]
22	1093	2-nonanone	MS, RI, STD	3.7	fruity, floral	200 [f]
23	1106	nonanal	MS, RI, STD	4.2	citrus	1 [f]
24	1160	(*E*)-2-nonenal	MS, RI, STD	4.3	beany, cucumber	0.08 [f]
25	1172	naphthalene	MS, RI, STD	2.5	naphthalene	5 [e]
26	1206	decanal	MS, RI, STD	2.2	citrus	2 [f]
27	1262	(*E*)-2-decenal	MS, RI, STD	2.3	fatty	0.4 [f]
28	1281	2-methylnaphthalene	MS, RI, STD	2.2	naphthalene	20 [e]
29	1289	indole	MS, RI, STD	3.5	sour fruit	140 [f]
30	1311	4-vinylguaiacol	MS, RI, STD	3.4	nutty	3 [f]
31	1315	(*E*,*E*)-2,4-decadienal	MS, RI, STD	3.8	fatty	0.07 [f]

[a] Retention index based on a series of *n*-hydrocarbons. [b] Method of identification: MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI, by comparison of RI with those from the literature; STD, by comparison of retention time, spectrum, odor description of an identified compound with those of an authentic compound. [c] Average intensity of compounds which were detected by all three assessors. [d] Odorants were described by assessors during GC-O. [e] Odor threshold in water by Van Gernert and Nettenbreijer (*32*). [f] Odor threshold in water by Buttery et al. (*14*). [g] Odor threshold in water by Fazzalari (*33*).

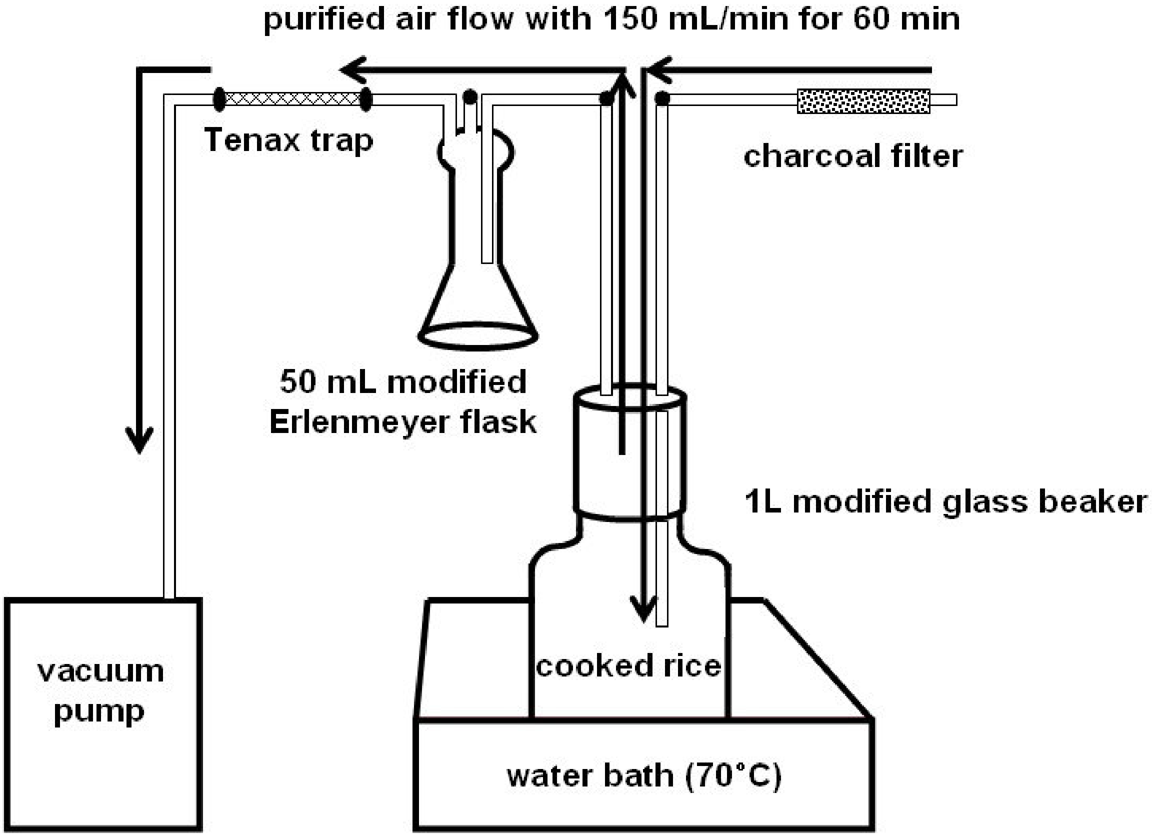

Figure 3.1. Diagram of dynamic headspace system with Tenax trapping for rice volatile collection.

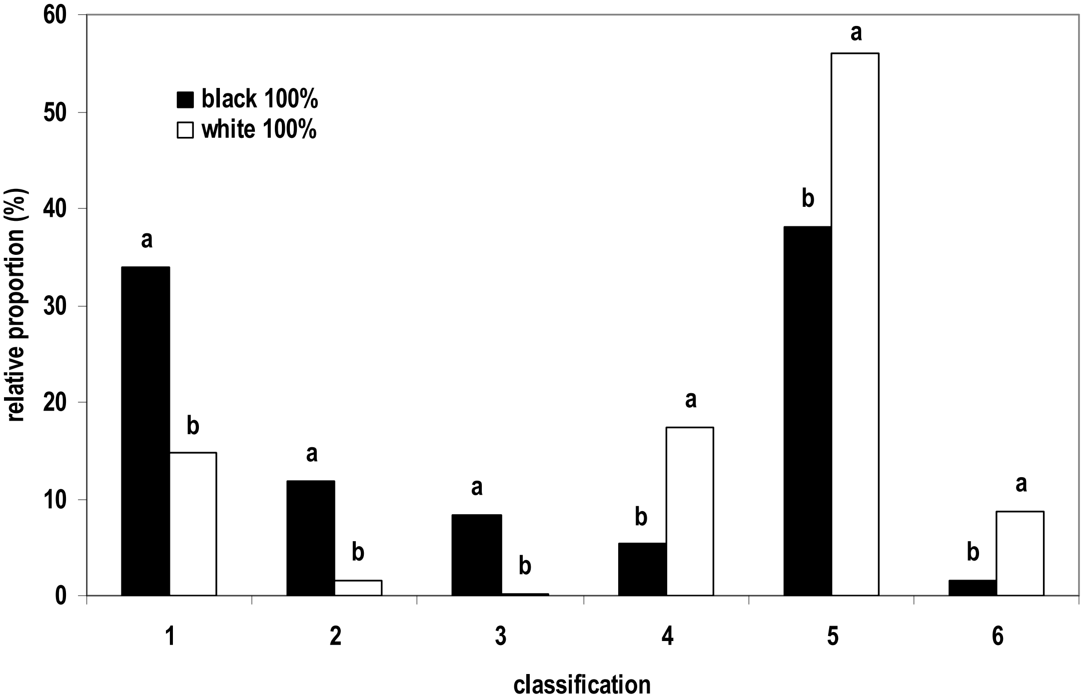

Figure 3.2. Relative proportion of the primary classes of volatile compounds emanating from a black (Geomjeong-ssal) and white (Jungilpum) cultivar. Vertical bars with different letters are significantly different ($p < 0.05$): (1) aromatics (2) aliphatic/alicyclic hydrocarbons (3) nitrogen-containing compounds (4) aliphatic alcohols (5) aliphatic aldehydes (6) aliphatic/alicyclic ketones.

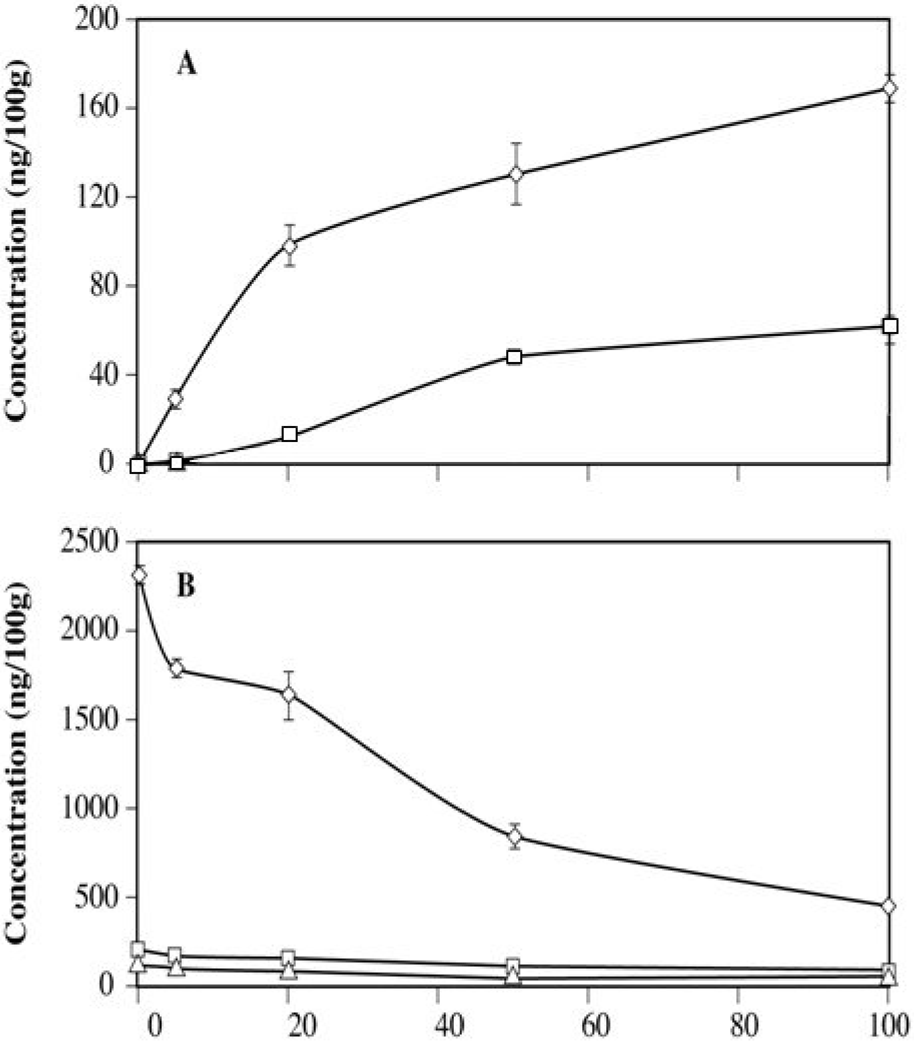

Figure 3.3. (A) Changes in concentration of 2-acetyl-pyrroline (◊) and guaiacol (□) and (B) changes in concentration of hexanal (◊), octanal (□), and (*E*)-2-nonenal (Δ) in varying ratios of black to white rice (1:0, 1:1, 1:4, 1:19, 0:1). Vertical bars represent the standard deviation.

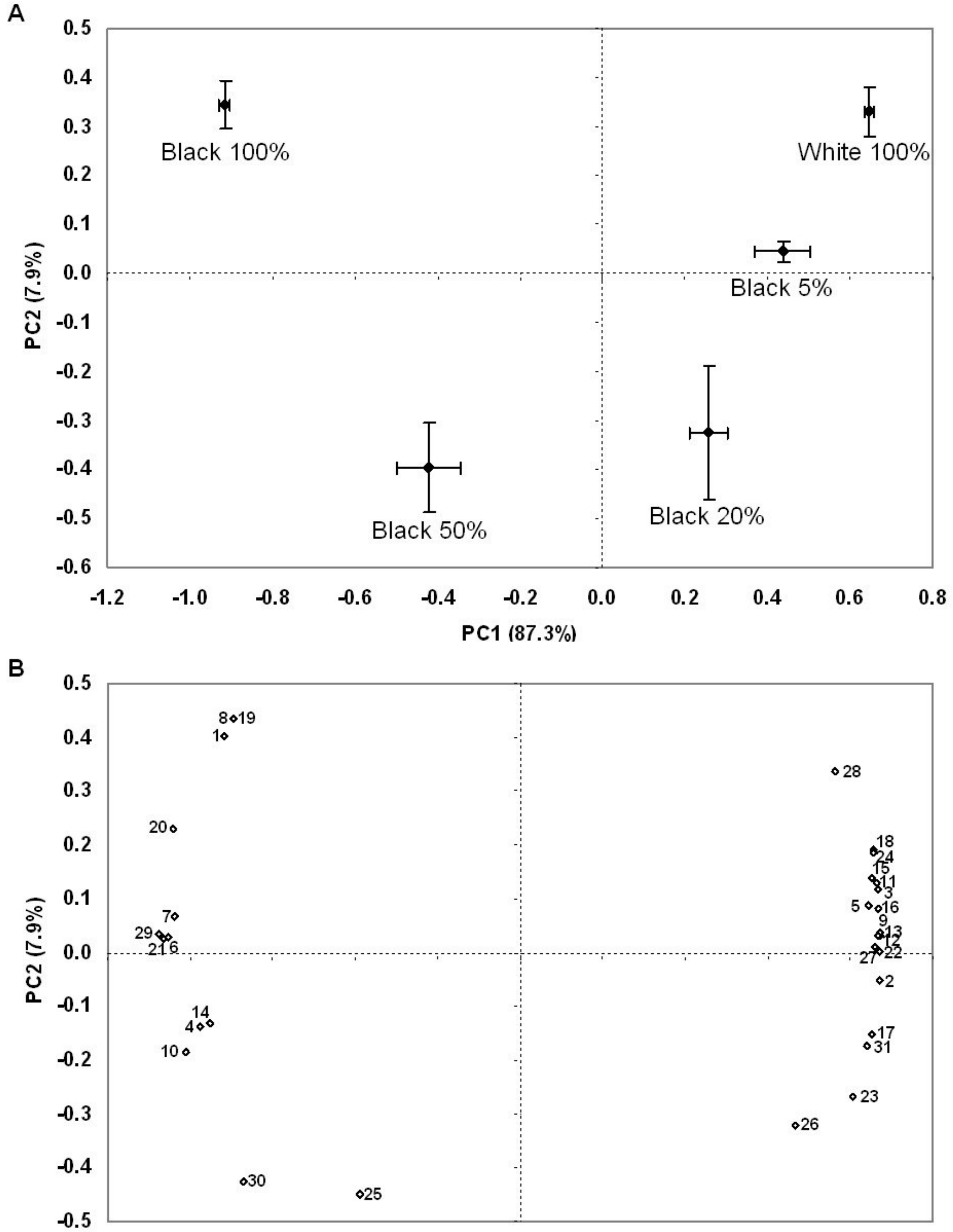

Figure 3.4. PCA plots from five cooked rice samples: (A) separation of different ratios of black pigmented and traditional white rice based on odor-active compounds; and (B) the distribution of 31 odor-active compounds (loadings) in relation to ratios of black to white rice. Vertical and horizontal bars represent the standard deviation. Numbers correspond to those in **Table 3.3**.

CHAPTER 4

COMPARISON OF ODOR-ACTIVE COMPOUNDS FROM SIX DISTINCTLY DIFFERENT RICE FLAVOR TYPES[1]

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[1] Yang, T.S., R.L. Shewfelt, K.S. Lee, and S.J. Kays. To be submitted to *Journal of Agricultural and Food Chemistry.*

ABSTRACT

Using a dynamic headspace system with Tenax trap, GC-MS, GC-olfactometry (GC-O), and multivariate analysis, the aroma chemistry of six distinctly different rice flavor types (basmati, jasmine, two Korean japonica cultivars, black rice and a non-aromatic rice) was analyzed. A total of 44 odorants from cooked samples were characterized by trained assessors. Twenty-nine odorants had an intermediate or greater intensity (odor intensity ≥ 3) and were considered major odor-active compounds. Their odor thresholds in air were determined using GC-O. 2-Acetyl-1-pyrroline (2-AP) had the lowest odor threshold (0.02 ng/L) followed by 11 aldehydes (ranging from 0.09 to 3.12 ng/L), guaiacol (1.49 ng/L), and 1-octen-3-ol (2.70 ng/L). Based on odor thresholds and odor activity values (OAVs), the importance of each major odor-active compound was assessed. OAVs for 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal comprised over 97 % of relative proportion of OAVs from each rice flavor type, even though the relative proportion varied among samples. Thirteen odor-active compounds, 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 among the six flavor types were the primary compounds explaining the differences in aroma. Multivariate analysis demonstrated that the individual rice flavor types could be separated and characterized using these compounds which may be of potential use in rice breeding programs focusing on flavor.

KEYWORDS: Odor-active compound; aromatic rice; black pigmented rice; dynamic headspace; Tenax trap; GC-O; principal component analysis; odor activity value (OAV)

INTRODUCTION

Rice (*Oryza sativa* L.) is the single most important food crop consumed by man. It is utilized as a staple by over a one-half of the world's population with approximately 95% of production being in Asia. Rice is also grown and consumed as an important non-staple food in America, Europe and Africa, where its popularity is progressively increasing (*1*). Because of its importance, rice breeders have developed cultivars with higher yields, insect resistance, herbicide tolerance and other agronomic traits. Grain quality is also a critical breeding objective in that quality has a significant impact on consumer preference. Of the various quality attributes of cooked rice, flavor is considered of primary importance in that superior flavor increases consumer satisfaction, overall acceptability and the probability of repeated purchase (*2*). Indian consumers consider aroma and taste the most critical quality traits while Asian consumers in the United States consider flavor one of the most important acceptance factors (*1*, *2*).

Flavor is comprised of taste and aroma, the latter of which is conferred by volatile compounds emanating from cooked rice that interact with olfactory receptors. While a relative large number of compounds from cooked rice have been identified (*3*-*5*), a much more limited number appear to make up the characteristic aroma. Qualitative and quantitative differences in these critical odor-active compounds are thought to collectively create the aroma perceived and account for differences among flavor types.

Rice cultivars can be separated very generally into aromatic and non-aromatic types. Aromatic rice has a relatively diverse range of aromas that are much more dominant than in non-aromatic cultivars. Within aromatic rice there is a cross-section of unique aromas. For example, Basmati 370 (India), Kha Dawk Mali 105 [Jasmine] (Thailand), Hieri (Japan) and Della (USA) are popular aromatic rice cultivars in the world market that command a higher price than non-aromatic cultivars due to their distinct aromas (*6*). In India the price of basmati was three times higher than that of non-aromatic rice (*7*).

The aroma of both aromatic and non-aromatic rice is comprised of a complex mixture of odor-active compounds. In aromatic rice, 2-acetyl-1-pyrroline (2-AP) is considered highly important and while found in some non-aromatic types, its concentration is very low to negligible (*8*). 2-AP is described as having a "popcorn-like" odor by American and "pandan-like" odor by Asian consumers (*9*). However, the unique aromas of basmati, jasmine, black and other rice flavor types can not be accounted for simply by differences in 2-AP. These differences are due to qualitative and quantitative variation in a diverse cross-section of odor active compounds.

Plant breeding is the primary avenue for altering rice flavor. The problem, however, is that sensory tests for flavor are expensive, time consuming, and labor intensive such that the number of progeny that can be assessed is only a very small percentage of the total. The net effect is that flavor is assessed very late in the selection process after the majority of the progeny have already been discarded. If flavor could be measured analytically, integrating progeny flavor chemistry with the chemistry of preference of targeted consumer populations, assessment could be moved much earlier in the selection process, greatly increasing the chance of finding new cultivars with truly superior flavor.

As a preliminary step toward this objective, we have identified and quantified the volatile compounds emanating from six different rice flavor types using GC-MS, identified and characterized the odor-active compounds using GC-olfactometry (GC-O), assessed the relative importance of each odor-active compound in the overall aroma using odor activity values (OAVs) and compared the distinct aroma of the various flavor types through the presence or absence of single odorants and their OAVs. Our intent in this study was not to identify all possible volatiles emanating from the cooked rice samples but rather to focus on the critical odor-active compounds that would be useful in distinguishing differences among progeny in rice breeding programs.

MATERIALS AND METHODS

Materials. Six rice cultivars, each displaying distinctly different cooked aromas, were selected for analysis. Five were aromatic and one a traditional non-aromatic rice cultivar. The aromatic cultivars were Hyangmibyeo 1 (H1, a medium grained Korean japonica), and Hyangmibyeo 2 (H2, a medium grained Korean japonica); Royal (BA, basmati, a long-grained Indian indica), Golden Elephant (JA, jasmine, a long-grained Thai indica) and Goemjeongssal (BP, a medium grained japonica black rice from Korean). Jeongilpum (TM) a traditional medium-grain, non-aromatic japonica from Korean was included for comparison. H1 and H2 were grown at the National Institute of Crop Science, Suwon, in 2006; the remainders indicated as a "New Crop 2007" on the package, were purchased from a local supermarket in October 2006. All rice samples were milled to removing the bran layer from brown rice except for the black rice cultivar (BP). The samples were sealed in glass containers and kept at -20°C until analysis.

Chemicals. Analytical standard utilized for identification were: benzaldehyde, decane, decanal, (*E*)-2-decenal, furfural, guaiacol, heptanal, heptane, 2-heptanone, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, undecane (Sigma-Aldrich Inc., St. Louis, MO); (*E*,*E*)-2,4-decadienal, 2-decanone, hexanal, (*E*)-2-hexenal, 1-hexanol, 1-nonanol, 2-nonanone, 1-methylnaphthalene, 2-methylnaphthalene,1-octanol, (*E*)-2-octenal, 1-octen-3-ol (Aldrich Chem.

Co., Milwaukee, WI); *p*-menthan-3-one, octanal, *p*-xylene (Fluka Chem. Co., Milwaukee, WI); naphthalene, toluene (J. T. Baker Inc., Phillipsburg, NJ); dodecane, 2-ethyl-1,4-dimethylbenzene, indole, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, 2-pentylfuran, and 4-vinylguaiacol (TCI America, Portland, OR); 1,2-diethylbenzene, 1,3-diethylbenzene, 3-octen-2-one (Alfa Aesar, Ward Hill, MA); and 4-methyloctane (Pfaltz & Bauer Inc., Waterbury, CT). 2,4,6-Trimethylpyridine (Aldrich Chem. Co., Milwaukee, WI) was used as an internal standard for 2-AP.

Dynamic Headspace Sampling. Dynamic headspace sampling of volatile componds in cooked rice was performed using a method described previously (*10*). Rice samples (100g) were cooked in distilled water (150 mL) for 30 min at 100 ºC in a specially constructed 1 L glass beaker with entry and exit ports. The entry and exit ports were wrapped with aluminum foil during cooking. Due to difference of water absorption characteristics, the black rice sample (100 g) was cooked in 100 mL of distilled water which was established based on texture after cooking by preliminary tests; the volatiles were collected as with the other samples. The glass beaker with freshly cooked rice was immediately placed in a hot water bath and was maintained at 70 °C during sampling. Headspace volatiles emanating from the cooked rice samples were collected on a Tenax trap using a vacuum sampling pump (Aircheck Sampler, Model 224-44XR, Eighty-Four, PA). Air purified using a charcoal filter (Alltech Assoc. Inc. Deerfield, IL; 1 cm i.d., 10 cm long Pyrex glass tube with a 7 cm bed of charcoal) connected to the entry port was passed through the beaker at 150 mL/min for 60 min. A 50 mL Erlenmeyer flask was placed between the exit port and the trap to collect any condensation. One mL of 18.34 mg/L trimethylpyridine (TMP) solution in 0.1 M HCl, used as an internal standard for 2-AP (*11*), was injected into the Erlenmeyer flask at the beginning of volatile collection using 1 mL syringe.

After sampling, the Tenax trap was connected to an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, Ringoes, NJ) on the injector port of the gas chromatograph/mass spectrometer (GC/MS, Model 6890N/5973, Agilent, Palo Alto, CA).The collected samples were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min and the analytes collected on the first 4 cm of the GC column using a CO_2 cooled cryofocus trap (-40 °C) (SIS 2" Cryo-Trap, Scientific Instrument Services, Ringoes, NJ). After desorption, the cryofocus trap was rapidly heated to 200 °C and the analytes separated using temperature programming.

GC/MS and GC-Olfactometry. The GC was equipped with a 30 m length, 0.25 mm i.d., 0.25 µm film thickness, fused silica capillary column (HP-5MS, Agilent, Palo Alto, CA). Injection port temperature was 225 °C with a split ratio of 0.5:1. He was used as the carrier gas with the flow rate of 2.0 mL/min. The column temperature was held at 40 °C for 1 min and then programmed 1.5 mL/min to 65 °C which was held for 1 min, 2 mL/min to 120 °C for 1 min, finally 15 mL/min to 280 °C for 5 min. Volatiles exiting the column were split between the mass spectrometer for identification and quantification and an Olfactory Detector Outlet for description and intensity assessment (ODO II, SGE Intl., Austin, TX). MS conditions were: ion source 230 °C; electron energy 70 eV; multiplier voltage 1247 V; GC/MS interface zone 280 °C; and a scan range of 35 to 350 mass units. Three assessors were trained by describing 15 materials with different odors: popcorn-like (popcorn), starchy (rice starch), woody (toothpicks), cooked grain (cream of wheat), corn (cream style corn), nutty (roasted peanut), floral (Jasmine scent), dairy (2% milk), hay (hay), barn (white pepper), buttery (butter), green (alfalfa), rancid (rancid vegetable oil), waxy (candle) and earthy (mushroom). Each of the assessors had considerable prior experience in GC-O. An aroma extract of each rice flavor type was characterized by describing the aroma of the individual components and assessing their intensity via GC-O. Odor intensity was classified on a 1 to 5 scale: 1 = very weak; 2 = weak; 3 = intermediate; 4 = strong; 5 = very strong. Odor intensity values were averaged for the assessors and odorants perceived by all assessors were accepted as odor-active compounds.

Identification and Quantification of Odorants. Each odorant was identified based on its mass spectra using NIST 02 and Wiley 7 libraries. Identifications were confirmed by comparing Kovats' retention indices (RI) and odor descriptions with those of authentic standards. Retention indices were calculated using a non-polar HP-5MS column and a series of *n*-hydrocarbons (C_7-C_{15}) and compared with those reported previously (http://webbook.nist.gov/). The identification of 2-acetyl-1-pyrroline (2-AP) was confirmed by mass spectra, RI, and its distinct descriptor (popcorn odor) in that an authentic standard was not available. Quantification was determined using standard curves for each odorant (i.e., 0, 5, 10, 20, 50, 100, 200, and 500 ppm in hexane). The quantification of 2-AP was expressed as TMP equivalents.

Odor Thresholds in Air and Odor Activity Values. Odor thresholds were determined using a modified Ulrich and Grosch GC-O method (*12*). A stock solution of each odorant (10 mg) dissolved in 10 mL of hexane was diluted stepwise (1:1 v/v) and 0.5 µL of each dilution was injected for GC-O. (*E*)-2-Decenal, which has an odor threshold in air of 2.7 ng/L, was used as an internal standard. The odor thresholds in air were calculated in relation to the odor threshold of (*E*)-2-decenal based on the detectable minimum concentration of (*E*)-2-decenal and the other odorants. Odor activity values (OAVs) were calculated by dividing the absolute concentration of each odor active compound by its odor threshold.

Data Analysis. Principal component analysis (PCA) was carried out on the odor-active compounds using SAS for Windows v8 to visualize the differences in odor among rice samples. Duncan's multiple-range test was used to compare odor activity values (OAV) of the odor-active compounds from rice samples.

RESULTS AND DISCUSSION

Odor-Active Compounds from Rice Flavor Types. Odor-active compounds emanating from six different aroma types of cooked rice were identified and quantified and their relative intensities and descriptors determined using trained assessors (**Table 4.1**). The 37 odorants detected in the rice samples were identified by mass spectra (NIST 02 and Wiley 7 libraries), retention indexes (RI) and odor description using authentic standards. Seven additional minor odorants were tentatively identified using GC-MS, RI, and odor descriptors from the literature.

A total of 34, 30, 20, 24, 34, and 28 odorants were detected in BA, JA, H1, H2, BP, and TM, respectively (**Table 4.1**). Aldehydes and aromatic compounds were the most abundant odor-active compounds in the aromatic rice types (BA, JA, H1, H2, and BP). In the non-aromatic rice (TM), aldehydes were the most abundant odor-active compounds. Of the total odorants detected across all samples, there were 9 aldehydes [hexanal, (*E*)-2-hexenal, heptanal, octanal, nonanal, (*E*)-2-nonenal, decanal, (*E*)-2-decenal, and (*E*,*E*)-2,4-decadienal], 4 aromatics (*p*-xylene, *o*-xylene, benzaldehyde, and 2-pentylfuran), 2 alcohols (1-pentanol and 1-octen-3-ol), and one nitrogen containing compound (2-acetyl-1-pyrroline). Odor intensities of the 16 compounds varied with concentration among the flavor types (data not shown). Pentanal and benzothiazole were detected in only BA, 1,2,3-trimethylbenzene in JA, 1-nonanol, 2-methylpyridine, guaiacol, indole and 4-methyl octane in BP, and 1-hexanol in TM. These compounds may contribute to the unique aroma of their respective cultivars, however, the average odor intensity was very low for all except 1-nonanol, guaiacol, indole and 4-methyl octane which had an intermediate or higher intensity (odor intensity ≥ 3).

Based on odor intensity, 15, 11, 7, 7, 21, and 19 odorants were found to be ≥ intermediate in intensity (i.e., ≥ 3) from BA, JA, H1, H2, BP, and TM, respectively (**Table 4.1**). Among these, 4, 2, 1, 0, 6, and 8 odorants were classified as ≥ high in intensity (i.e., ≥ 4) from BA, JA, H1, H2, BP, and TM, respectively. Hexanal (odor intensity = 4.9), 2-AP (4.3), and heptanal (4.3) in BA; hexanal (4.7), 2-AP (4.0), and (*E*)-2-octenal (3.8) in JA; 2-AP (4.0), nonanal (3.5), and hexanal (3.5) in H1; hexanal (3.9), 2-AP (3.5), and nonanal (3.5) in H2; 2-AP (4.4), (*E*)-2-nonenal (4.3), and nonanal (4.2) in BP; and (*E*)-2-octenal (4.6), and hexanal (4.4), and heptanal (4.3) in TM were found to be the most potent odorants in each of the flavor types. 2-AP, a central component in the aroma of aromatic rice (*8*), was the most potent odorant in the five aromatic rice cultivars (BA, JA, H1, H2, BP). Hexanal, which has been reported to be produced nonenzymatically or by an unknown pathway from linoleic aicds *via* 9-D-hydroperoxy-10,12-(*E*,*Z*)-octadecadienoic acid (*13*), was a significant odorant in all rice samples except BP.

Assessment of the Relative Importance of Each Odor-Active Compound to the Overall Aroma. To assess the importance of individual odor-active compounds to the overall aroma, 29 compounds with an odor intensity score of equal to or greater than intermediate (i.e., ≥ 3) were considered potentially significant contributors and their odor threshold values in air determined (**Table 4.2**). The odor threshold of 2-AP was the lowest (0.02 ng/L) of the major odor-active compounds. Eleven aldehydes [(*E*)-2-nonenal, (*E*,*E*)-2,4-nonadinenal, octanal, heptanal, hexanal, (*E*,*E*)-2,4-decadienal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-decenal, and (*E*)-2-hexenal] had odor thresholds ranging from 0.09 to 3.12 ng/L. These compounds also have relatively low thresholds in water (*14*). The odor of guaiacol, described as "smoky", had an odor threshold of 1.49 ng/L and was detected only in the black rice sample (BP). Guaiacol has been reported as the key odorant in black rice due to its unique odor and low odor threshold (*14*); it is also responsible for the smoked odor in smoked salmon (*15*). In contrast, 1-octen-3-ol, described as "mushroom", had an odor threshold of 2.70 ng/L and was found in each of the rice cultivars, ranging in intensity from 1.8 to 3.5. 1-Octen-3-ol is a significant odorant in cheese due to its low odor threshold (*16*).

Odor activity values (OAVs), obtained by dividing each compound's concentration by its odor threshold, were used to assess the relative importance of individual odorants to the overall aroma (**Table 4.2**). To quantify the odor-active compounds, standard curves for 28 odor-active compounds were established using authentic standards (i.e., 8 concentrations for each compound). The accuracy of the standard curves was high as indicated by coefficients of determination (R^2) which ranged from 0.9979 to 0.9999 and relative standard deviations (RSDs) values which ranged 0.71 to 2.97 % (data not shown). The most potent odor active compound in BA was hexanal (OAV: 236.7, relative proportion: 75.9 %) followed by (*E*)-2-nonenal (25.2, 7.0 %), octanal (17.0, 4.7 %), 2-AP (16.6, 4.6 %), heptanal (12.7, 3.5 %), and nonanal (5.0, 1.4 %) (**Table 4.2, Figure 4.1**). To date, 2-AP has generally been considered to be the most critical odorant in aromatic rice cultivars, however, this is clearly not always the case. For example, hexanal (169.9, 79.3 %) had the highest OAV in TM followed by (*E*)-2-nonenal (16.2, 7.5 %), octanal (8.5, 4.0 %), 2-AP (5.8, 2.7 %), heptanal (5.6, 2.6 %), and nonanal (2.0, 0.9 %). The OAVs of these five odor-active compounds except 2-AP in BA, were significantly higher than those in TM. The most potent compound in H2 also was hexanal (44.3, 60.5 %), however, 2-AP (8.0, 10.9 %), heptanal (6.3, 8.6 %), (*E*)-2-nonenal (5.7, 7.8 %), octanal (4.5, 6.1 %), and nonanal (2.3, 3.1 %) followed. The most potent compound in JA, H1, and BP was 2-AP (191.0, 57.4 %; 153.3, 76.5 %; 246.2, 90.6 %) followed by hexanal (119.3, 35.8 %; 31.8, 15.9 %; 16.7, 6.1 %) and (*E*)-2-nonenal (7.8, 2.3 %; 5.0, 2.5 %; 2.7, 1.0 %), respectively. The OAV of guaiacol (0.3) coupled with its unique odor made it a significant contributor to the distinct aroma of BP (*10*). OAVs and relative proportion of 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal were different among the rice flavor types, however, they had the first to sixth highest OAV in all rice samples (**Table 4.2**, **Figure 4.1**). With the exception of 2-AP, aldehydes were thought not to be particularly important odorants in rice (*14*), however, we found they made up over 97% of the OVAs in the rice cultivars studied, indicating a critical role in the overall aroma. It has also been reported that hexanal, (*E*)-2-nonenal, and octanal contribute to off-flavors in rice that develop during storage (*17*). Changes in the concentration of these compounds may have a pronounced effect on flavor.

Comparison of Flavor Types. The concentration of 2-AP has been used as an indicator of aroma in selection of aromatic lines (*8*, *18*, *19*). Several techniques have been developed for identification and quantification of 2-AP (*20-22*). Our data do not support this contention (**Figure 4.2**). While three of the aromatic cultivars had high levels of 2-AP [i.e., BP (4.9 ng/g), JA (3.8 ng/g), H1 (3.1 ng/g)], two [BA (0.3 ng/g), H2 (0.2 ng/g)] had concentrations on par with the non-aromatic cultivar [TM (0.1 ng/g)]. 2-AP is controlled by a single gene (*23*), the expression of which does not appear to be closely tied with that of a number of the other critical odorants in rice. In addition, while 2-AP confers a popcorn-like odor, other distinct odors (e.g., earthy, nutty, roasty and green) are present in aromatic rice indicating that the overall aroma is made up of a cross-section of compounds (*9*, *24*).

PCA of the OAVs for the 29 major odor-active compounds from the six flavor types in **Table 4.2** is presented in **Figure 4.3**. The five aromatic rice cultivars (BA, JA, H1, H2, BP) segregated distinctly from the non-aromatic cultivar (TM) with 62.1 % and 15.9 % of the total variance accounted for by the first (PC 1) and second (PC 2) principal components, respectively (**Figure 4.3A**). BA was positioned on the positive side of JA, H1, H2, and BP on the negative side of PC 1. JA was separated from H1 and H2 mainly in PC 1. BP distinctly segregated from JA, H1 and H2 in the PC 2, and BA in PC 1 and PC 2. The indica aromatic cultivars (BA and JA) were segregated from each other as well as from the japonica aromatic cultivars (H1 and H2). The results indicate that the indica/japonica classification is not an adequate indication of flavor.

It is evident that PCA using all 29 odor-active compounds gives a distinct separation of the various rice flavor types. However, when assessing the flavor of large numbers of progeny, it would be advantageous if the number of odor active compounds that need to be quantified could be reduced while maintaining adequate discrimination potential. A PCA plot utilizing the 13 most important odorants [i.e., 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], based upon their OAV values, is presented in **Figure 4.3B**. Guaiacol was included in that it is unique to black rice. While reducing the number of compounds from 29 to 13 did not give as good of separation, it was adequate for segregating the representative flavor types tested. The aromatic rice cultivars (BA, JA, H1, H2, BP) were clearly separated from non-aromatic TM, accounting for 87.4 % of the total variance [PC 1 (76.9 %) + PC2 (10.5 %)]. The primary compounds contributing to PC 1 were 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, and decanal. OAVs for these compounds were significantly different in each rice sample. In contrast, the major compounds contributing to PC 2 were 2-AP and guaiacol which are critical to the overall aroma of BP. Reducing the number further to the 7 most important odorants [i.e., 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, nonanal, guaiacol] resulted in a PCA plot with less than adequate separation (**Figure 4.3C**). PCA using 29 odor-active compounds required three components to account for 87.6 % of the total variance, while 13 odor-active compounds accounted for 87.4 % using only the first two components (**Figure 4.4**). It would appear that 13 odor-active compounds can potentially be used to segregate flavor types though this needs to be substantiated with additional cultivars within each flavor.

Initial rice flavor selection decisions are currently made in some breeding programs by chewing individual grains or sniffing the aroma of leaf tissue or grains after either heating in water or reacting with KOH or I_2-KI (*25*). The technique is not quantitative and only allows classifying progeny as scented, moderately scented and non-scented. Decisions are made primarily on the amount of a single volatile compound (2-AP), even though 29 odor-active compounds contribute to the aroma, especially 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal. Likewise, use of molecular markers associated with 2-AP can indicate the presence of the gene but do not indicate the level of expression (*26*-*27*). Coupled with this is the apparent absence of concurrent changes in other odorants with changes in 2-AP. Current sensory and chemical tests, therefore, do not appear to be adequate due to numerical, accuracy and other limitations.

An analytical method for flavor assessment has the potential to facilitate the selection of lines with superior flavor attributes. Wang and Kays (*28*) used a principal component reference standard, which was developed using multivariate analysis of sweetness and the 17 most important odor-active compounds, as a marker for selecting sweetpotatoes for flavor. If applied to rice, this approach would allow the accurate classification of progeny for aroma, the simultaneous selection of multiple flavor types, and the development of superior new cultivars for a wide cross-section of flavors without using sensory tests. Our data indicate that a reference standard with 13 odor-active compounds should allow accurate characterization of rice aroma.

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Table 4.1. Odor Intensity and Description of Odor-Active Compounds in Cooked Rice Cultivars Basmati (BA), Jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), Black Pigmented Rice (BP), and Traditional Medium Rice (TM)

		odor intensity [b]							
R.I. [a]	odorant	BA	JA	H1	H2	BP	TM	odor description [c]	identification [d]
732	pentanal	2.9	nd	nd	nd	nd	nd	nutty, sweet	MS, RI
760	toluene	nd	nd	2.2	2.5	2.4	nd	caramel, paint	MS, RI, STD
766	1-pentanol	4.0	3.7	2.8	2.4	3.4	2.8	plastic	MS, RI, STD
803	hexanal	4.9	4.7	3.5	3.9	4.0	4.4	green tomato, green	MS, RI, STD
816	2-methylpyridine	nd	nd	nd	nd	1.0	nd	ash	MS, RI
842	chlorobenzene	2.6	2.8	2.0	2.3	nd	nd	nutty, burnt	MS, RI
857	(*E*)-2-hexenal	2.3	1.4	1.8	2.2	1.0	3.3	green, apple	MS, RI, STD
852	4-methyl octane	nd	nd	nd	nd	3.2	nd	nutty	MS, RI, STD
859	*p*-xylene	3.1	2.8	3.0	3.1	3.6	3.2	medicinal	MS, RI, STD
870	1-hexanol	nd	nd	nd	nd	nd	2.5	green	MS, RI, STD
887	*o*-xylene	2.1	2.6	2.5	1.2	2.3	2.6	peanut	MS, RI
895	2-heptanone	3.3	1.9	nd	2.2	nd	3.5	fruity, sweet	MS, RI, STD
903	heptanal	4.3	3.6	3.1	2.8	3.9	4.6	floral	MS, RI, STD
918	2-acetyl-1-pyrroline	4.3	4.0	4.0	3.5	4.4	3.6	popcorn	MS, RI
952	benzaldehyde	3.8	3.6	2.4	2.7	3.5	4.2	almond	MS, RI, STD
969	1-heptanol	1.0	nd	nd	nd	0.6	2.8	green	MS, RI, STD
984	1-octen-3-ol	2.6	1.9	1.8	2.2	3.7	3.5	mushroom	MS, RI, STD
992	2-pentylfuran	2.5	2.4	2.1	2.1	2.5	3.2	floral, fruit	MS, RI, STD
1005	octanal	2.1	3.2	3.1	3.0	3.8	4.0	citrus	MS, RI, STD
1015	1,2,3-trimethylbenzene	nd	1.3	nd	nd	nd	nd	plastic	MS, RI, STD
1026	3-ethyl-2-methyl-1,3-hexadiene	1.0	nd	nd	nd	nd	2.6	nutty	MS, RI
1036	3-octen-2-one	3.5	2.7	nd	2.8	4.0	3.5	rose	MS, RI, STD
1050	1,3-diethylbenzene	3.2	2.4	nd	nd	1.0	2.6	oil	MS, RI, STD
1056	1,2-diethylbenzene	3.3	1.3	nd	nd	4.1	3.6	nut	MS, RI, STD
1058	(*E*)-2-octenal	2.9	3.8	nd	3.2	2.9	4.3	nutty, cooked flour	MS, RI, STD
1072	2-ethyl-1,4-dimethylbenzene	nd	1.3	nd	nd	2.4	nd	benzene	MS, RI, STD
1075	1-octanol	2.8	nd	nd	nd	nd	2.9	citrus	MS, RI, STD
1086	guaiacol	nd	nd	nd	nd	3.2	nd	black rice-like, smoke	MS, RI, STD
1093	2-nonanone	2.1	3.3	nd	2.8	3.7	4.2	fruity, flora	MS, RI, STD
1106	nonanal	3.6	2.9	3.5	3.5	4.2	4.1	citrus, fatty	MS, RI, STD
1152	*p*-menthan-3-one	3.3	nd	2.6	nd	3.3	4.1	mint	MS, RI, STD
1160	(*E*)-2-nonenal	3.6	2.5	2.8	2.4	4.3	2.9	beany, cucumber	MS, RI, STD
1172	naphthalene	nd	1.8	2.1	1.9	2.5	nd	naphthalene	MS, RI, STD
1175	1-nonanol	nd	nd	nd	nd	3.0	nd	fatty	MS, RI, STD
1194	2-decanone	1.5	1.5	nd	nd	nd	nd	fruity, fatty	MS, RI, STD
1206	decanal	3.0	3.0	2.8	1.6	2.2	3.3	citrus	MS, RI, STD
1212	(*E*,*E*)-2,4-nonadienal	1.4	2.2	nd	nd	3.7	2.4	nutty, fatty	MS, RI, STD
1213	benzothiazole	1.7	nd	nd	nd	nd	nd	nutty, rubber	MS, RI
1262	(*E*)-2-decenal	3.0	3.1	1.7	2.3	2.3	3.1	fatty	MS, RI, STD

1281	2-methylnaphthalene	2.5	nd	nd	0.7	2.2	nd	naphthalene	MS, RI, STD
1289	indole	nd	nd	nd	nd	3.5	nd	sour fruit	MS, RI, STD
1296	1-methylnaphthalene	1.5	1.9	nd	nd	nd	nd	naphthalene	MS, RI, STD
1311	4-vinylguaiacol	1.7	1.5	nd	nd	3.4	nd	nutty	MS, RI, STD
1315	(*E*,*E*)-2,4-decadienal	2.8	3.1	3.3	3.1	3.8	3.5	fatty	MS, RI, STD

[a] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [b] Average intensity of compounds which were detected by all three assessors.; nd = not detected. [c] Odorants were described by assessors during GC-O. [d] Method of identification: MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI, by comparison of RI with those from the literature; STD, by comparison of retention time, spectrum, and odor description of an identified compound with those of an authentic compound.

Table 4.2. Odor Activity Values (OAVs) and Odor Threshold Values of Major Odor-Active Compounds in Cooked Rice Cultivar Basmati (BA), Jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), Black Pigmented Rice (BP), and Traditional Medium Rice (TM)

		OAV [b] (n=3)						odor threshold
R.I. [a]	odorant	BA	JA	H1	H2	BP	TM	in air (ng / L)
766	1-pentanol	0.002 c	0.010 a	0.004 bc	0.006 b	0.003 bc	0.004 bc	152.97
803	hexanal	236.702 a	119.290 c	1.804 d	44.245 d	16.661 d	169.929 b	1.08
857	(*E*)-2-hexenal	0.300 a	0.085 b	0.039 b	0.057 b	0.060 b	0.248 a	3.12
852	4-methyl octane	nd	nd	nd	nd	0.001 a	nd	155.64
859	*p*-xylene	0.005 cd	0.015 b	0.014 b	0.024 a	0.009 c	0.001 d	46.89
895	2-heptanone	0.910 a	0.545 b	nd	0.453 b	nd	0.679 ab	3.48
903	heptanal	12.680 a	3.255 d	4.085 cd	6.280 b	1.201 e	5.572 bc	0.87
918	2-acetyl-1-pyrroline	16.611 d	191.028 b	153.337 c	7.996 d	246.228 a	5.774 d	0.02[c]
952	benzaldehyde	0.105 a	0.049 b	0.014 c	0.016 c	0.007 c	0.061 b	84.80
984	1-octen-3-ol	1.824 a	0.750 c	0.312 d	0.354 d	0.330 d	1.253 b	2.70
992	2-pentylfuran	0.242 a	0.279 a	0.083 b	0.097 b	0.120 b	0.099 b	19.27
1005	octanal	16.986 a	5.602 bc	3.286 cd	4.448 cd	1.588 d	8.503 b	0.44
1036	3-octen-2-one	0.150 a	0.053 c	nd	nd	0.022 d	0.120 b	6.73
1050	1,3-diethylbenzene	0.006 a	0.001 b	nd	nd	0.002 b	0.008 a	209.78
1056	1,2-diethylbenzene	nd	nd	nd	nd	0.017 b	0.059 a	13.99
1058	(*E*)-2-octenal	1.719 a	0.903 c	nd	0.563 cd	0.216 d	1.300 b	2.69
1075	1-octanol	0.044 a	nd	nd	nd	nd	0.031 a	22.18
1086	guaiacol	nd	nd	nd	nd	0.312 a	nd	1.49
1093	2-nonanone	0.018 a	0.005 c	nd	0.007 c	0.004 c	0.012 b	30.78
1106	nonanal	5.030 a	1.392 c	1.694 bc	2.246 b	1.385 c	1.967 bc	2.64
1152	*p*-menthan-3-one	1.101 a	nd	0.268 b	nd	0.032 b	0.254 b	4.75
1160	(*E*)-2-nonenal	25.148 a	7.809 c	4.984 cd	5.726 cd	2.699 d	16.155 b	0.09
1175	1-nonanol	nd	nd	nd	nd	0.006 a	nd	17.81
1206	decanal	0.534 a	0.166 bc	0.206 bc	0.272 b	0.108 c	0.227 bc	2.65
1212	(*E*,*E*)-2,4-nonadinenal	1.479 a	1.038 b	nd	nd	0.572 c	1.234 ab	0.18
1262	(*E*)-2-decenal	0.431 a	0.247 bc	0.068 d	0.105 cd	0.040 d	0.320 ab	2.70
1289	indole	nd	nd	nd	nd	0.044 a	nd	8.08
1311	4-vinylguaiacol	0.136 a	0.087 b	nd	nd	0.077 b	nd	2.80
1315	(*E*,*E*)-2,4-decadienal	0.799 a	0.365 bc	0.184 cd	0.222 cd	0.117 d	0.429 b	2.30

[a] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [b] OAV is obtained by dividing the concentration of an odor-active compound by its odor threshold and means of three replicates per sample; means separation within rows by Duncan's multiple range test at $P < 0.05$.; nd = not detected. [c] Data from Schieberle (*19*).

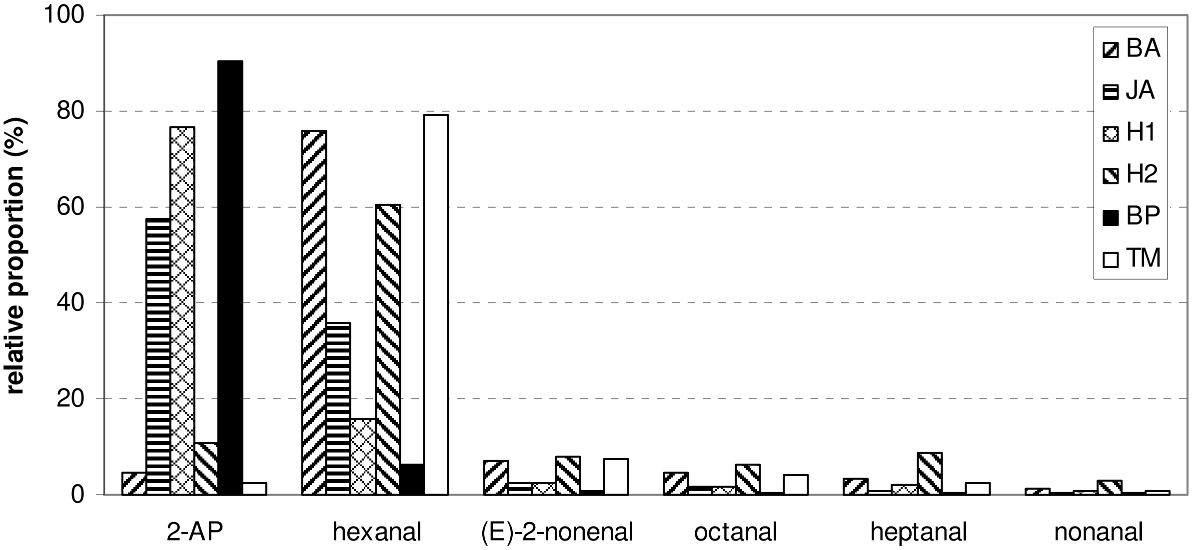

Figure 4.1. Relative proportion (percent) of OAVs of the primary odor-active compounds in cooked rice cultivars basmati (BA), jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), black pigmented rice (BP), and traditional medium rice (TM).

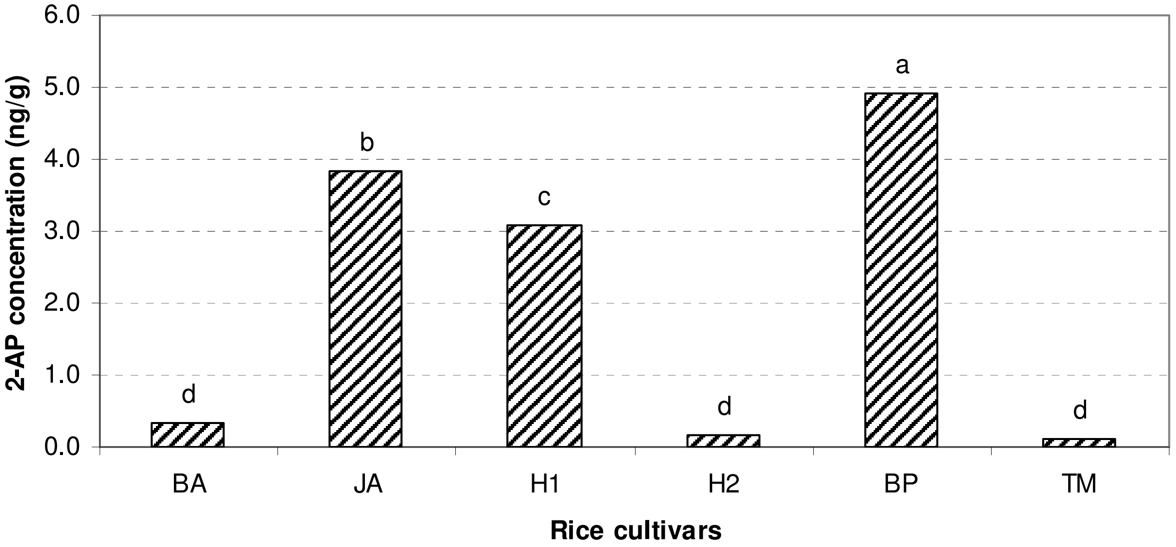

Figure 4.2. Comparison of concentration of 2-acetyl-1-pryrroline (2-AP) in cooked rice cultivars basmati (BA), jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), black pigmented rice (BP), and traditional medium rice (TM). Value was calculated in ng/g equivalent of 2,4,6-trimethylpryridine (TMP) as relative data. Vertical bars with different letters are significantly different ($p < 0.05$).

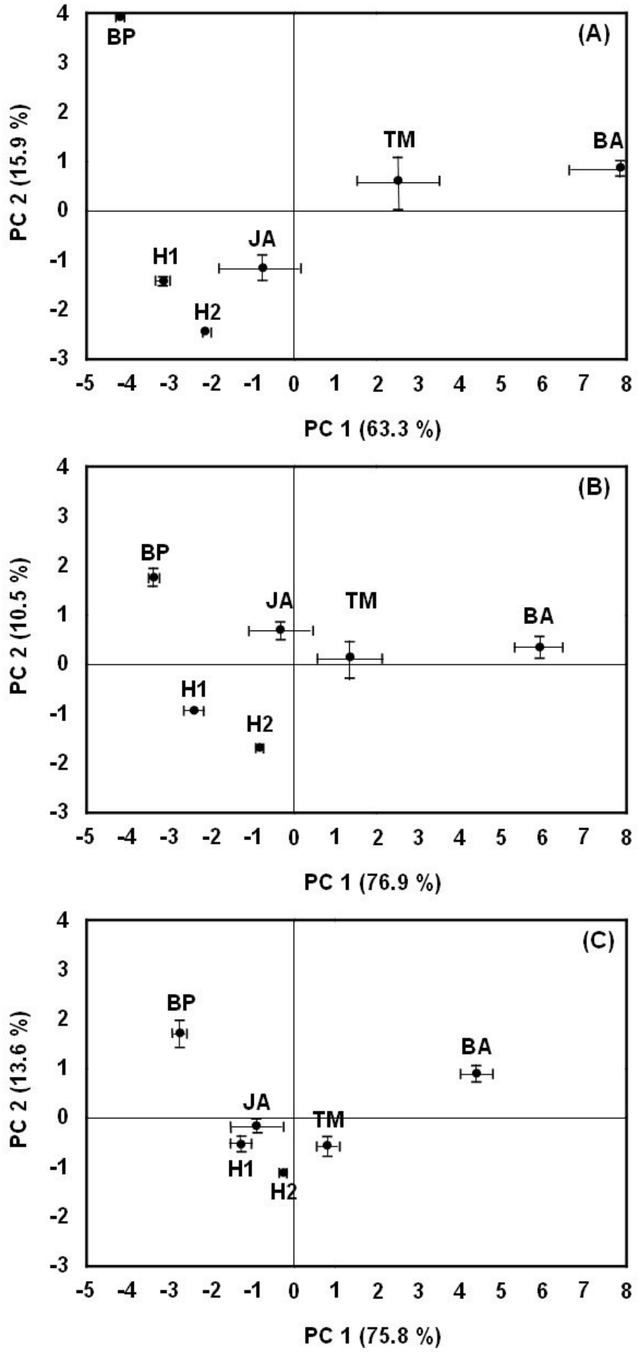

Figure 4.3. PCA Plot using OAVs of (A) 29 odor-active compounds, (B) 13 odor-active compounds and (C) 7 odor-active compounds in cooked rice cultivars basmati (BA), jasmine (JA), Hyangminbyeo 1 (H1), Hyangmibyeo 2 (H2), black pigmented Rice (BP), and traditional medium rice (TM), respectively. Vertical and horizontal bars represent the standard deviation.

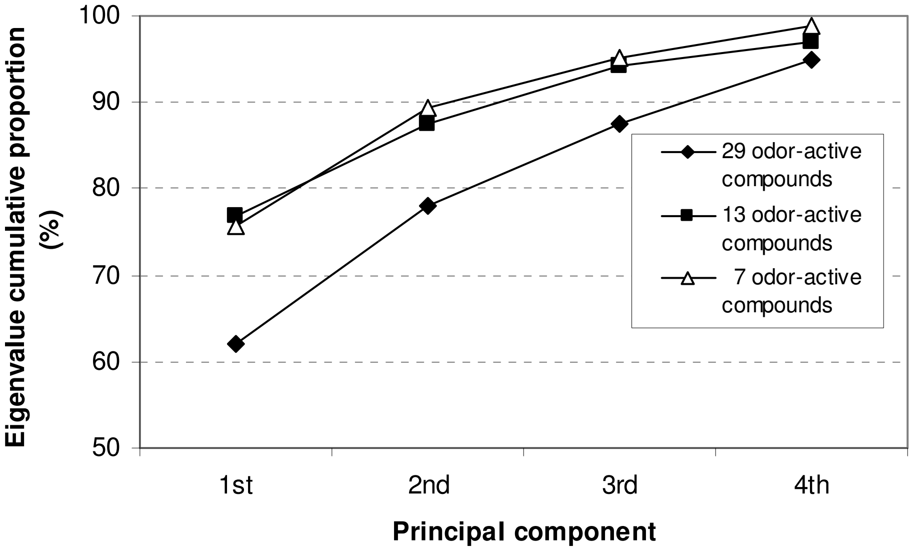

Figure 4.4. Eigenvalue cumulative proportion of first four principal components from PCA using 29 odor-active compounds, 13 odor-active compounds and 7 odor-active compounds in cooked rice cultivars

CHAPTER 5

DISCRIMINATION OF PREMIUM-QUALITY, BLACK-PIGMENTED, AND WAXY RICE BASED ON ODOR-ACTIVE COMPOUNDS [1]

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[1] Yang, T.S. and S.J. Kays. To be submitted to *Journal of Agricultural and Food Chemistry.*

ABSTRACT

Premium-quality, black-pigmented, and waxy rice types were discriminated based on six odor-active compounds [(*E*)-2-hexenal, heptanal, (*E*)-2-octenal, guaiacol, (*E*)-2-nonenal, and decanal] that were selected in two steps. Initially 21, 23, and 22 odorants in six premium-quality, three black-pigmented, and two waxy rice cultivars were detected using GC-O, respectively. Among the odorants, 18 primary odor-active compounds were selected based on their odor activity values (OAVs) by principal component analysis (PCA). The odorants, which including 10 aldehydes, 2 alcohols, 1 ketone, 4 aromatic compounds, and 1 nitrogen-containing compound, were subsequently reduced to 6 odor-active compounds by variable selection for canonical discriminant analysis (CDA). CDA clearly discriminated three rice types with the first (CDF 1) and second (CDF 2) canonical discriminant functions, accounting for 94.6 % and 5.4 % of the total variance, respectively. Two discriminant equations obtained by CDA identified (*E*)-2-hexenal, guaiacol, (*E*)-2-nonenal, and decanal as influencing CDF 1 and heptanal, (*E*)-2-nonenal, and decanal CDF 2. The two discriminant equations provided a predictive model for discriminating the three types of specialty rice. Comparison of cultivars within a specialty types demonstrated excellent separation which may be useful to rice breeders in making progeny selection decisions for improved flavor quality.

KEYWORDS: Specialty rice; *Oryza sativa*; dynamic headspace; Tenax trap; principal component analysis (PCA); canonical discriminant analysis (CDA); odor activity value (OAV); odor-active compounds; GC-O

INTRODUCTION

"Specialty rice" is a term used to distinguish cultivars that have unique properties (e.g., flavor, color, nutrition, chemical composition) from traditional rice. The demand for specialty rice has been increasing progressively and they are sold for as much as 50 % more than traditional rice due to their unique properties (*1*). Aromatic rice is an example of a type of specialty rice in which the cultivars have unique aromas. They are widely produced in India, Pakistan, and Thailand, and are popular in the orient, Middle East, Europe, and the United States (*2*). Other examples of types of specialty rice are premium-rice, which has superior flavor (*3*), black rice which has a unique color and flavor (*4*), and waxy rice has a very low amylose content and superior processing quality (*1*).

In Korea, six primary quality traits, considered critical in premium rice, are utilized as selection criteria in breeding programs (*3*). These are: 1) grain length/width ratio prior to milling (1.7-2.0); 2) appearance (size 172 g/1000 milled grains), very low chalkiness, translucency and gloss; 3) physicochemical properties (amylose content below 20 %, protein content of brown rice 7-9 %, high alkali digestion value); 4) appearance and palatability when cooked (glossy appearance, intact grains, weakly aromatic fragrance, sticky, good taste and texture); 5) slow retrogradation of cooked rice; and 6) milling recovery from rough to polished rice above 75 % and the head rice ratio above 90 %. The high quality afforded by premium rice increases consumer demand and sales.

In pigmented rice, the color varies from red to black. The color of black rice is due to anthocyanins. The higher protein and anthocyanin content in black rice increases its nutritional and antioxidant properties, which are beneficial to health (*5*, *6*). Black rice is popular in Asian countries, especially so in China, Sri Lanka, Indonesia, India, and Bangladesh where their rice germplasm collections contains359, 50, 42, 30, 25, and 24 accessions, respectively (*1*). Red rice contains four anthocyanin pigments (i.e., cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin-diglucoside, and cyanidin 3-rutinoside); the concentration of cyanidin 3-glucoside is exceptionally high in black rice (*7*). The pigmented rices are used as functional foods and as food colorants in that a portion of the water soluble anthocyanins alter the color of other components with which they are cooked (*1*).

Waxy rice is characterized by very low levels of amylose (0-5 %) and higher than normal amounts of amylopectin in the endosperm (*1*). Amylose content alters cooking and processing requirements, and the edibility of the product. Cooked low amylose rice is stickier and more tender, cohesive, and glossy than high amylose rice that is firmer and fluffier, and the grains do not stick together (*8*). Low amylose rice is widely grown in Laos, Northeast Thailand, and certain other Asian countries, where it is used in rice cakes and desserts due to its stickiness and for alcoholic beverages due to it conversion efficiency during fermentation (*1*).

Flavor is one of the most important factors in defining the character and quality of rice and in determining consumer preference. As a consequence, considerable interest has been focused on the identification and quantification of the volatile compounds emanating from cooked rice. Over 300 volatiles have been reported using a cross-section of isolation techniques: solvent extraction (*9*), distillation [modified Likens-Nickerson simultaneous distillation-extraction (*10*), and steam distillation (*11*)], and headspace sampling [solid-phase microextraction (*12*), and Tenax trapping (*13*)]. Dynamic headspace trapping onto Tenax TA (2,6-diphenylene oxide-based porous polymer), in particular, is widely used for studying organic volatiles in air, food or plants due to its ability to adsorb and desorb a wide-range of compounds (*14*). Tenax TA, which has a low affinity for water, is used for collecting volatiles from high moisture samples such as cooked foods. A dynamic headspace system using a Tenax trap in conjunction with short path thermal desorption can detect volatile organic compounds in the ppb range (*15*).

Among the volatile compounds identified in cooked rice, approximately 20 odor-active compounds [e.g., 2-acetyl-1-pyrroline, hexanal, (*E*)-2-nonenal, 2-amino acetophenone] have been reported using odor units and aroma extract dilution analysis and are known to contribute to the unique flavor of a cross-section of rice cultivars (*10*, *13*, *16*). For example, the popcorn-like odor in aromatic rice is conferred by 2-acetyl-1-pyrroline, while hexanal has been associated with the development of a rancid odor in aged rice (*9*). Recently the odor-active compounds in black-pigmented rice were determined; 2-acetyl-1-pyrroline and guaiacol were the primary components of its unique aroma (*4*).

Differences in odor-active compounds among of black-pigmented, premium-quality and waxy rice and cultivars of each have not been characterized. A more thorough understanding of what constitutes flavor quality would be advantageous in breeding superior cultivars of specialty rice. The objectives of this study were to: 1) identify and quantify the odor-active compounds emanating from three different types of specialty rice (premium-quality, black-pigmented, and waxy); 2) assess the importance of each odor-active compound in the overall aroma; and 3) determine the most important odor-active compounds contributing to the aroma of each.

MATERIALS AND METHODS

Materials. Six premium-quality (*Oryza sativa* L. *japonica* cv. Hwaseongbyeo, Ilpumbyeo, Gopumbyeo, Taebongbyeo, Chucheongbyeo, and Samkwangbyeo), three black-pigmented (*O. sativa* L. *japonica* cv. Heugjinjubyeo, Heugkwangbyeo, and Heugnambyeo), and two waxy cultivars (*O. sativa* L. *japonica* cv. Hwaseonchalbyeo, and *O. sativa* L. *indica* cv. Hangangchalbyeo) were grown at the National Institute of Crop Science, Suwon, South Korea in 2006. After harvest, the rice was sealed in glass and held at -20°C until analysis.

Chemicals. Analytical standard products utilized for identification and quantification were: benzaldehyde, decane, decanal, (*E*)-2-decenal, guaiacol, heptanal, heptane, 2-heptanone, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, undecane (Sigma-Aldrich Inc., St. Louis, MO); (*E*,*E*)-2,4-decadienal, hexanal, (*E*)-2-hexenal, 2-nonanone, (*E*)-2-octenal, 1-octen-3-ol (Aldrich Chem. Co., Milwaukee, WI); *p*-menthan-3-one, octanal, *p*-xylene (Fluka Chem. Co., Milwaukee, WI); naphthalene (J. T. Baker Inc., Phillipsburg, NJ); dodecane, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, and 2-pentylfuran (TCI America, Portland, OR); 3-octen-2-one (Alfa Aesar, Ward Hill, MA). 2,4,6-Trimethylpyridine (TMP) was purchased from Aldrich Chem. Co. (Milwaukee, WI) and used as a internal standard to quantify concentration of 2-acetyl-1-pyrroline.

Preparation of Cooked Rice. Rice samples (100g) were cooked in distilled water (150 mL) for 30 min at 100 ºC in a specially constructed 1 L glass beaker with entry and exit ports. The entry and exit ports were tightly sealed with aluminum foil during cooking. Due to differences in water absorption, 100 mL of distilled water was added to black rice samples (100 g) and similarly cooked for 30 min as described previously by Yang and others (2007a). Three replications of each cultivar were prepared and analyzed.

Dynamic Headspace Using Tenax Trap. Isolation of volatile compounds in cooked rice was performed using a method described previously (*4*). The glass beaker with freshly cooked rice was immediately placed in a hot water bath and maintained at 70 °C during sampling. Headspace volatiles emanating from cooked rice samples were collected using a Tenax trap and a vacuum sampling pump (Aircheck Sampler, Model 224-44XR, Eighty-Four, PA) that swept headspace over the sample. The air was purified by passing it through a charcoal filter (Alltech Assoc. Inc. Deerfield, IL; 1 cm i.d., 10 cm long Pyrex glass tube with a 7 cm bed of charcoal) connected to the entry port of the glass beaker at a rate of 150 mL/min for 60 min. A 50 mL Erlenmeyer flask was placed between the exit port and the trap to collect any condensation. One mL of 18.34 mg/L TMP solution in 0.1 M HCl used as an internal standard (*17*), was injected into the Erlenmeyer flask at the beginning of volatile collection.

After sampling, the Tenax trap was connected to an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, NJ) on the injector port of the gas chromatograph/mass spectrometer (GC/MS, Model 6890N/5973, Agilent, Palo Alto, CA). The volatiles were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min and the analytes collected on the first 4 cm of the GC column using a CO_2 cooled cryofocus trap (-40 °C) (SIS 2” Cryo-Trap, Scientific Instrument Services, NJ). After desorption, the cryofocus trap was rapidly heated to 200 °C and condensed analytes were separated utilizing GC temperature programming.

GC/MS and GC-Olfactometry (GC-O). Desorbed odorants were split between the mass spectrometer for identification and quantification and an Olfactory Detector Outlet used to describe the individual odorants and to assess their intensity (ODO II, SGE Intl., Austin, TX). The GC was equipped with a 30 m length, 0.25 mm i.d., 0.25 µm film thickness (5 % phenyl methyl silicone), fused silica capillary column (HP-5MS, Agilent, Palo Alto, CA). Injection port temperature was 225 °C with a split ratio of 0.5:1. He was used as the carrier gas with a flow rate of 2.0 mL/min. The column temperature was held at 40 °C for 1 min and then programmed 1.5 degrees/min to 65 °C and was held for 1 min, then 2 degrees/min to 120 °C and held for 1 min, and finally 15 degrees/min to 280 °C and held for 5 min. MS conditions were: ion source 230 °C; electron energy 70 eV; multiplier voltage 1247 V; GC/MS interface zone 280 °C; and a scan range of 35 to 350 atom mass units.

GC-O analysis was made by three assessors who had considerable previous experience in sensory tests. The assessors were trained by describing 15 materials with different odor: popcorn-like (popcorn), starchy (rice starch), woody (toothpicks), cooked grain (cream of wheat), corn (cream style corn), nutty (roasted peanut), floral (Jasmine scent), dairy (2% milk), hay (hay), barn (white pepper), buttery (butter), green (alfalfa), rancid (vegetable oil), waxy (candle) and earthy (mushroom) odors. An aroma extract of the sample was characterized by describing the aroma of the individual components and assessing their odor intensity on a 1 to 5 scale: 1 = very weak; 2 = weak; 3 = intermediate; 4 = strong; 5 = very strong. Odorants perceived by all 3 panelists were considered odor-active compounds (odor description and intensity data were not shown).

Identification and Quantification of Odorants. Odorants were identified based on their mass spectra using NIST 02 and Wiley 7 libraries. Identification was confirmed using Kovats' retention indices (RI) and odor descriptors with authentic standards. RIs were determined using a non-polar HP-5MS column and a series of *n*-hydrocarbons (C_7-C_{15}) and compared with those reported previously and at http://webbook.nist.gov/. In the absence of an authentic standard, the identification of 2-acetyl-1-pyrroline (2-AP) was confirmed by mass spectra, RI, and its distinctive popcorn odor. The concentration of each odorant was quantified using standard curves for each compound in hexane (0, 5, 10, 20, 50, 100, 200, and 500 ppm). The quantification of 2-AP was expressed as TMP equivalents.

Odor Thresholds in Air and Odor Activity Values. Odor thresholds in air were determined by GC-O to assess the importance of individual odorants (*18*). A solution of each odorant (10 mg) was prepared in 10 mL of hexane and 0.5 µL of the solution was injected into the GC and sniffed using an Olfactory Detector Outlet on the GC. The solution was diluted stepwise (1:1 v/v) until the odorant was not detected (minimum detectable concentration). The odor threshold in air for each odorant was calculated relative to minimum detectable concentration of (*E*)-2-decenal in air (2.7 ng/L). The odor threshold for 2-AP was reported previously (*19*). Odor activity values (OAVs) were calculated by dividing the absolute concentration of each odorant in a sample by its odor threshold.

Statistical Data Analysis. Principal component analysis (PCA) and stepwise canonical discriminate analysis (CDA) were carried out using the SAS system for Windows v8. PCA was performed with OAVs of the primary odor-active compounds which allowed visualization of differences in flavor among samples and determining which odor-active compounds had the highest loadings and contributed the most to differences in aroma among the samples. Odor-active compounds with the highest loadings were used for the subsequent computations. CDA was applied to the concentration of the main odor-active compounds selected by PCA to obtain the predictive equation by which specialty rice samples could be classified. For variable selection, Wilks' Lambda stepwise method was used recommending entering a variable when the level of significance is at most 0.05 and removing a variable when the level of significance is greater than 0.10.

RESULTS AND DISSCUTION

Odor-Active Compounds Emanating from Cooked Premium-Quality Cultivars. Among the premium cultivars (Hwaseongbyeo, Ilpumbyeo, Gopumbyeo, Taebongbyeo, Chucheongbyeo, and Samkwangbyeo) there were 21 odorants identified that included 12 aldehydes, 2 alcohols, 3 ketones, and 4 aromatic compounds (**Table 5.1**). Hexanal was quantitatively the most abundant odor-active compound in all six cultivars: e.g., Hwaseongbyeo (concentration: 16.8 ng/g; relative proportion: 59.3 %), Ilpumbyeo (14.5; 54.7), Gopumbyeo (24.3; 60.1), Taebongbyeo (34.2; 63.3), Chucheongbyeo (28.8; 65.2), and Samkwangbyeo (38.1; 66.0). In Gopumbyeo, Taebongbyeo, Chucheongbyeo, and Samkwangbyeo, nonanal (2.74 ng/g, 4.44 ng/g, 2.70 ng/g, and 3.89 ng/g), (*E*)-2-heptenal (1.95, 2.28, 1.82, and 2.48), 2-pentylfuran (1.46, 1.77, 1.50, and 1.88), and heptanal (1.30, 1.59, 1.24, and 1.86) followed hexanal in abundance, respectively. In Hwaseongbyeo, nonanal, 2-pentylfuran, (*E*)-2-heptenal, and heptanal followed hexanal and in Ilpumbyeo, nonanal, heptanal, naphthalene, and 2-pentylfuran. Therefore, aldehydes like hexanal, nonanal, (*E*)-2-heptenal, and heptanal were quantitatively the main odor-active compounds in the six premium-quality cultivars. 1-Pentanol (plastic odor), 2-heptanone (fruity odor), and 2-pentylfuran (floral odor) were the primary odor-active alcohol, ketone, and aromatic compounds, respectively.

Odor-Active Compounds from Cooked Black-Pigmented Cultivars. Twenty-three odorants were identified in the black-pigmented cultivars Heugjinjubyeo and Heugnambyeo and 22 in Heugkwnagbyeo. Eleven 11 aldehydes, 2 alcohols, 2 ketones, 6 aromatics and 1 nitrogen containing compound were found in all cultivars (**Table 5.2**). 2-Nonanone was present in Heugjinjubyeo and Heugnambyeo but not Heugkwnagbyeo. Hexanal was quantitatively the most abundant odor-active compound in black rice. 2-AP, which confers a popcorn-like odor, was also a significant component in the aroma of the cultivar. 2-AP used as an indicator of aromatic rice during selection in breeding program was present in black rice at substantially greater level than in commercial basmati and jasmine rice (*20*). The concentration of 2-AP varied substantially among three black rice cultivars from a low of 0.05 ng/g in Heugnambyeo to a high of 11.38 ng/g in Heugjinjubyeo (**Table 5.2**). Guaiacol was detected only in black rice [e.g., Heugjinjubyeo (1.67 ng/g), Heugkwangbyeo (1.59 ng/g), and Heugnambyeo (1.13 ng/g)], where it is a key aroma compound that confers a "smoked" odor and has a low odor threshold (*4*, *20*).

Odor-Active Compounds from Cooked Waxy Cultivars. Hwaseonchalbyeo and Hangangchalbyeo cultivars have glutinous endosperms due to low amylose (i.e., less than 5 %) which confers a soft, waxy and sticky texture and a low gel consistency and gelatinization temperature (*3*, *21*). Twenty-two odorants were identified in Hwaseonchalbyeo and 21 in Hangangchalbyeo including 12 aldehydes, 1 alcohol, 3 ketones, 1 hydrocarbon, and 4 aromatic compounds. 1-Pentanol was present only in Hwaseonchalbyeo (**Table 5.2**). The hexanal level in Hwanseonchalbyeo and Hangangchalbyeo was 340.8 and 483.3 ng/g, respectively, 8.9 and 12.7 times higher than that in Samkwangbyeo which showed the highest hexanal level in premium-quality cultivars (**Table 5.1**). The very high hexanal concentration appears to be due to the higher lipid content in waxy rice and its subsequent degradation resulting in the formation of hexanal (*22*). The odor of hexanal is described as green or green tomato and has been associated with rancidity and consumer rejection of rice (*9*). Dodecane was detected in only the two waxy cultivars and was not found in either the premium or black-pigmented cultivars. Its low concentration and high odor threshold in air indicates that it makes only a minor contribution to the aroma.

Assessment of the Importance of Each Odor-Active Compound. The odor threshold in air of each of the odorants was determined using GC-O and a stepwise dilution with authentic standards. The odor activity value (OAV) was calculated for each odorant in each sample by dividing its concentration by its odor threshold (**Table 5.3**). The OAV gives an indication of the importance of each odor-active compound to the overall aroma of each sample. Based on the OAV, hexanal was the dominant odorant, followed by (*E*)-2-nonenal, octanal, heptanal, (*E*,*E*)-2,4-nonadienal, and nonanal in six premium-quality cultivars. Collectively they accounted for over 95 % of the total OAV and were key aroma compounds in premium rice. These cultivars had lower OAVs than the more aromatic black rice, indicating that the aroma of these premium cultivars was less pronounced. In the black rice cultivars Heugjinjubyeo and Heugkwangbyeo, 2-AP had the highest OAV (e.g., 569.2 and 285.6, respectively) followed by hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal. In the cultivar Heugnambyeo, hexanal was the highest, followed by (*E*)-2-nonenal, octanal, heptanal, nonanal, and 2-AP. Guaiacol had OAV of 1.12, 1.07, and 0.76 in Heugjinjubyeo, Heuckkwangbyeo, and Heugnambyeo, respectively. Even though their OAVs were not as high as hexanal, guaiacol and 2-AP contributed to the unique aroma of black rice due to their unique odors and low odor threshold values. In waxy rice, hexanal in Hwaseonchalbyeo (315.5), a japonica type and Hangangchalbyeo (447.5), an indica type, had the highest OAVs, however, the cultivars differed in the pattern of the other odor-active compounds present. Japonica and indica waxy rice cultivars have different physicochemical properties (e.g., water binding capacity of starch, swelling power, hardness) which may be related to the formation and emission of volatile compounds (*23*).

Principal Component Analysis of Odor-Active Compounds in Specialty Rice. PCA was applied using the OAV of the odor-active compounds in each sample to determine if there are general differences in odor among the types of specialty rice. Premium-quality (P1 to P6), black-pigmented (B1 to B3), and waxy (W1 and W2) types were clearly separated with the first (PC 1) and second (PC 2) principal components accounting for 53.5 % and 20.7 % of the total variance, respectively (**Figure 5.1**). The premium-quality cultivars were positioned on the negative side, the waxy cultivars on the positive side, and the black-rice cultivars near the zero value of PC 1. The main compounds influencing PC 1 were, in decreasing order of their contribution, (*E*)-2-nonenal (19)[1], hexanal (2), 2-pentylfuran (11), 2-heptanone (5), heptanal (6), 3-octen-2-one (13), (*E*)-2-octenal (14), 1-octen-3-ol (10), (*E*)-2-heptenal (8), octanal (12), (*E*,*E*)-2,4-nonadienal (23), and (*E*)-2-hexenal (3); while dodecane (21) had a high loading, it was removed from the main compounds due to its high odor threshold (202.4 ng/L). In contrast, the black-pigmented cultivars positioned on the positive value of PC 2, were clearly separated from the premium-

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[1] Numbers for compounds in the text and Figure 5.1 correspond to those in Table 5.3.

quality and waxy cultivars. The main compounds influencing the differences on PC 2 were, in decreasing order of contribution, guaiacol (15), benzaldehyde (9), decanal (22), *p*-xylene (4), 2-AP (7), and nonanal (17). Therefore, 18 odor-active compounds from PC 1 and PC 2, gave excellent separation of three types of specialty rice.

Discrimination of Specialty Rices Using Stepwise Canonical Discriminant Analysis. Canonical discriminant analysis (CDA) is a useful statistical tool for determining the variables that can discriminate between more than two groups and suggesting the best predictive model with the variables (*24*). Among the 18 main odor-active compounds obtained from the previous PCA, stepwise variable selection was used to determine the variables for CDA. Six odor-active compounds were selected [(*E*)-2-hexenal, heptanal, (*E*)-2-octenal, guaiacol, (*E*)-2-nonenal, and decanal] and CDA applied using the concentration of each. Two statistically significant canonical discriminant functions were obtained and tested the significance using the Wilks' lambda statistic (**Figure 5.2**). Based on the extremely low value (0.00002186), it was concluded that the three groups were significantly different (*25*). The separation of three groups is readily evident with 94.6 % and 5.4 % of the total variance accounted for by the first (CDF 1) and second canonical discriminant functions (CDF 2), respectively. CDF 1 separated black-pigmented cultivars from premium-quality and waxy cultivars, while CDF 2 separated the premium-quality from the waxy cultivars. CDF 1 and CDF 2 obtained by CDA of six odor-active compounds, could readily distinguish the three groups of specialty rice. Discriminant equations for CDF 1 and CDF 2 (**Figure 5.2**) allowed determining which compounds were the most important. The following equation is for CDF 1:

D_1 = 64.27 [(*E*)-2-hexenal] + 7.11 [heptanal] + 3.48 [(*E*)-2-octenal] + 95.02 [guaiacol] – 94.65 [(*E*)-2-nonenal] + 50.5 [decanal] – 61.10

Discrimination of black-pigmented cultivars from premium-quality and waxy cultivars was explained by D_1. (*E*)-2-Hexenal, guaiacol, and decanal with positive coefficients have a greater contribution to black-pigmented cultivars on the positive side of the *x*-axis in **Figure 5.2**, while (*E*)-2-nonenal with negative coefficients has a greater contribution to premium-quality and waxy cultivars. In contrast, the equation for CDF 2 was:

D_2 = -1.48 [(*E*)-2-hexenal] + 9.79 [heptanal] + 13.19 [(*E*)-2-octenal] - 3.70 [guaiacol] - 35.28 [(*E*)-2-nonenal] - 10.91 [decanal] -16.73

Discrimination of waxy cultivars from premium-quality cultivars was explained by D_2. Heptanal and (*E*)-2-octenal with positive coefficients contributed to the waxy cultivars on the *y*-axis in **Figure 5.2**, while (*E*)-2-nonenal and decanal with negative coefficients were important in discriminating premium-quality cultivars. If these discriminant equations are validated by other cultivars, they can be considered a good predictive model with strong discriminant power.

Difference of Odor-Active Compounds within Each Rice Type. **Figure 5.1** shows the PCA pattern for three rice types, while **Figure 5.3** and **Figure 5.4** shows the pattern for the odor-active compounds within a rice type (i.e., premium-quality and black-pigmented cultivars). The different pattern within waxy rice shown in **Figure 5.1** was explained by the different physicochemical properties between japonica (Hwaseonchalbyeo) and indica (Hangangchalbyeo) ecotypes. Premium rice cultivars were separated with PC 1 and PC 2, accounting for 82.9 % of the total variance (**Figure 5.3**). Samkwangbyeo (P6) and Taebongbyeo (P4) were positioned on the positive side of the *x*-axis (PC 1), however, Ilpumbyeo (P2) and Hwaseongbyeo (P1) were positioned on the negative side of PC 1. Gopumbyeo (P3) and Chucheongbyeo (P5) were near the zero of PC 1. Based on the PC 1, premium cultivars were divided into three groups, accounting for 67.7 % of total variance. The main compounds influencing PC 1 were, in decreasing order of their contribution, (*E*)-2-nonenal (19), (*E*)-2-heptenal (8), hexanal (2), 2-pentrylfuran (11), (*E*)-2-decenal (24), (*E*,*E*)-2,4-decadienal (25), 1-octen-3-ol (10), (*E*)-2-octenal (14), heptanal (6), decanal (22), *p*-xylene (4), 2-heptanone (5), (*E*,*E*)-2,4-nonadienal (23), octanal (12), 2-nonanone (16), and nonanal (17). PC 2 separated two groups (Samkwangbyeo and Taebongbyeo; Ilpumbyeo and Hwaseongbyeo) with similar values on PC 1, accounting for 15.2 % of total variance. Taebongbyeo and Hwaseongbyeo positioned on the positive side of *y*-axis (PC 2) were separated from Samkwangbyeo and Ilpumbyeo on the negative side of PC 2, respectively. (*E*)-2-hexenal and 1-pentanol largely influenced their separation. Within premium rice cultivars, only Chucheongbyeo and Gopumbyeo appeared to be similar aromas; the other cultivars appeared to possess differing aromas.

The three black cultivars were clearly separated with PC 1 and PC 2, accounting for 68.9 % and 31.1 % of the total variance, respectively (**Figure 5.4**). Heugjinjubyeo (B1) was positioned on the negative side, and Heugnambyeo (B3) and Heugkwangbyeo (B2) were positioned on the positive side of the PC 1. Heugkwangbyeo (B2) was positioned on the positive side of PC2 while Heugnambyeo (B3) was positioned on the negative side of PC2. The main compounds contributing to separating the three cultivars were: Heugjinjubyeo [2-acetyl-1-pyrroline (7), hexanal (2), 1-octen-3-ol (10), (*E*)-2-octenal (14), 2-pentrylfuran (11), guaiacol

(15), naphthalene (20), 2-nonanone (16), and 1-pentanol (1)]; Heugkwangbyeo [(*E*,*E*)-2,4-nonadienal (23), 2-heptanone (5), *p*-xylene (4), and *p*-menthan-3-one (18)]; and Heugnambyeo [(*E*,*E*)-2,4-decadienal (25), octanal (12), (*E*)-2-decenal (24), nonanal (17), heptanal (6), (*E*)-2-nonenal (19), 3-octen-2-one (13), decanal (22), benzaldehyde (9), and (*E*)-2-hexenal].

As demonstrated in **Figure 5.2**, the ability to discriminate between rice types will facilitate our understanding of differences in flavor requirements. In addition, comparisons among progeny within a type of specialty rice will potentially facilitate making selection decisions in rice breeding programs, accelerating the development of superior new cultivars.

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Table 5.1. Concentration of Odor-Active Compounds in Six Premium-Quality Rice Cultivars[a]

			concentration (ng/g)[b]						
no.	odorant	R.I.[c]	P1	P2	P3	P4	P5	P6	ID[d]
1	1-pentanol	766	0.79 ± 0.22	0.38 ± 0.11	1.26 ± 0.17	1.07 ± 0.19	1.16 ± 0.02	0.58 ± 0.47	A
2	hexanal	803	16.78 ± 3.00	14.51 ± 3.47	24.29 ± 1.31	34.23 ± 5.18	28.80 ± 0.20	38.08 ± 7.59	A
3	(*E*)-2-hexenal	857	0.16 ± 0.04	0.08 ± 0.05	0.15 ± 0.03	0.17 ± 0.03	0.17 ± 0.03	0.14 ± 0.07	A
4	*p*-xylene	859	0.30 ± 0.02	0.33 ± 0.05	0.36 ± 0.07	0.36 ± 0.04	0.34 ± 0.02	0.44 ± 0.06	A
5	2-heptanone	895	0.55 ± 0.06	0.34 ± 0.20	0.68 ± 0.06	0.86 ± 0.11	0.79 ± 0.07	0.83 ± 0.11	A
6	heptanal	903	0.94 ± 0.14	1.19 ± 0.27	1.30 ± 0.13	1.59 ± 0.25	1.24 ± 0.04	1.86 ± 0.27	A
9	(*E*)-2-heptenal	954	1.21 ± 0.37	0.92 ± 0.46	1.95 ± 0.29	2.28 ± 0.31	1.82 ± 0.09	2.48 ± 0.23	A
10	1-octen-3-ol	984	0.37 ± 0.10	0.36 ± 0.13	0.56 ± 0.10	0.73 ± 0.13	0.54 ± 0.02	0.62 ± 0.07	A
11	2-pentrylfuran	992	1.28 ± 0.14	0.99 ± 0.15	1.46 ± 0.17	1.77 ± 0.30	1.50 ± 0.04	1.88 ± 0.23	A
12	octanal	1005	0.67 ± 0.08	0.98 ± 0.22	0.95 ± 0.13	1.45 ± 0.23	0.96 ± 0.03	1.38 ± 0.27	A
13	3-octen-2-one	1036	0.16 ± 0.05	0.04 ± 0.02	0.27 ± 0.12	0.37 ± 0.06	0.05 ± 0.03	0.15 ± 0.02	A
14	(*E*)-2-octenal	1058	0.82 ± 0.18	0.65 ± 0.17	1.06 ± 0.10	1.12 ± 0.15	0.88 ± 0.03	1.14 ± 0.15	A
16	2-nonanone	1093	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	A
17	nonanal	1106	1.71 ± 0.28	3.01 ± 0.64	2.74 ± 0.55	4.44 ± 0.62	2.70 ± 0.17	3.89 ± 0.77	A
18	*p*-menthan-3-one	1152	0.08 ± 0.04	0.12 ± 0.02	0.19 ± 0.15	0.06 ± 0.04	0.20 ± 0.14	0.19 ± 0.10	A
19	(*E*)-2-nonenal	1160	0.30 ± 0.07	0.32 ± 0.10	0.41 ± 0.12	0.52 ± 0.08	0.42 ± 0.07	0.57 ± 0.10	A
20	naphthalene	1172	0.79 ± 0.24	1.03 ± 0.26	1.13 ± 0.33	1.01 ± 0.27	0.87 ± 0.14	1.10 ± 0.32	A
22	decanal	1206	0.33 ± 0.08	0.41 ± 0.13	0.42 ± 0.11	0.61 ± 0.14	0.43 ± 0.05	0.69 ± 0.10	A
23	(*E*,*E*)-2,4-nonadienal	1212	0.16 ± 0.06	0.14 ± 0.06	0.23 ± 0.07	0.22 ± 0.06	0.19 ± 0.06	0.22 ± 0.05	A
24	(*E*)-2-decenal	1262	0.18 ± 0.06	0.18 ± 0.05	0.24 ± 0.08	0.26 ± 0.06	0.24 ± 0.07	0.35 ± 0.07	A
25	(*E*,*E*)-2,4-decadienal	1315	0.54 ± 0.17	0.36 ± 0.15	0.58 ± 0.20	0.82 ± 0.13	0.70 ± 0.09	0.92 ± 0.08	A

[a] P1, Hwaseongbyeo; P2, Ilpumbyeo; P3, Gopumbyeo; P4, Taebongbyeo; P5, Chucheongbyeo; P6, Samkwangbyeo. [b] All of the values are given as the mean of three samples with the standard deviation. [c] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [d] Method of idenrification: A, Compounds were identified by comparison of mass spectrum and retention index of an perceived compound with those of an authentic compound.; B, Compounds were tentatively identified by comparison of mass spectrum, odor description, and RI with those from the literature when reference compounds were not available.

Table 5.2. Concentration of Odor-Active Compounds in Three Black-Pigmented [a] and Two Waxy Rice Cultivars [b]

			concentration (ng/g)[c]					
no.	odorant	RI.[d]	B1	B2	B3	W1	W2	ID[e]
1	1-pentanol	766	2.01 ± 0.06	0.93 ± 0.15	1.56 ± 0.34	3.29 ± 0.25	nd[d]	A
2	hexanal	803	95.60 ± 2.43	39.54 ± 5.26	40.22 ± 9.17	340.76 ± 2.73	483.34 ± 5.86	A
3	(*E*)-2-hexenal	857	0.31 ± 0.03	0.33 ± 0.06	0.59 ± 0.11	0.46 ± 0.08	0.92 ± 0.02	A
4	*p*-xylene	859	0.90 ± 0.03	2.96 ± 0.27	1.01 ± 0.20	0.46 ± 0.06	1.24 ± 0.01	A
5	2-heptanone	895	2.36 ± 0.06	3.22 ± 0.34	1.75 ± 0.35	3.98 ± 0.53	7.97 ± 0.03	A
6	heptanal	903	2.37 ± 0.07	3.48 ± 0.42	4.37 ± 0.86	4.54 ± 0.64	6.39 ± 0.05	A
7	2-acetyl-1-pyrroline	918	11.38 ± 1.34	5.71 ± 1.62	0.05 ± 0.01	nd	nd	B
8	benzaldehyde	952	1.01 ± 0.29	4.46 ± 0.83	4.25 ± 0.83	nd	nd	A
9	(*E*)-2-heptenal	954	nd	nd	nd	6.95 ± 0.79	9.97 ± 0.21	A
10	1-octen-3-ol	984	1.38 ± 0.11	0.65 ± 0.32	0.74 ± 0.17	3.21 ± 0.46	7.38 ± 0.62	A
11	2-pentrylfuran	992	2.91 ± 0.06	2.00 ± 0.21	2.16 ± 0.44	5.80 ± 0.91	10.28 ± 0.58	A
12	octanal	1005	1.70 ± 0.05	2.21 ± 0.32	2.54 ± 0.47	4.94 ± 0.70	2.99 ± 0.05	A
13	3-octen-2-one	1036	0.36 ± 0.05	0.56 ± 0.10	0.87 ± 0.19	0.94 ± 0.17	1.84 ± 0.12	A
14	(*E*)-2-octenal	1058	1.60 ± 0.16	1.51 ± 0.22	1.15 ± 0.24	4.16 ± 0.55	3.33 ± 1.13	A
15	guaiacol	1086	1.67 ± 0.23	1.59 ± 0.23	1.13 ± 0.31	nd	nd	A
16	2-nonanone	1093	0.18 ± 0.02	nd	0.06 ± 0.05	0.17 ± 0.03	0.25 ± 0.02	A
17	nonanal	1106	6.25 ± 0.41	7.77 ± 1.05	8.99 ± 1.99	9.19 ± 1.40	5.96 ± 0.09	A
18	*p*-menthan-3-one	1152	0.12 ± 0.02	0.46 ± 0.24	0.23 ± 0.09	0.12 ± 0.03	1.55 ± 0.13	A
19	(*E*)-2-nonenal	1160	0.49 ± 0.10	0.66 ± 0.13	0.68 ± 0.17	0.94 ± 0.13	1.20 ± 0.09	A
20	naphthalene	1172	1.21 ± 0.21	1.18 ± 0.29	0.98 ± 0.26	0.74 ± 0.25	0.89 ± 0.20	A
21	dodecane	1200	nd	nd	nd	0.18 ± 0.04	0.24 ± 0.07	A
22	decanal	1206	0.71 ± 0.07	0.99 ± 0.12	1.46 ± 0.29	0.86 ± 0.11	0.57 ± 0.07	A
23	(*E*,*E*)-2,4-nonadienal	1212	0.30 ± 0.06	0.32 ± 0.05	0.28 ± 0.10	0.43 ± 0.14	0.33 ± 0.10	A
24	(*E*)-2-decenal	1262	0.19 ± 0.05	0.25 ± 0.09	0.27 ± 0.05	0.60 ± 0.12	0.31 ± 0.07	A
25	(*E*,*E*)-2,4-decadienal	1315	0.32 ± 0.11	0.52 ± 0.18	0.64 ± 0.17	0.77 ± 0.22	0.80 ± 0.10	A

[a] B1, Heugjinjubyeo; B2, Heugkwangbyeo; B3, Heugnambyeo. [b] W1, Hwaseonchalbyeo; W2, Hangangchalbyeo. [c] All of the values are given as the mean of three samples with the standard deviation. [d] Retention index based on HP-5MS column using a series of *n*-hydrocarbons. [e] Method of idenrification: A, Compounds were identified by comparison of mass spectrum and RI of an perceived compound with those of an authentic compound.; B, Compounds were tentatively identified by comparison of mass spectrum, odor description, and RI with those from the literature when reference compounds were not available. [d] nd: not detected.

Table 5.3. Odor Activity Value (OAV)[a] of Odor-Active Compounds in Six Premium-Quality, Three Black-Pigmented, and Two Waxy Rice Cultivars

		premium-quality rice[b]						black-pigmented rice[c]			waxy rice[d]		
no	odorant	P1	P2	P3	P4	P5	P6	B1	B2	B3	W1	W2	OT[e]
1	1-pentanol	0.01	tr[f]	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.02	nd[g]	152.97
2	hexanal	15.53	13.43	22.49	31.70	26.67	35.26	88.52	36.61	37.24	315.52	447.53	1.08
3	(*E*)-2-hexenal	0.05	0.03	0.05	0.05	0.06	0.04	0.10	0.11	0.19	0.15	0.30	3.12
4	*p*-xylene	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.06	0.02	0.01	0.03	46.89
5	2-heptanone	0.16	0.10	0.20	0.25	0.23	0.24	0.68	0.93	0.50	1.14	2.29	3.48
6	heptanal	1.08	1.37	1.50	1.82	1.43	2.14	2.73	4.00	5.03	5.22	7.35	0.87
7	2-acetyl-1-pyrroline	nd	nd	nd	nd	nd	nd	569.24	285.60	2.24	nd	nd	0.02[h]
8	(*E*)-2-heptenal	0.09	0.07	0.15	0.17	0.14	0.19	nd	nd	nd	0.52	0.74	13.42
9	benzaldehyde	nd	nd	nd	nd	nd	nd	0.01	0.05	0.05	nd	nd	84.80
10	1-octen-3-ol	0.14	0.13	0.21	0.27	0.20	0.23	0.51	0.24	0.27	1.19	2.73	2.70
11	2-pentrylfuran	0.07	0.05	0.08	0.09	0.08	0.10	0.15	0.10	0.11	0.30	0.53	19.27
12	octanal	1.52	2.22	2.16	3.30	2.18	3.13	3.87	5.02	5.78	11.24	6.80	0.44
13	3-octen-2-one	0.02	0.01	0.04	0.05	0.01	0.02	0.05	0.08	0.13	0.14	0.27	6.73
14	(*E*)-2-octenal	0.31	0.24	0.39	0.42	0.33	0.42	0.60	0.56	0.43	1.55	1.24	2.69
15	guaiacol	nd	nd	nd	nd	nd	nd	1.12	1.07	0.76	nd	nd	1.49
16	2-nonanone	tr	tr	tr	tr	tr	tr	0.01	nd	tr	0.01	0.01	30.78
17	nonanal	0.65	1.14	1.04	1.68	1.02	1.47	2.37	2.94	3.40	3.48	2.26	2.64
18	*p*-menthan-3-one	0.02	0.02	0.04	0.01	0.04	0.04	0.03	0.10	0.05	0.03	0.33	4.75
19	(*E*)-2-nonenal	3.32	3.58	4.51	5.77	4.66	6.37	5.46	7.29	7.50	10.42	13.38	0.09
20	naphthalene	0.02	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.02	0.03	33.00
21	dodecane	nd	nd	nd	nd	nd	nd	nd	nd	nd	tr	tr	202.37
22	decanal	0.12	0.15	0.16	0.23	0.16	0.26	0.27	0.38	0.55	0.33	0.22	2.65
23	(*E*,*E*)-2,4-nonadienal	0.89	0.80	1.27	1.23	1.04	1.22	1.64	1.79	1.54	2.38	1.86	0.18
24	(*E*)-2-decenal	0.07	0.07	0.09	0.10	0.09	0.13	0.07	0.09	0.10	0.22	0.11	2.70
25	(*E*,*E*)-2,4-decadienal	0.24	0.16	0.25	0.36	0.30	0.40	0.14	0.23	0.28	0.34	0.35	2.30

[a] OAVs are given as the mean of three sampes. [b] P1, Hwaseongbyeo; P2, Ilpumbyeo; P3, Gopumbyeo; P4, Taebongbyeo; P5, Chucheongbyeo; P6, Samkwangbyeo. [c] B1, Heugjinjubyeo; B2, Heugkwangbyeo; B3, Heugnambyeo. [d] W1, Hwaseonchalbyeo; W2, Hangangchalbyeo. [e] OT, odor threshold in air (ng / L). [f] tr, trace value (< 0.01) [g] nd, not detected. [h] Data from (*22*).

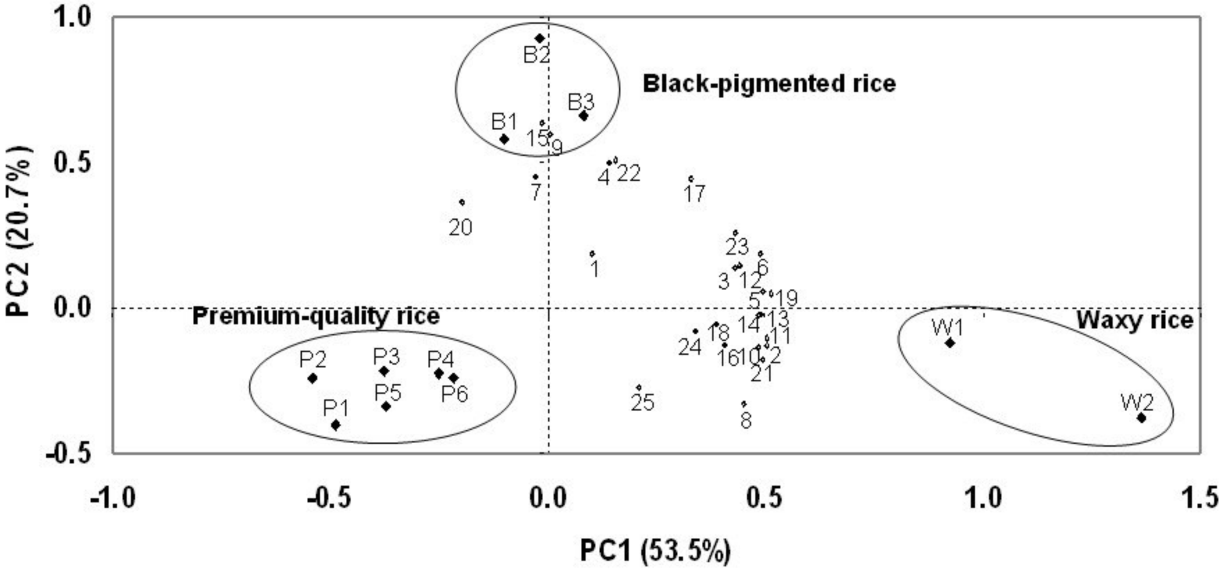

Figure 5.1. PCA biplot of odor-active compounds in cooked specialty rices based on their odor active values (OAVs). Numbers correspond to compounds listed in **Table 5.3**.

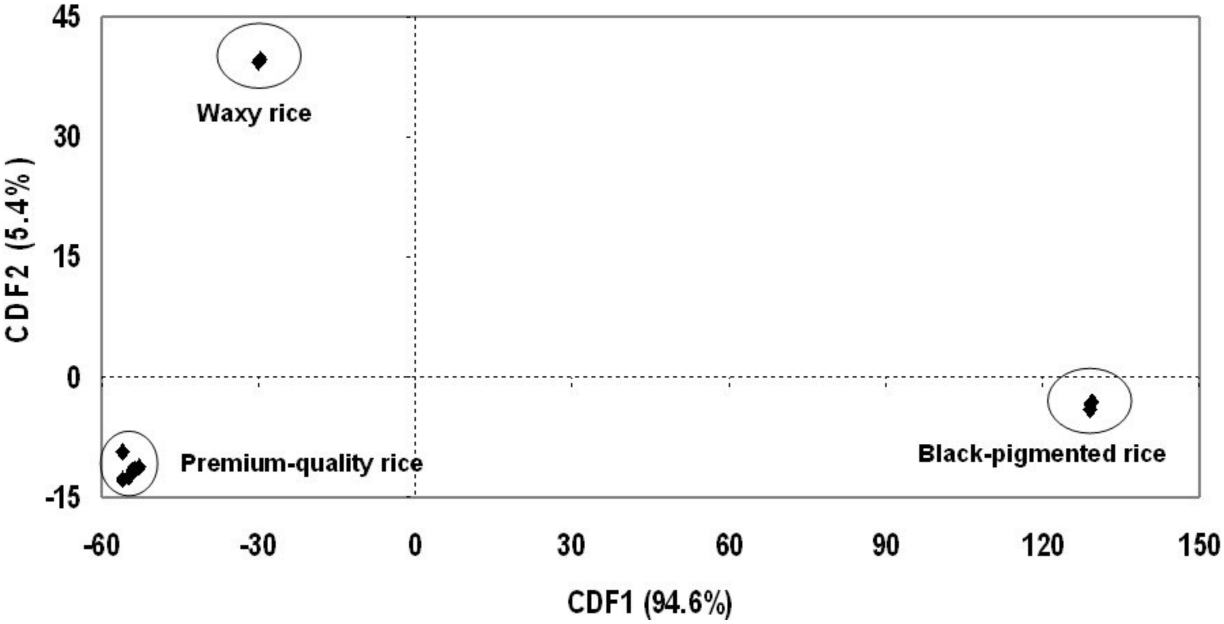

Figure 5.2. Separation of rice specialty type using scatter plot for scores of odor-active compounds based on the first two canonical discriminant functions.

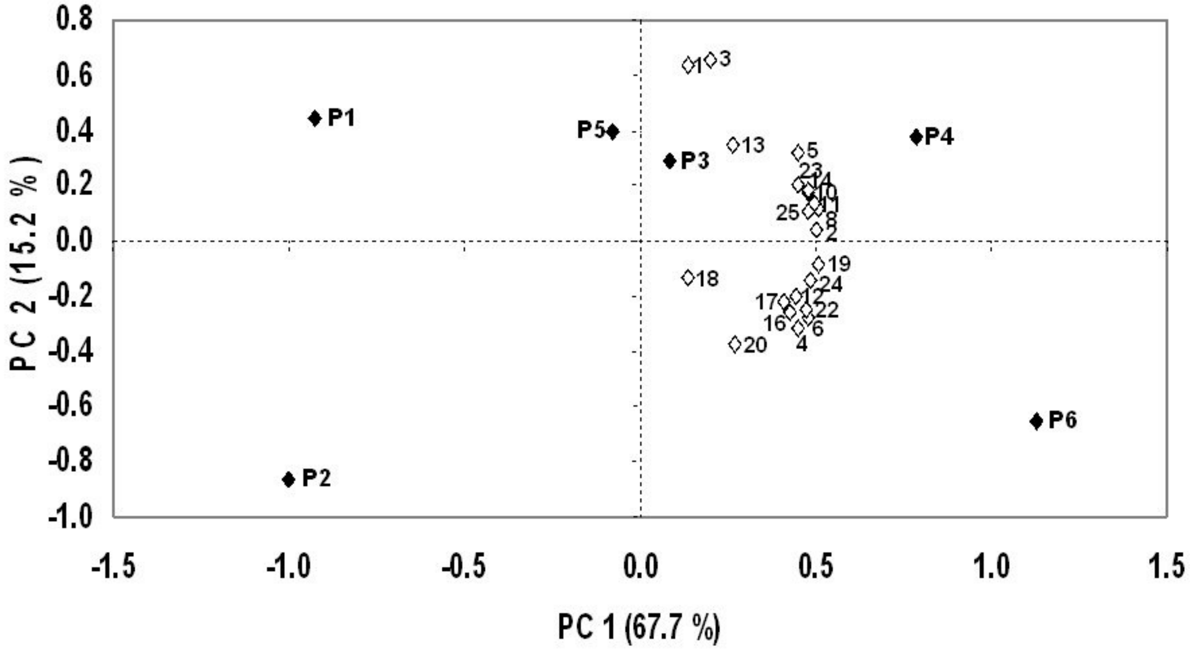

Figure 5.3. PCA biplot of odor-active compounds in premium-quality rice cultivars (P1, Hwaseongbyeo; P2, Ilpumbyeo; P3, Gopumbyeo; P4, Taebongbyeo; P5, Chucheongbyeo; P6, Samkwangbyeo) based on their concentration. Numbers correspond to compounds listed in **Table 5.3**.

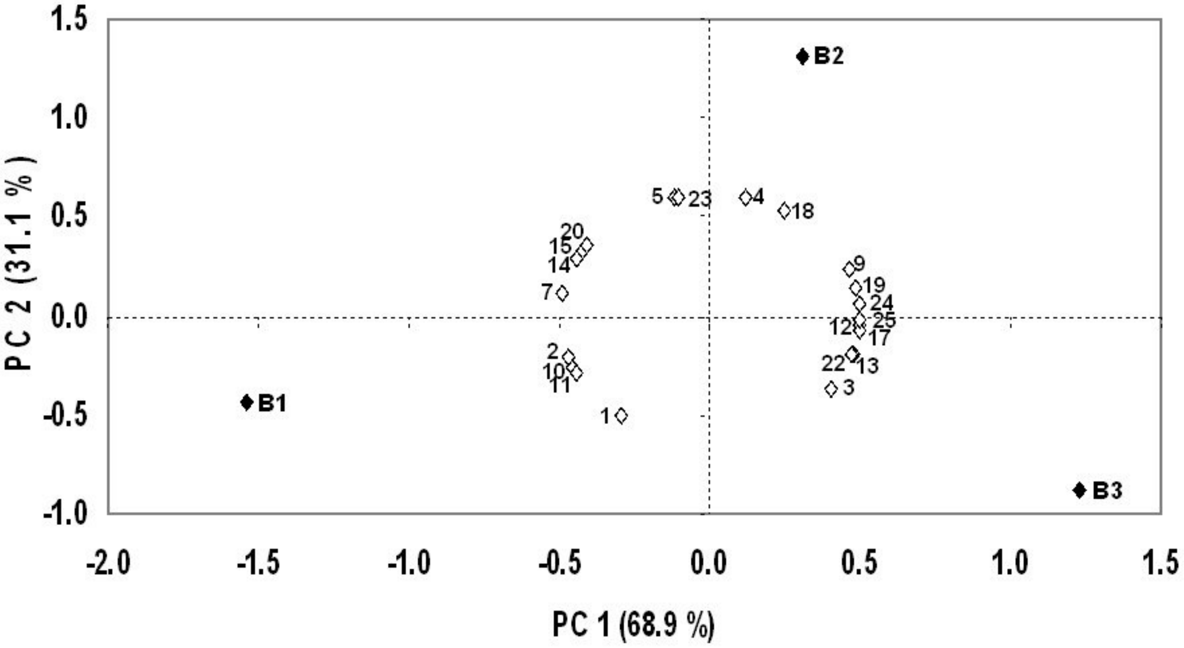

Figure 5.4. PCA biplot of odor-active compounds in black-pigmented rice cultivars (B1, Heugjinjubyeo; B2, Heugkwangbyeo; B3, Heugnambyeo) based on their concentration. Numbers correspond to compounds listed in **Table 5.3**.

CHAPTER 6

RICE VOLATILE COMPOUNDS – SITE OF ORIGIN [1]

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[1] Yang, T.S. and S.J. Kays. To be submitted to *Food Chemistry.*

ABSTRACT

Volatile compounds emanating from 3 cultivars of cooked rice milled to different degrees (0, 6 and 8 % by weight) were compared to ascertain their site of origin and the effect of pigmentation on synthesis. The cultivars tested were Ilpumbyeo (traditional white rice), Heugjinjubyeo (black pigmented), and Jeogjinjubyeo (red pigmented). A total of 41, 51, and 38 volatile compounds were identified in dehulled, unmilled Ilpumbyeo, Heugjinjubyeo, and Jeogjinjubyeo, respectively. Significant quantitative and qualitative differences were found among the cultivars in their volatile profiles. 2-Acetyl-1-pyrroline, guaiacol, 1-nonanol, 3-octen-2-one, 2-ethyl-1,4-dimethylbenzene, 1,2-dimethoxybenzene, pyridine, and pyrrole were found only in Heugjinjubyeo and (1-methylethyl)benzene and benzenacetaldehyde only in Jeogjinjubyeo. Milling qualitatively affected the volatiles emanating from the samples indicating the bran layer as their site of origin. Milling eliminated safranal, (*E*,*E*)-2,4-nonadienal, toluene, and 2-acethylthiazole from Ilpumbyeo; safranal, (*E*,*E*)-2,4-nonadienal, toluene, 1-nonanol, 1,2-dimethoxybenzene, 1,2,3,4-tetrahydronaphthalene, indole from Heugjinjubyeo; and safranal and benzyl alcohol from Jeogjinjubyeo. Removal of the bran also quantitatively affected the volatile compounds formed with certain volatiles increasing, indicating the endosperm as their primary site of origin and a second group decreasing, indicating the pericarp and aleurone layer (bran) as the primary site of origin. Differences in product chemistry with location within the grain appear to account for the quantitative and qualitative variation in volatile synthesis.

KEYWORDS: Rice flavor; volatile compound; colored rice; pigment; milling; rice bran; starch lipid; dynamic headspace.

1. Introduction

The flavor of rice varies widely, especially so among types of specialty rice selected for a diverse range of attributes. Aroma is an especially important component of the overall flavor in a variety of rice flavor types. For example, there are pronounced differences in aroma between black and traditional white rice (Yang, Lee, Jeong, Kim, & Kays, 2007).

Upon harvest rough rice is dehulled producing brown rice which is subsequently milled to remove the surface pigmented bran (pericarp and aleurone layer) yielding white rice (Zhou, Robards, Helliwell, & Blanchard, 2002a). As the degree of milling increases, additional portions of the rice grain are removed (e.g., 9 % – pericarp and aleurone layer, 9-15 % – outer endosperm, 15-25 % – middle endosperm) (Lamberts, Bie, Vandeputte, Veraverbeke, Derycke, Man, & Delcour, 2007). Most rice is consumed as white rice (Itani, Tamaki, Arai, & Horino, 2002), however, with pigmented rice the coloration normally extends beyond the pericarp and aleurone layer. For example, the pigmentation of many black and red rice cultivars is found not only in the surface bran but also in the outer and parts of the inner endosperm. While white rice can be produced from most pigmented rice cultivars with >20 % milling (Itani et al., 2002), pigmented rice generally is not milled and contains the surface bran. Therefore, the chemical composition of rice varies not only among cultivars but also with the degree of milling. “Golden rice” is a transgenic line, created through the insertion of three *β*-carotene biosynthesis genes (*psy*, *lyc*, and *crt1*) that creats yellow coloration throughout the endosperm (Paine, Shipton, Chaggar, Howells, Kennedy, Vernon, Wright, Hinchliffe, Adams, Silverstone, & Drake, 2005). While milling alters the chemical composition of “golden rice”, it remains pigmented.

Black and red rice cultivars are popular in Asia where they are consumed for their nutritional, color and unique flavor attributes. For example, red and black rice are used as food colorants in cakes, breads, ice cream, and liquor and as functional foods due to their antioxidant activity conferred by high levels of polyphenols (Choi, 2002; Itani & Ogawa, 2004; Yoshinaga, 1986). The color of black and red rice is derived from anthocyanin pigments (cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin-diglucoside, and cyanidin 3-rutinoside) (Abdel-Aal, Young, & Rabalski, 2006). In black rice, cyanidin 3-glucoside and peonidin 3-glucoside are primary pigments with the former being 136× higher in black than in red rice. The presence of pigments impacts the flavor of the rice. In black rice, 2-acetyl-1-pyrroline and guaiacol confer a unique aroma impacting the overall flavor (Yang et al., 2007). Red rice contains up to 50× higher tannin content than the common brown rice which increases its astringency (Goffman & Bergman, 2004).

The primary chemical components of rice are lipids, protein, and starch. Lipids and protein are found in the highest concentration in the bran while starch is predominantly found in the endosperm (Zhou et al., 2002a). Other components [e.g., ash, thiamine, minerals (e.g., P, Mg, K, and Mg), pigments, nutraceutical lipids (e.g., tocol, phytosterol, γ-oryzanol, octacosanol, and squalene), and total lipids] are concentrated in the outer bran layer. The bran, therefore, affects not only the nutritional attributes of rice but also its eating and cooking quality (Ha, Ko, Lee, Kim, Chung, Kim, Yoon, & Kim, 2006; Itani et al., 2002; Villareal, Maranville, & Juliano, 1991). As a consequence, the quality of finished rice is strongly modulated by the degree of milling (Itani et al., 2002).

The contribution of the surface layers and inner portion of pigmented rice to their unique aroma has not been assessed. We identified and characterized the volatile compounds emanating from pigmented and white rice cultivars before and after different degrees of milling to determine the contribution of the surface layers and inner portion to the overall aroma of cooked rice.

2. Materials and methods

2.1. *Plant Materials*

Three Korean japonica rice cultivars, Ilpumbyeo, Heugjinjubyeo, and Jeogjinjubyeo, were tested. Ilpumbyeo is the leading white rice cultivar in Korea, Heugjinjubyeo is a black pigmented, and Jeogjinjubyeo a red pigment cultivar. The cultivars were grown at the National Institute of Crop Science, Suwon in 2006. After harvest, samples were dehulled using a rice huller (THU 35A, Satake, Tokyo, Japan) to obtain brown rice. The brown rice of each cultivar was milled (MCM-250, Satake, Tokyo, Japan) to three different degrees (0, 6 and 8 % based on brown rice) as calculated using the equation (Ha et al., 2006):

$$\text{Degree of milling} = [1 - (\text{weight of milled rice}/\text{weight of brown rice})] \times 100$$

The brown rice and milled rice were sealed in glass containers and stored at -20°C until analysis.

2.2. *Chemicals*

Analytical standard utilized for identification were: benzaldehyde, decane, decanal, (*E*)-2-decenal, furfural, guaiacol, heptanal, heptane, nonane, nonanal, octane, pentadecane, 1-pentanol, tridecane, tetradecane, undecane (Sigma-Aldrich Inc., St. Louis, MO); (*E*,*E*)-2,4-decadienal, hexanal, 1-hexanol, (*E*)-2-hexenal, *d*-limonene, 1-methylnaphthalene, 2-methylnaphthalene, 1-nonanol, 2-nonanone, (*E*)-2-octenal, 1-octen-3-ol (Aldrich Chem. Co., Milwaukee, WI); δ-carvone, octanal, *α*-pinene, *p*-xylene (Fluka Chem. Co., Milwaukee, WI); naphthalene, toluene (J. T. Baker Inc., Phillipsburg, NJ); 2-ethyl-1,4-dimethylbenzene, indole, dodecane, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, and 2-pentylfuran (TCI America, Portland, OR); 1,3-diethylbenzene, 3-octen-2-one (Alfa Aesar, Ward Hill, MA); pyridine (ICN Biomedicals, Aurora, OH).

2.3. *Dynamic headspace using Tenax trap*

Rice samples (70 g) were added to 105 mL of distilled water and cooked for 30 min at 100 ºC in a specially constructed 1 L glass beaker with entry and exit ports. The entry and exit ports were wrapped with aluminum foil to minimize diffusive losses during cooking. Three replications of each rice sample were analyzed. Due to difference of water absorption characteristics, the black rice sample (70 g) was cooked in 70 mL of distilled water which was established based on the texture after cooking by preliminary tests; the volatiles were collected as with the other samples. Immediately after cooking the beaker containing the cooked rice was placed in a hot water bath (70 °C) and purified air was passed through the beaker (150 mL/min for 60 min) exiting into a Tenax trap where the volatiles were collected. The 10 cm long, 6 mm o.d., 4 mm i.d. stainless-steel sorbent trap (Scientific Instrument Services, Inc., Ringoes, NJ) contained 150 mg 60/80 mesh Tenax-TA® (Alltech Assoc. Inc. Deerfield, IL) was held in place with silated glass wool. The Tenax trap was preconditioned at 280 °C for 2 hr with purified He at 20 mL/min. A 50 mL glass flask was placed between the exit port and the trap to collect any condensation. Using a vacuum sampling pump (Aircheck Sampler, Model 224-44XR, Eighty-Four, PA), the rice volatiles were swept from the exit port of the beaker into the Tenax trap using air purified by passing it through an activated charcoal filter (Alltech Assoc. Inc. Deerfield, IL) [Pyrex glass tube (10 cm × 1 cm i.d.) with 7 cm of 2.5 g of charcoal].

2.4. *Short path thermal desorption*

The volatile samples were desorbed at 250 °C for 5 min with He at a flow rate of 10 mL/min onto the gas chromatograph column (GC/MS, Model 6890N/5973, Agilent, Palo Alto, CA) using an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, Ringoes, NJ). Analytes were collected on the first 4 cm of the GC column using a CO_2 cooled cryofocus trap (-40 °C) (SIS 2" Cryo-Trap, Scientific Instrument Services, Ringoes, NJ). After desorption, the cryofocus trap was rapidly heated up to 200 °C and the GC separation program started.

2.5. *GC/MS analysis*

Identification and quantification of the volatile compounds were performed using a GC/MS equipped with a 30 m length, 0.25 mm i.d., 0.25 µm film thickness (5 % phenyl methyl silicone), fused silica capillary column (HP-5MS, Agilent, Palo Alto, CA). Injection port temperature was 225 °C with a split ratio of 0.5:1. Helium was used as the carrier gas at a flow rate of 2.0 °C/min. The column temperature was held at 40 °C for 1 min and then programmed at 1.5 °C/min to 65 °C which was held for 1 min, increased at 2 °C/min to 120 °C, held for 1 min, and increased at 15 °C/min to 280 °C and held for 5 min. MS conditions were: ion source 230 °C; electron energy 70 eV; multiplier voltage 1247 V; GC/MS interface zone 280 °C; and a scan range of 35 to 350 mass units.

2.6. *Identification and quantification*

The volatiles were identified based on comparison of their mass spectra and relative abundances with NIST 02 and Wiley 7 spectral libraries. The identity of a compound was confirmed by comparison of its Kovats' retention index (RI) and mass spectra with authentic standards. Kovats' RI was calculated using a non-polar HP-5MS column with a series of *n*-hydrocarbons (C_7-C_{15}) and compared with those reported previously (http://webbook.nist.gov/). The levels of individual volatiles were expressed as δ-carvone equivalents (response factors were assumed to be 1). The concentrations are considered relative since recovery and calibration factors were not determined. The internal standard was introduced by placing 5 mL in a sealed 1 L Erlenmyer flask. After 24 hrs, 10 mL of air saturated with δ-carvone was removed and injected into the glass beaker containing cooked rice at the beginning of volatile collection. The concentration of the internal standard in the trapped sample was determined *via* direct GC injection of a range of concentrations in hexane. The results were expressed as the average of three replicates.

2.7. *Color measurements*

A Gardner XL-20 Tristimulus Colorimeter (Gardner Laborator Inc., Bethesda, MD) was used to measure the Hunter *L*, *a*, and *b* values of reflected color in the rice samples with three different degrees of milling (0, 6, and 8%; 5 replications). *L* indicates brightness from black (0) to white (100). A positive value of *a* indicates redness, and a negative value greenness, while a positive *b* value indicates yellowness and a negative value blueness. Prior to color measurements, *L*, *a*, and *b* values were calibrated with a white calibration tile. Samples were placed in a clear Petri dish covered with a white plate.

2.8. *Statistical data analysis*

Analysis of variance (ANOVA) and principal component analysis (PCA) (Khattree & Naik, 2000) were carried out by using the SAS system for Windows v8 to assess differences in volatile compounds from cooked rice due pigmentation and degree of milling.

3. Results and disccusion

3.1. *Color of brown, black, and red rice and changes with milling*

The color of the individual cultivars expressed as *L*, *a*, and *b* values are presented in Figure 6.1 for unmilled samples 0 % and after 6 % and 8 % of the weight had been abrasively removed from the surface. Prior to milling, the cultivars were brown (Ilpumbyeo), black

(Heugjinjubyeo), and red (Jeogjinjubyeo) in color. Ilpumbyeo at 6 % and 8 % became white. While there were significant differences with milling of the colored rice cultivars (Heugjinjubyeo and Jeogjinjubyeo), milling did not appreciably alter the coloration (best illustrated by the *a* value for Jeogjinjubyeo and *b* value for Heugjinjubyeo in Figure 6.1) indicating the pigmentation was present substantially beyond the surface layers of cells. Complete removal of the color necessitates milling >20 % of the grain weight (Itani et al, 2002).

3.2. *Volatiles emanating from unmilled and milled rice*

A total of 41 volatile compounds were identified in the unmilled, cooked Ilpumbyeo cultivar which included 3 alcohols, 11 aldehydes, 2 ketones, 17 aromatics, 5 hydrocarbons, 2 terpenoids, and 1 sulfur compound. After milling (6 and 8 %), the number decreased to 37 compounds (Table 6.1). (*E*,*E*)-2,4-Nonadienal, toluene, safranal, and 2-acethylthiazole present in the unmilled rice were eliminated with milling. In the unmilled rice, aldehydes were quantitatively the most abundant class of volatile compounds, followed by aromatics, alcohols, ketones, and hydrocarbons (Figure 6.2.A). After milling (6 and 8 %) the aldehyde increased 1.9 and 1.7 fold ($p < 0.05$), respectively and the alcohols and aromatics decreased slightly.

In Heugjinjubyeo, a total of 51, 44, and 44 volatile compounds were identified in 0, 6, and 8 % milling samples, respectively (Table 6.2). There were 5 alcohols, 11 aldehydes, 3 ketones, 20 aromatics, 5 hydrocarbons, 4 nitrogen compounds, and 3 terpenoids in the unmilled rice. Seven compounds, [1-nonanol, (*E*,*E*)-2,4-nonadienal, toluene, 1,2-dimethoxybenzene, 1,2,3,4-tetrahydronaphthalene, indole, and safranal], were eliminated with milling (6 and 8 % samples). In unmilled Heugjinjubyeo, aldehydes were quantitatively the most abundant class of volatiles, followed by aromatics, alcohols, nitrogen compounds, hydrocarbons, and ketones (Figure 6.2.B). Milling (6 and 8 %) significantly increased the concentration of the alcohols, aldehydes, ketones, aromatics, and terpenoids present compared to unmilled samples.

A total of 38 volatile compounds (3 alcohols, 10 aldehydes, 1 ketones, 15 aromatics, 5 hydrocarbons, 1 nitrogen compound, and 3 terpenoids) were identified emanating from cooked, unmilled Jeogjinjubyeo, while only 36 volatile compounds were identified in the milled samples (6 and 8 %). Benzyl alcohol and safranal were eliminated with the removal of the surface components from the grains (Table 6.3). Aldehydes were quantitatively the most abundant class of volatile compounds in the unmilled sample (as in Ilpumbyeo and Heugjinjubyeo), followed by aromatics, hydrocarbons, alcohols, ketones, and alcohols (Figure 6.2.C). Alcohols, aldehydes, ketones, and aromatics at milled Jeogjinjubyeo (6 and 8 %) were statistically higher than those in unmilled samples (0 %); the 6 and 8 % milling samples were not significantly different.

There were significant differences among cultivars in their volatile profiles which were quantitatively and qualitatively affected by milling. For example, 2-acetyl-1-pyrroline, guaiacol, 1-nonanol, 3-octen-2-one, 2-ethyl-1,4-dimethylbenzene, 1,2-dimethoxybenzene, pyridine, and pyrrole were found only in Heugjinjubyeo, while (1-methylethyl)benzene and benzenacetaldehyde were present only in Jeogjinjubyeo. Safranal identified in the unmilled samples of each cultivar, was not present after milling.

Changes in the volatile profiles with the removal of the surface bran layer are apparently due to differences in the chemical composition between the surface and interior portion of the grain. The interior (endosperm) is comprised largely of starch (Itani et al., 2002) and in Heugjinjubyeo and Jeogjinjubyeo cultivars anthocyanins. There were also differences among the cultivars after the surface was removed, again reflecting differences in chemistry. For example, Ilpumbyeo has a low amylose (18 %) and lipid (0.44 %) content (Kang, Hwang, Kim, & Choi, 2003). Heugjinjubyeo has high anthocyanin, potassium, and vitamin B_2 (Lee, Oh, Choi, & Kim, 1988). 2-Arylbenzofuran, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester, and oryzafuran which have strong antioxidative activity, were also isolated in the bran of Heugjinjubyeo (Han, Ryu, & Kang, 2004). Jeogjinjubyeo, which was developed for processed food products, contains catechin and catchol-tannins (Kim, Han, & Yoon, 1999), however, due to their complexity, details on the pigment chemistry of the cultivar have not been reported. There are distinct differences in chemical composition among the three cultivars tested and their degrees of milling, resulting in qualitatively and quantitatively impacting the volatiles emanating from the cooked product.

3.3. *Origin of volatile compounds in Ilpumbyeo*

PCA was applied with volatile compounds of Ilpumbyeo according to different degrees of milling to investigate the site of origin of the volatiles. Differences in the volatile composition of Ilpumbyeo with milling accounted for 69.6 % and 22.6 % of the total variance in the first (PC 1) and second (PC 2) principal components, respectively (Figure 6.3). Volatile compounds in the PCA plot were divided into three groups (Figure 6.3). The first group is volatile compounds positioned on the positive side of the *x*-axis. As the degree of milling increased from 0 % to 8 %, the bran was progressively removed. The primary volatiles after milling were toluene (20)[2], 1-octen-3-ol (4), (*E*,*E*)-2,4-nonadienal (14), 2-acethylthiazole (54), *p*-xylene (23), 1,2,3,4-tetrahydronaphthalene (38), ethylbenzene (22), safranal (49), *o*-xylene (25), furfural (21), (*E*,*E*)-2,4-decadienal (16), 2-pentylfuran (29), nonane (42), (*E*)-2-hexenal (7), (*E*)-2-decenal (15),

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[2] Numbers for compounds in the text and Figure 6.3 correspond to those in Table 6.1.

benzyl alcohol (32), 1-hexanol (3), and propylbenzene (27). Their concentrations were not significantly different between 6 % and 8 % milling with the exception of (*E*)-2-decenal (Table 6.1). These volatiles appeared to be predominantly from the bran region of the grain as indicated by the significant difference between milled and unmilled rice. A second group of volatiles are positioned near zero on the *x*-axis. The main compounds were 1,2,3-trimethylbenzene (30), 6-methyl-5-hepten-2-one (17), decane (43), 2-methylnaphthalene (40), and dodecane (45). Their concentrations were lowest at 8 % milling when the bran was completely removed. They originated predominantly in the bran. A third group of volatiles were positioned on the negative side of the *x*-axis and were higher in milled rice. In this group, the main compounds were benzaldehyde (28), naphthalene (39), nonanal (11), hexanal (6), 2-nonanone (19), 1-pentanol (2), octanal (9), (*E*)-2-nonenal (10), heptanal (8), (*E*)-2-octenal (10), styrene (24), decanal (13), and tridecane (46), indicating that the endosperm is their primary site of origin. The concentration of several compounds [i.e., hexanal (6), heptanal (8), decanal (13), (*E*)-2-octenal (10), styrene (24), and tridecane (46)] increased with limited milling (6 %) but subsequently decreased (8 %).

3.4. *Origin of volatile compounds in Heugjinjubyeo*

Heugjinjubyeo with 0, 6, and 8 % milling were clearly separated, accounting for 79.1 % and 14.6 % of the total variance by PC 1 and PC 2, respectively (Figure 6.4). PCA analysis of the effect of milling on the volatiles indicated two groups. PC 1 was positioned on the negative side of the *x*-axis and PC 2 on the positive. PC 1 had higher amounts of guaiacol (36)[3], toluene (20), 1-nonanol (5), indole (53), safranal (49), (*E*,*E*)-2,4-nonadienal (14), 1,2-dimethoxybenzene (37), 1,2,3,4-tetrahydronaphthalene (38), (*E*,*E*)-2,4-decadienal (16), 2-nonanone (19), ethylbenzene

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[3] Numbers for compounds in the text and Figure 6.4 correspond to those in Table 6.2.

(22), *p*-xylene (23), decane (43), and nonane (42) in unmilled rice (Figure 6.4). Seven of these compounds [toluene, 1-nonanol, indole, safranal, (*E*,*E*)-2,4-nonadienal, 1,2-dimethoxybenzene, and 1,2,3,4-tetrahydronaphthalene] were found only in ummilled rice; the others decreased with increased milling. In PC 2, 38 compounds were positioned on the positive side of the *x*-axis and had higher amounts in milled than in unmilled rice. The main compounds with higher loadings were 1-octen-3-ol (4), 3-octen-2-one (18), 6-methyl-5-hepten-2-one (17), *d*-limonene (48), (*E*)-2-octenal (10), benzaldehyde (28), tridecane (46), 2-methylnaphthalene (40), (*E*)-2-nonenal (12), pyrrole (51), 1-methylnaphthalene (41), naphthalene (39), 1-methyl-2-(1-methylethyl)benzene (31), hexanal (6), 2-acetyl-1-pyrroline (52). Their amounts increased significantly with increased milling, indicating a greater formation in the endosperm rather than the bran. Guaiacol and 2-acetyl-1-pyrroline which were considered the key aroma compounds in black-pigmented rice (Yang et al., 2007), differed in their primary site of origin; 2-acetyl-1-pyrroline was predominantly from the endosperm and guaiacol from the bran region. Bergman, Delgado, Bryant, Grimm, Cadwallader, and Webb (2000) also reported that 2-acetyl-1-pyrroline was found within the endosperm.

3.5. *Origin of volatile compounds in Jeogjinjubyeo*

Milling of Jeogjinjubyeo accounted for 86.8 % (PC 1) and 7.9 % (PC 2) of the total variance (Figure 6.5). Two groups of volatiles were clearly separated as in Heugjinjubyeo. The first group was positioned on the negative side of the *x*-axis and appeared to have originated primarily from the bran (i.e., higher amounts in unmilled rice), while the second group was positioned on the positive side and appeared to have originated more so from the endosperm. The primary compounds in the first group were nonane (42)[4], benzyl alcohol (32), safranal (49), decane (43), and benzeneacetaldehyde (33) and in the second group benzaldehyde (28), 1-hexanol (3), 1-pentanol (2), hexanal (6), 1,2,3-trimethylbenzene (30), octanal (9), naphthalene (39), 6-methyl-5-hepten-2-one (17), tridecane (46), 2-pentylfuran (29), styrene (24), propylbenzene (27), 1-octen-3-ol (4), *d*-limonene (48), undecane (44), (*E*,*E*)-2,4-decadienal (16), (1-methylethyl)benzene (26), (*E*)-2-decenal (15), nonanal (11), and dodecane (45).

With the transition from the exterior of the rice grain toward the interior, there are distinct changes in chemistry of the cells (Zhou et al., 2002a). As the surface layers are removed, more volatile compounds quantitatively are produced (Figure 6.2.A-C). Among the volatiles that increased in concentration with milling, there are a number of lipid-derived compounds e.g., Ilpumbyeo [e.g., hexanal, heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal, 1-pentanol, 2-nonanone, tridecane]; Heugjinjubyeo [e.g., hexanal, (*E*)-2-octenal, (*E*)-2-nonenal, 1-octen-3-ol, tridecane]; and Jeogjinjubyeo [e.g., 1-hexanol, hexanal, octanal, nonanal, (*E*)-2-decenal, (*E*,*E*)-2,4-decadienal, 2-pentylfuran, 1-pentanol, 1-octen-3-ol, undecane, dodecane, tridecane]. The endosperm contains starch as the major constituent (~ 90 % of the dwt), while lipids associated with starch granules in milled rice represent around 0.5 to 1.0 % and are in greater abundance than non-starch lipids (Kawashima & Kiribuchi, 1980). The lipid portion of the starch-lipids complexes present in the endosperm may contribute to the formation of rice volatile compounds. The starch-lipids complexes are melted and dissociated during cooking because their melting temperature range (93 to 96 ℃) is below their cooking temperature (Morrison, 1988). The dissociation of starch-lipids complexes also facilitates the degradation of the lipids, resulting in the formation of free fatty acids such as oleic and linoleic acids, which may contribute to the formation of rice volatiles (Ito, Sato, & Fujino, 1979; Kitahara, Tanaka,

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[4] Numbers for compounds in the text and Figure 6.5 correspond to those in Table 6.3.

Suganuma, & Nagahama, 1997). The oxidation of non-starch lipids and formation of off-flavors during storage is well documented (e.g., hexanal, octanal, 2-nonenal) (Lam & Proctor, 2003; Zhou, Robards, Helliwell, & Blanchard, 2002b). During cooking the formation of volatiles is more complex in that some of the qualitative and quantitative differences in volatiles are due to differences in the chemical composition with location in the grain and differences in composition among cultivars. In particular, 2-acethylthiazole, benzyl alcohol, 1,2-dimethoxybenzene, indole, (*E*,*E*)-2,4-nonadienal, 1-nonanol, 1,2,3,4-teterahydronaphthalene, toluene, and safranal were derived from the bran. In addition, dissociation of the starch lipid complex during cooking appears to enhance the formation of a cross-section of lipid derived volatiles even though removal of the bran significantly decreases the amount of the total lipids. Therefore, characteristic compounds in rice bran and chemical components such as lipid and protein associated with starch in the endosperm contribute to the overall aroma.

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Table 6.1. Concentration of Volatiles Identified in Cooked Ilpumbyeo at Different Degrees of Milling [a]

no [b]	RI [c]	compound	ID [d]	0 %	6 %	8 %
		alcohols				
2	787	1-pentanol	A	8.6 ± 2.0 b	15.6 ± 1.6 a	16.0 ± 1.7 a
3	878	1-hexanol	A	16.3 ± 4.5 a	12.5 ± 1.2 ab	10.3 ± 1.5 b
4	981	1-octen-3-ol	A	43.4 ± 3.7 a	15.1 ± 0.8 b	14.6 ± 0.0 b
		aldehydes				
6	835	hexanal	A	141.5 ± 18.4 c	339.5 ± 16.7 a	299.4 ± 10.2 b
7	865	(*E*)-2-hexenal	A	4.3 ± 1.5 a	3.2 ± 0.1 ab	2.4 ± 0.2 b
8	902	heptanal	A	18.6 ± 2.0 c	38.5 ± 2.3 a	28.4 ± 0.6 b
9	1003	octanal	A	32.1 ± 3.9 b	49.9 ± 3.1 a	43.3 ± 1.6 a
10	1056	(*E*)-2-octenal	A	12.9 ± 1.6 c	26.3 ± 0.6 a	17.6 ± 1.1 b
11	1107	nonanal	A	117.0 ± 25.9 b	219.2 ± 20.9 a	205.2 ± 3.9 a
12	1159	(*E*)-2-nonenal	A	7.4 ± 1.5 b	14.4 ± 1.9 a	14.8 ± 2.1 a
13	1206	decanal	A	16.8 ± 0.3 c	21.1 ± 0.0 a	18.5 ± 0.2 b
14	1211	(*E*,*E*)-2,4-nonadienal	A	1.7 ± 0.7 a	ND [e]	ND
15	1261	(*E*)-2-decenal	A	11.0 ± 1.9 a	7.0 ± 0.4 b	4.0 ± 0.3 c
16	1315	(*E*,*E*)-2,4-decadienal	A	23.6 ± 0.7 a	19.6 ± 1.0 b	19.0 ± 0.4 b
		ketones				
17	988	6-methyl-5-hepten-2-one	A	41.9 ± 13.5 a	38.4 ± 4.2 a	27.4 ± 3.7 b
19	1093	2-nonanone	A	1.3 ± 0.4 b	3.6 ± 0.1 a	3.8 ± 0.6 a
		aromatics				
20	760	toluene	A	5.9 ± 0.7 a	ND	ND
21	853	furfural	A	0.8 ± 0.1 a	0.4 ± 0.1 b	0.3 ± 0.0 b
22	869	ethylbenzene	B	14.5 ± 1.2 a	4.5 ± 0.2 b	3.4 ± 0.1 b
23	875	*p*-xylene	A	53.6 ± 4.4 a	13.3 ± 0.7 b	9.2 ± 0.3 b
24	891	styrene	B	3.0 ± 0.7 c	6.9 ± 0.1 a	4.2 ± 0.2 b
25	893	*o*-xylene	B	29.7 ± 3.7 a	15.6 ± 0.2 b	12.8 ± 0.4 b
27	947	propylbenzene	B	1.2 ± 0.3 a	0.8 ± 0.1 b	0.6 ± 0.1 b
28	953	benzaldehyde	A	27.6 ± 2.1 b	45.9 ± 0.3 a	45.9 ± 1.8 a
29	991	2-pentylfuran	A	70.2 ± 5.1 a	59.2 ± 3.6 b	54.5 ± 3.3 b
30	1016	1,2,3-trimethylbenzene	B	4.2 ± 0.9 a	4.7 ± 0.3 a	2.9 ± 0.2 b
31	1019	1-methyl-2-(1-methylethyl)benzene	B	1.4 ± 0.3 b	2.1 ± 0.4 a	1.7 ± 0.2 ab
32	1033	benzylalcohol	B	16.9 ± 2.4 a	11.9 ± 1.7 b	13.9 ± 0.5 ab
34	1046	1,3-diethylbenzene	A	2.1 ± 0.6 b	3.7 ± 0.7 a	2.5 ± 0.3 b
38	1152	1,2,3,4-tetrahydronaphthalene	B	1.7 ± 0.1 a	0.5 ± 0.2 b	0.7 ± 0.0 b
39	1172	naphthalene	A	8.6 ± 0.8 b	19.9 ± 0.4 a	17.9 ± 0.9 a
40	1281	2-methylnaphthalene	A	11.1 ± 0.9 b	14.6 ± 0.4 a	9.3 ± 0.1 c
41	1296	1-methylnaphthalene	A	4.9 ± 0.7 a	4.2 ± 0.2 a	3.0 ± 0.1 b
		hydrocarbons				
42	900	nonane	A	3.7 ± 0.5 a	2.7 ± 0.2 b	2.7 ± 0.2 b
43	1000	decane	A	1.5 ± 0.5 ab	1.9 ± 0.1 a	1.1 ± 0.2 b
44	1100	undecane	A	3.2 ± 0.2 a	2.9 ± 0.1 b	1.4 ± 0.1 c
45	1200	dodecane	A	3.5 ± 0.8 b	5.2 ± 0.6 a	2.7 ± 0.3 b
46	1300	tridecane	A	5.3 ± 1.0 c	14.6 ± 1.2 a	8.0 ± 0.6 b
		terpenoids				
47	927	α-pinene	A	0.4 ± 0.1 b	0.9 ± 0.0 a	0.5 ± 0.1 b
49	1194	safranal	B	5.4 ± 1.7 a	ND	ND
		sulfur compound				

54	1014	2-acethylthiazole	B	2.6 ± 0.3 a	ND	ND

[a] Values expressed as δ-carvone equivalent (ng/100g) and given as average ± standard deviation (n=3); Means with the different letter in the same row are significantly different ($P < 0.05$). b Numbers correspond to those Table 2 and 3 and Figure 3, 4, and 5. [c] Retention index based on a series of *n*-hydrocarbons. [d] Method of identification: A, by comparison of RI, and the mass spectrum of an identified compound with those of an authentic compound.; B: tentatively identified by comparison of the mass spectrum with the NIST/Wiley mass spectral library, and RI with those from the literature. [e] ND = not detected.

Table 6.2. Concentration of Volatiles Identified in Cooked Heugjinjubyeo at Different Degrees of Milling[a]

no[b]	RI[c]	compound	ID[d]	0 %	6 %	8 %
		alcohols				
1	683	1-penten-3-ol	B	4.8 ± 0.3 b	5.6 ± 1.0 ab	6.8 ± 0.6 a
2	787	1-pentanol	A	33.5 ± 9.1 b	43.2 ± 14.2 b	69.9 ± 5.5 a
3	878	1-hexanol	A	18.4 ± 3.3 b	22.6 ± 3.0 b	32.3 ± 2.4 a
4	981	1-octen-3-ol	A	23.8 ± 0.1 c	43.2 ± 7.1 b	53.0 ± 1.2 a
5	1174	1-nonanol	A	4.7 ± 0.9 a	ND[e]	ND
		aldehydes				
6	838	hexanal	A	723.3 ± 3.8 c	1158.7 ± 29.5 b	1802.0 ± 33.2 a
7	865	(*E*)-2-hexenal	A	6.9 ± 1.0 a	6.6 ± 0.6 b	8.8 ± 0.6 a
8	902	heptanal	A	43.6 ± 1.3 b	46.8 ± 7.3 b	59.8 ± 3.2 a
9	1004	octanal	A	43.8 ± 6.2 b	49.1 ± 2.7 b	63.7 ± 0.1 a
10	1057	(*E*)-2-octenal	A	21.1 ± 0.4 b	38.6 ± 4.0 b	52.1 ± 0.9 a
11	1108	nonanal	A	242.9 ± 16.9 c	260.8 ± 1.8 b	307.2 ± 8.7 a
12	1160	(*E*)-2-nonenal	A	11.3 ± 1.4 b	15.7 ± 3.0 ab	19.9 ± 0.9 a
13	1206	decanal	A	33.9 ± 3.5 b	33.9 ± 4.2 a	36.5 ± 6.3 a
14	1209	(*E*,*E*)-2,4-nonadienal	A	1.6 ± 0.4 a	ND	ND
15	1261	(*E*)-2-decenal	A	3.9 ± 0.6 c	6.1 ± 0.3 b	14.9 ± 0.6 a
16	1315	(*E*,*E*)-2,4-decadienal	A	10.9 ± 1.1 a	9.3 ± 0.6 a	9.2 ± 0.6 a
		ketones				
17	988	6-methyl-5-hepten-2-one	A	29.4 ± 2.7 c	54.6 ± 7.0 b	66.1 ± 1.0 a
18	1038	3-octen-2-one	A	5.6 ± 0.1 c	14.4 ± 1.1 b	19.9 ± 1.0 a
19	1093	2-nonanone	A	8.1 ± 0.6 a	5.4 ± 0.9 b	5.7 ± 0.6 b
		aromatics				
20	760	toluene	A	3.6 ± 1.4 a	ND	ND
21	853	furfural	A	1.7 ± 0.2 b	33.9 ± 12.7 a	36.2 ± 10.7 a
22	869	ethylbenzene	B	16.9 ± 2.0 a	9.1 ± 0.4 b	11.4 ± 0.3 b
23	875	*p*-xylene	A	54.7 ± 3.1 a	30.9 ± 1.5 c	39.8 ± 0.6 b
24	891	styrene	B	6.2 ± 1.1 c	9.1 ± 1.2 b	12.7 ± 0.7 a
25	893	*o*-xylene	B	48.4 ± 5.6 b	50.1 ± 6.5 b	70.9 ± 2.3 a
27	947	propylbenzene	B	1.5 ± 0.1 b	1.6 ± 0.1 ab	1.8 ± 0.1 a
28	953	benzaldehyde	A	39.1 ± 2.4 b	64.6 ± 5.4 a	75.3 ± 0.8 a
29	991	2-pentylfuran	A	97.8 ± 1.2 b	103.3 ± 1.4 b	148.1 ± 4.8 a
30	1016	1,2,3-trimethylbenzene	B	4.3 ± 0.4 c	5.1 ± 0.6 b	6.1 ± 0.2 a
31	1019	1-methyl-2-(1-methylethyl)benzene	B	2.4 ± 0.2 c	3.7 ± 0.8 b	5.0 ± 0.0 a
32	1033	benzylalcohol	B	15.4 ± 1.8 a	16.1 ± 3.0 a	16.6 ± 2.0 a
34	1045	1,3-diethylbenzene	A	2.6 ± 0.2 c	3.9 ± 1.0 b	7.6 ± 0.0 a
35	1072	2-ethyl-1,4-dimethylbenzene	A	4.5 ± 0.5 a	5.3 ± 0.3 b	4.7 ± 1.6 a
36	1087	guaiacol	A	13.2 ± 5.6 a	4.5 ± 0.2 b	3.2 ± 0.4 b
37	1149	1,2-dimethoxybenzene	B	4.1 ± 0.5 a	ND	ND
38	1152	1,2,3,4-tetrahydronaphthalene	B	0.3 ± 0.1 a	ND	ND
39	1172	naphthalene	A	15.7 ± 0.8 c	30.7 ± 0.1 b	32.7 ± 0.5 a
40	1281	2-methylnaphthalene	A	7.0 ± 0.8 b	15.9 ± 1.4 a	18.6 ± 1.4 a
41	1296	1-methylnaphthalene	A	2.1 ± 0.2 b	3.6 ± 0.3 a	4.1 ± 0.3 a
		hydrocarbons				
42	900	nonane	A	11.7 ± 1.3 a	9.4 ± 0.7 b	10.6 ± 0.9 ab
43	1000	decane	A	8.4 ± 1.5 a	5.7 ± 0.6 b	6.9 ± 0.1 ab
44	1100	undecane	A	6.2 ± 0.8 b	6.2 ± 1.2 b	8.4 ± 0.4 a

45	1200	dodecane	A	12.0 ± 0.9 b	13.8 ± 1.3 ab	15.9 ± 0.4 a
46	1300	tridecane	A	9.4 ± 0.1 c	24.5 ± 1.6 b	29.0 ± 1.5 a
		terpenoids				
47	927	*α*-pinene	A	1.0 ± 0.1 b	2.4 ± 0.4 a	2.9 ± 0.4 a
48	1022	*d*-limonene	A	19.7 ± 5.4 c	63.4 ± 3.7 b	94.1 ± 3.6 a
49	1194	safranal	B	4.6 ± 0.5 a	ND	ND
		nitrogen compounds				
50	736	pyridine	A	10.4 ± 1.5 b	15.5 ± 4.8 b	26.0 ± 0.8 a
51	765	pyrrole	B	4.2 ± 0.7 b	6.5 ± 1.7 ab	9.1 ± 0.9 a
52	919	2-acetyl-1-pyrroline	B	38.2 ± 8.8 b	75.4 ± 8.2 a	79.2 ± 5.8 a
53	1289	inidole	A	7.0 ± 0.7 a	ND	ND

[a] Values expressed as δ-carvone equivalent (ng/100g) and given as average ± standard deviation (n=3); Means with the different letter in the same row are significantly different ($P < 0.05$). b Numbers correspond to those Table 1 and 3 and Figure 3, 4, and 5. [c] Retention index based on a series of *n*-hydrocarbons. [d] Method of identification: A, by comparison of RI, and the mass spectrum of an identified compound with those of an authentic compound.; B: tentatively identified by comparison of the mass spectrum with the NIST/Wiley mass spectral library, and RI with those from the literature. [e] ND = not detected.

Table 6.3. Concentration of Volatiles Identified in Cooked Jeogjinjubyeo at Different Degrees of Milling[a]

no[b]	RI[c]	compound	ID[d]	0 %	6 %	8 %
		alcohols				
2	787	1-pentanol	A	3.1 ± 0.6 c	13.7 ± 0.4 b	14.2 ± 0.3 a
3	877	1-hexanol	A	1.9 ± 0.1 b	8.7 ± 0.2 a	8.9 ± 0.3 a
4	978	1-octen-3-ol	A	2.4 ± 0.1 b	16.4 ± 0.2 a	20.9 ± 1.0 a
		aldehydes				
6	834	hexanal	A	68.2 ± 1.7 b	331.3 ± 19.1 a	365.1 ± 0.3 a
7	865	(*E*)-2-hexenal	A	2.1 ± 0.6 b	3.4 ± 0.1 a	3.2 ± 0.3 a
8	901	heptanal	A	25.4 ± 0.3 b	33.1 ± 4.1 ab	35.9 ± 2.7 a
9	1003	octanal	A	32.2 ± 0.1 b	54.3 ± 3.4 a	54.7 ± 1.4 a
10	1056	(*E*)-2-octenal	A	5.7 ± 1.2 a	7.9 ± 1.1 a	7.6 ± 1.5 a
11	1108	nonanal	A	242.9 ± 11.1 b	297.1 ± 10.7 a	293.1 ± 9.1 a
12	1159	(*E*)-2-nonenal	A	5.6 ± 1.2 b	15.2 ± 1.3 b	22.3 ± 2.1 a
13	1206	decanal	A	45.3 ± 4.0 c	44.5 ± 1.1 a	43.0 ± 1.5 a
15	1261	(*E*)-2-decenal	A	4.9 ± 0.2 b	6.2 ± 0.2 a	6.0 ± 0.2 a
16	1315	(*E*,*E*)-2,4-decadienal	A	6.9 ± 1.0 b	13.3 ± 0.2 a	12.2 ± 0.6 a
		ketones				
17	986	6-methyl-5-hepten-2-one	A	10.5 ± 1.7 b	28.5 ± 2.2 a	27.9 ± 0.4 a
		aromatics				
21	852	furfural	A	0.7 ± 0.1 c	17.8 ± 2.1 b	44.3 ± 3.6 a
22	869	ethylbenzene	B	9.5 ± 1.8 c	19.6 ± 2.5 a	14.0 ± 2.3 b
23	875	*p*-xylene	A	28.1 ± 0.7 c	71.5 ± 0.3 a	51.7 ± 1.4 b
24	891	styrene	B	2.8 ± 0.5 b	15.1 ± 2.3 a	14.7 ± 1.2 a
25	893	*o*-xylene	B	13.8 ± 0.1 c	44.9 ± 0.7 a	35.9 ± 2.8 b
26	919	(1-methylethyl) benzene	B	0.2 ± 0.1 b	1.0 ± 0.1 a	0.9 ± 0.1 a
27	947	propylbenzene	B	0.7 ± 0.1 b	2.3 ± 0.4 a	2.2 ± 0.0 a
28	953	benzaldehyde	A	18.4 ± 1.0 b	48.1 ± 0.8 a	47.5 ± 1.0 a
29	990	2-pentylfuran	A	23.3 ± 1.9 b	51.9 ± 4.4 a	52.2 ± 2.0 a
30	1016	1,2,3-trimethylbenzene	B	2.0 ± 0.4 b	9.7 ± 1.0 a	9.9 ± 0.7 a
31	1019	1-methyl-2-(1-methylethyl) benzene	B	0.7 ± 0.2 c	6.6 ± 0.9 b	15.9 ± 4.8 a
32	1033	benzylalcohol	B	15.3 ± 4.0 a	ND[e]	ND
33	1039	benzeneacetaldehyde	B	11.6 ± 1.1 a	9.4 ± 0.5 b	9.1 ± 0.6 b
39	1172	naphthalene	A	5.9 ± 0.3 b	38.3 ± 1.3 a	40.6 ± 3.0 a
40	1281	2-methylnaphthalene	A	2.4 ± 0.4 c	18.1 ± 1.4 b	29.4 ± 1.9 a
		hydrocarbons				
42	900	nonane	A	35.9 ± 2.9 a	14.7 ± 1.1 b	9.6 ± 1.1 c
43	1000	decane	A	14.2 ± 1.5 a	10.5 ± 0.5 b	8.6 ± 0.5 c
44	1100	undecane	A	5.7 ± 0.7 b	18.3 ± 1.6 a	18.7 ± 2.9 a
45	1200	dodecane	A	10.1 ± 1.0 b	15.2 ± 1.3 a	14.7 ± 0.0 a
46	1300	tridecane	A	4.3 ± 0.4 b	26.8 ± 2.3 a	26.7 ± 2.8 a
		terpenoids				
47	927	*α*-pinene	A	0.4 ± 0.1 c	3.5 ± 0.1 b	4.5 ± 0.3 a
48	1022	*d*-limonen	A	3.6 ± 0.5 b	145.7 ± 4.8 a	144.1 ± 1.4 a
49	1194	safranal	B	1.8 ± 0.4 a	ND	ND
		nitrogen compound				
53	1289	indole	A	6.0 ± 1.2 a	8.7 ± 0.8 a	7.5 ± 0.8 a

[a] Values expressed as δ-carvone equivalent (ng/100g) and given as average ± standard deviation (n=3); Means with the different letter in the same row are significantly different ($P < 0.05$). b Numbers correspond to those Table 1 and 2 and Figure 3, 4, and 5. [c] Retention index based

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on a series of *n*-hydrocarbons. [d] Method of identification: A, by comparison of RI, and the mass spectrum of an identified compound with those of an authentic compound.; B: tentatively identified by comparison of the mass spectrum with the NIST/Wiley mass spectral library, and RI with those from the literature. [e] ND = not detected.

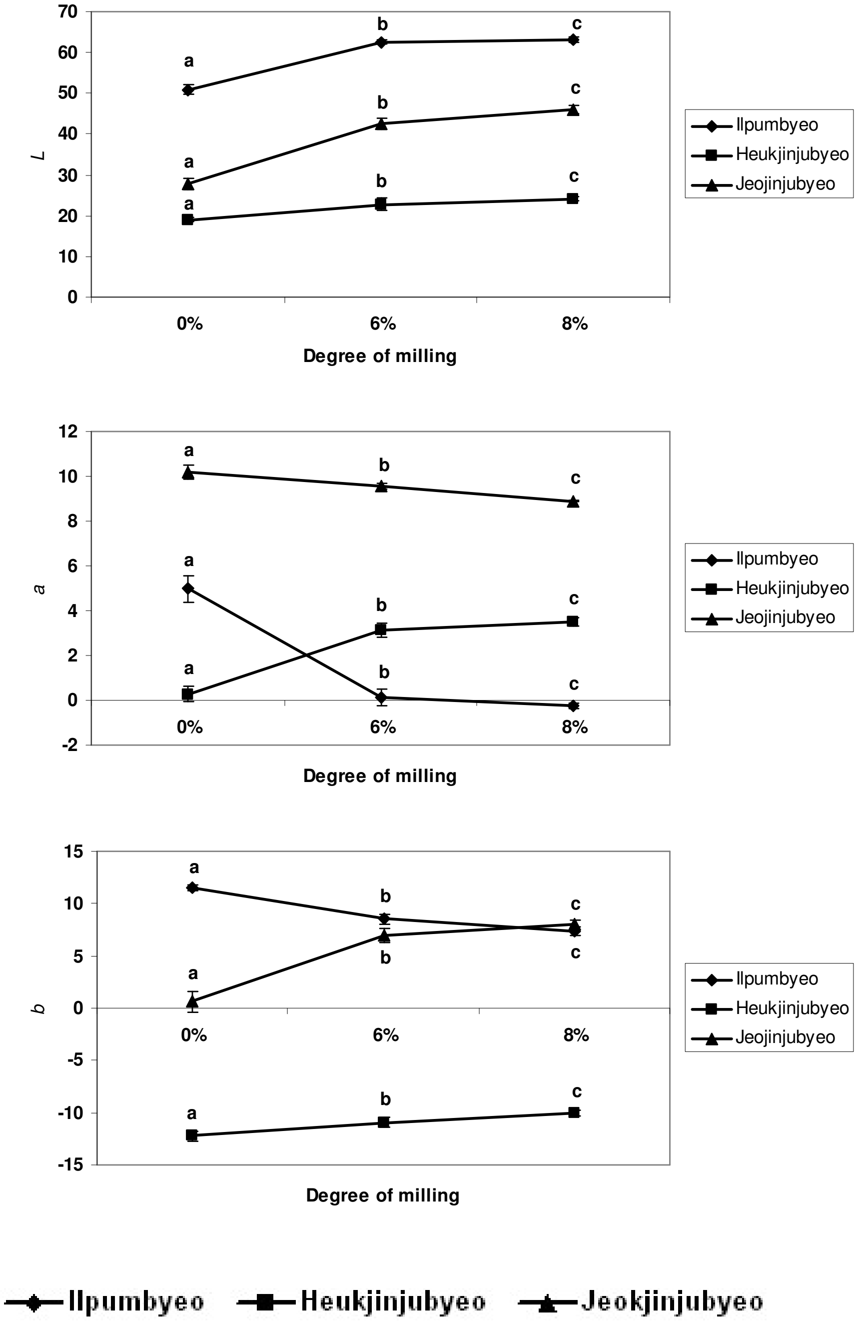

Figure 6.1. The color of Ilpumbyeo, Heukjinjubyeo and Jeokjinjubyeo rice cultivars milled to different degrees (0, 6, and 8%) as indicated by *L* (brightness), *a* (redness), and *b* (yellowness) values. Vertical bars represent the standard deviation. Different letters are significantly different ($p < 0.05$).

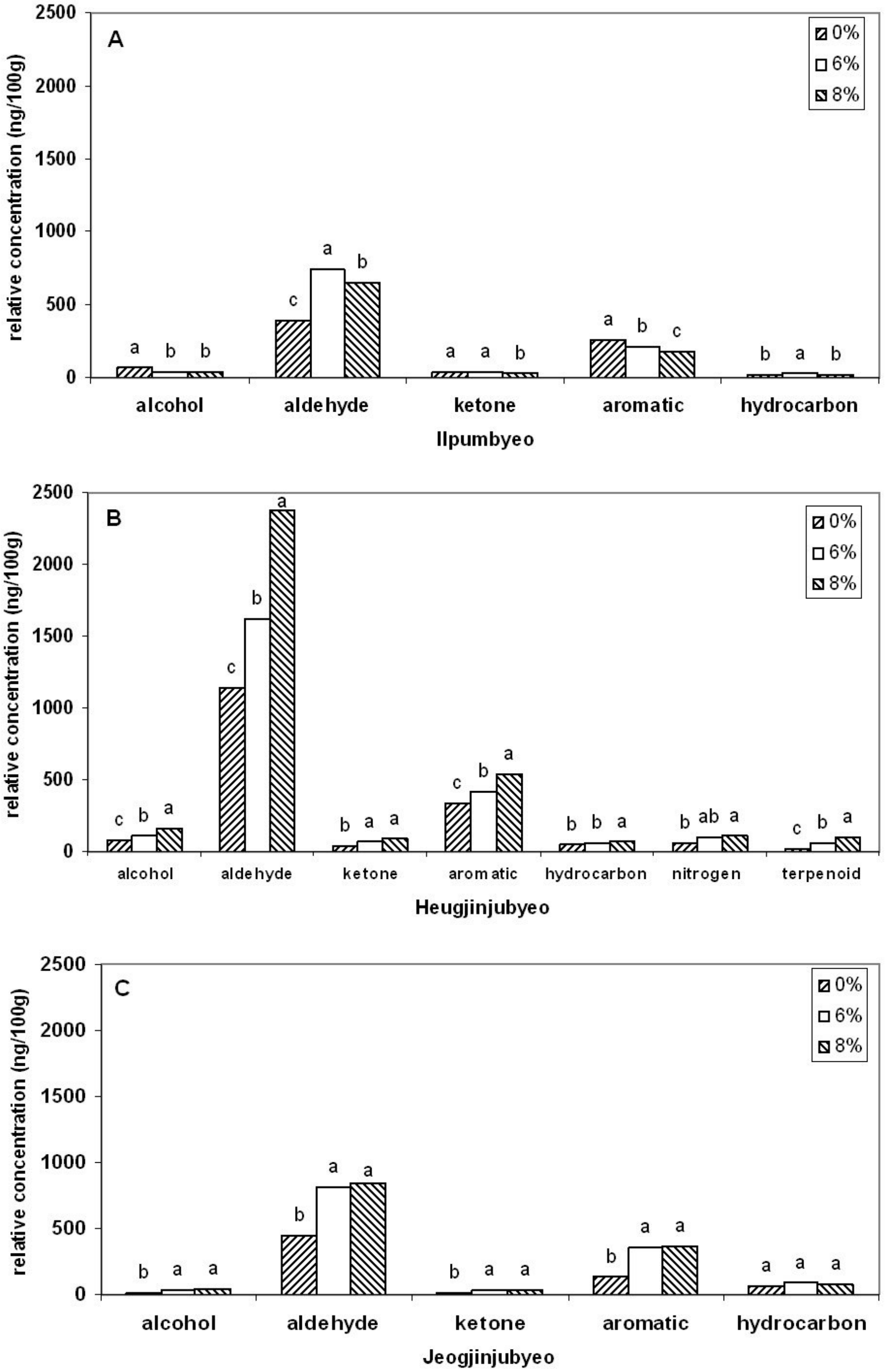

Figure 6.2. Relative concentration of the primary classes of volatile compounds emanating from cooked (A) Ilpumbyeo, (B) Heugjinjubyeo, and (C) Jeogjinjubyeo at different degrees of milling. Vertical bars with different letters are significantly different ($p < 0.05$).

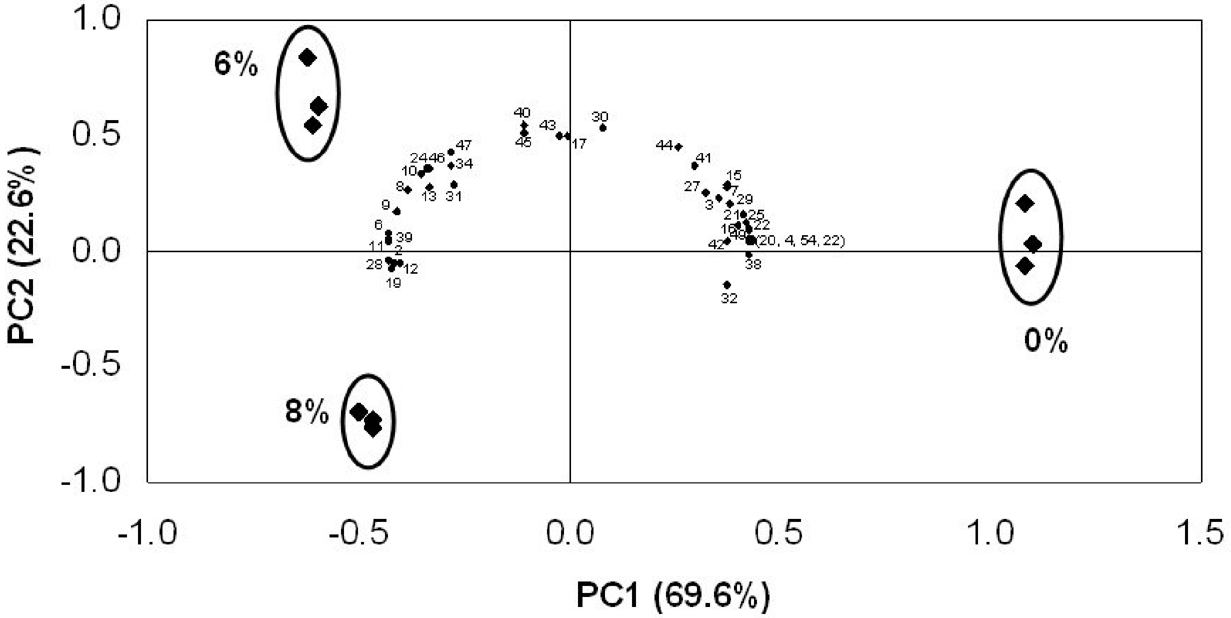

Figure 6.3. PCA biplot of volatile compounds emanating from cooked Ilpumbyeo with different degrees of milling (0, 6, and 8 %). Numbers correspond to compounds listed in Table 6.1.

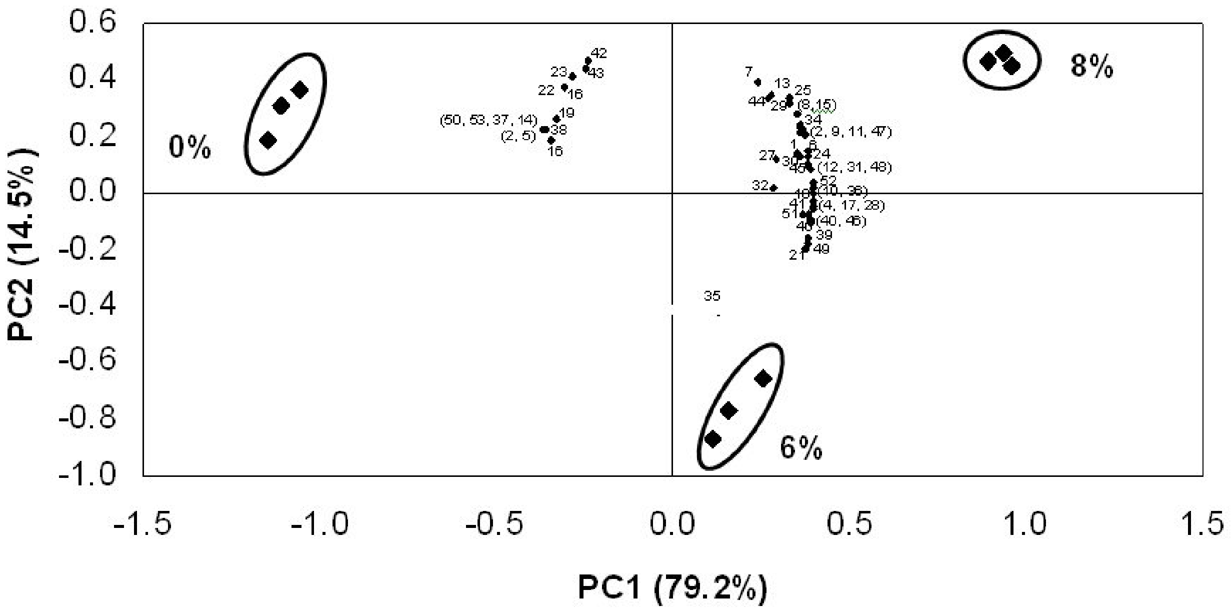

Figure 6.4. PCA biplot of volatile compounds emanating from cooked Heugjinjubyeo with different degrees of milling (0, 6, and 8 %). Numbers correspond to compounds listed in Table 6.2.

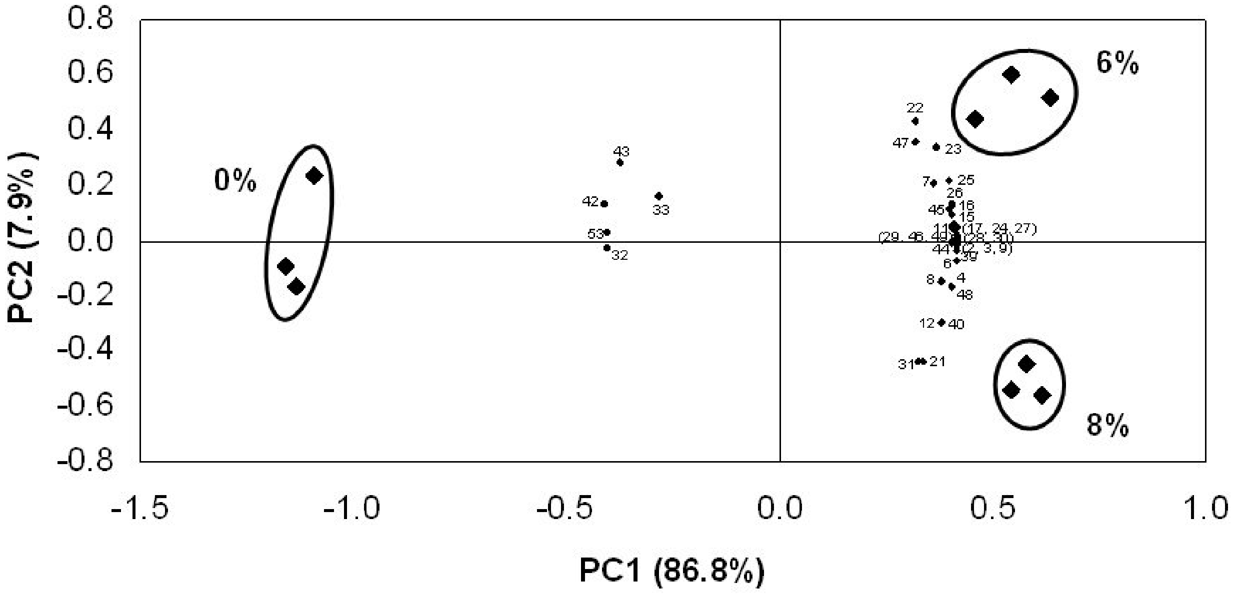

Figure 6.5. PCA biplot of volatile compounds emanating from cooked Jeogjinjubyeo with different degrees of milling (0, 6, and 8 %). Numbers correspond to compounds listed in Table 6.3.

CHAPTER 7

CONCLUSIONS

Rice (*Oryza sativa* L.) is the single most important food crop consumed by man with 628 million tons production from over 100 countries in 2005. It is utilized as a staple be over a one-half of the world's population with approximately 95 % of production being in Asia. Rice is also grown and consumed as an important non-staple food in America, Europe, and Africa, where its popularity is progressively increasing. Because of its importance, rice breeders have developed cultivars with higher yields, insect resistance, herbicide tolerance and other agronomic traits. Grain quality is also a critical breeding objective in that quality has a significant impact on consumer preference. A diverse cross-section of specialty rice types have been developed that have unique flavor, nutritional, textural, esthetic or other properties that often garner higher prices in the market place. Of the various quality attributes of cooked rice, flavor is considered of primary importance in that superior flavor increases consumer satisfaction, overall acceptability and the probability of repeated purchase.

Dynamic headspace system with Tenax trapping and gas chromatography-mass spectrometry (GC-MS) were used to identify and quantify volatile compounds emanating from cooked rice samples. For their characterization, assessment, and discrimination on the different rice flavor types, gas chromatography-olfactometry (GC-O), odor activity values (OAVs) and multivariate analysis were used. This dissertation was composed of four parts.

First, the aroma chemistry of black rice, an aromatic specialty rice popular in Asia was studied by determining volatile profiles of cooked black rice and characterizing the cooked rice with odor-active compounds. Fifty-eight volatile compounds were identified by GC-MS using a dynamic headspace system with Tenax trapping. Aromatics and aldehydes were quantitatively in the greatest abundance, accounting for 72.1 % of total relative concentration of volatiles. The concentration of 2-acetyl-1-pyrroline (2-AP) was high, exceeded only by hexanal, nonanal, and 2-pentylfuran. A total of 31 odor-active compounds determined by GC-O, was applied to principal component analysis (PCA) demonstrating significant differences between a black and a traditional white rice cultivar in terms of aroma, explaining 95.2 % of the total variation. 2-AP, guaiacol, indole, and *p*-xylene largely influenced the difference between the aroma in cooked black and white rice. 2-AP and guaiacol were major contributors to the unique character of black rice based on odor thresholds, relative concentrations, and olfactometry.

Second, we have identified and quantified the volatile compounds emanating from six different rice flavor types using GC-MS, identified and characterized the odor-active compounds using GC-O, assessed the relative importance of each odor-active compound in the overall aroma using odor activity values (OAVs) and compared the distinct aroma of the various flavor types through the presence or absence of single odorants and their OAVs. Our intent in this study was not to identify all possible volatiles emanating from the cooked rice samples but rather to focus on the critical odor-active compounds that would be useful in distinguishing differences among progeny in rice breeding programs. A total of 44 odorants from cooked samples were characterized by trained assessors. Twenty-nine odorants had an intermediate or greater intensity (odor intensity ≥ 3) and were considered major odor-active compounds. Their odor thresholds in air were determined using GC-O. 2-AP had the lowest odor threshold (0.02 ng/L) followed by 11 aldehydes (ranging from 0.09 to 3.12 ng/L), guaiacol (1.49 ng/L), and 1-octen-3-ol (2.70 ng/L). Based on odor thresholds and OAVs, the importance of each major odor-active compound was assessed. OAVs for 2-AP, hexanal, (*E*)-2-nonenal, octanal, heptanal, and nonanal comprised over 97 % of relative proportion of OAVs from each rice flavor type, even though the relative proportion varied among samples. Thirteen odor-active compounds, 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 among the six flavor types were the primary compounds explaining the differences in aroma. Multivariate analysis demonstrated that the individual rice flavor types could be separated and characterized using these compounds which may be of potential use in rice breeding programs focusing on flavor.

Third, premium-quality, black-pigmented, and waxy rice types were discriminated based on six odor-active compounds [(*E*)-2-hexenal, heptanal, (*E*)-2-octenal, guaiacol, (*E*)-2-nonenal, and decanal] that were selected in two steps. Initially 21, 23, and 22 odorants in six premium-quality, three black-pigmented, and two waxy rice cultivars were detected using GC-O, respectively. Among the odorants, 18 primary odor-active compounds were selected based on their OAVs by PCA. The odorants, which including 10 aldehydes, 2 alcohols, 1 ketone, 4 aromatic compounds, and 1 nitrogen-containing compound, were subsequently reduced to 6 odor-active compounds by variable selection for canonical discriminant analysis (CDA). CDA clearly discriminated three rice types with the first (CDF 1) and second (CDF 2) canonical discriminant functions, accounting for 94.6 % and 5.4 % of the total variance, respectively. Two discriminant equations obtained by CDA identified (*E*)-2-hexenal, guaiacol, (*E*)-2-nonenal, and decanal as influencing CDF 1 and heptanal, (*E*)-2-nonenal, and decanal CDF 2. The two discriminant equations provided a predictive model for discriminating the three types of specialty rice. Comparison of cultivars within a specialty types demonstrated excellent separation which may be useful to rice breeders in making progeny selection decisions for improved flavor quality.

Fourth, volatile compounds emanating from 3 cultivars of cooked rice milled to different degrees (0, 6 and 8 % by weight) were compared to ascertain their site of origin and the effect of pigmentation on synthesis. The cultivars tested were Ilpumbyeo (traditional white rice), Heugjinjubyeo (black pigmented), and Jeogjinjubyeo (red pigmented). A total of 41, 51, and 38 volatile compounds were identified in dehulled, unmilled Ilpumbyeo, Heugjinjubyeo, and Jeogjinjubyeo, respectively. Significant quantitative and qualitative differences were found among the cultivars in their volatile profiles. 2-AP, guaiacol, 1-nonanol, 3-octen-2-one, 2-ethyl-1,4-dimethylbenzene, 1,2-dimethoxybenzene, pyridine, and pyrrole were found only in Heugjinjubyeo and (1-methylethyl)benzene and benzenacetaldehyde only in Jeogjinjubyeo. Milling qualitatively affected the volatiles emanating from the samples indicating the bran layer as their site of origin. Milling eliminated safranal, (*E*,*E*)-2,4-nonadienal, toluene, and 2-acethylthiazole from Ilpumbyeo; safranal, (*E*,*E*)-2,4-nonadienal, toluene, 1-nonanol, 1,2-dimethoxybenzene, 1,2,3,4-tetrahydronaphthalene, indole from Heugjinjubyeo; and safranal and benzyl alcohol from Jeogjinjubyeo. Removal of the bran also quantitatively affected the volatile compounds formed with certain volatiles increasing, indicating the endosperm as their primary site of origin and a second group decreasing, indicating the pericarp and aleurone layer (bran) as the primary site of origin. Differences in product chemistry with location within the grain appear to account for the quantitative and qualitative variation in volatile synthesis.