Aroma Chemistry of Wild Rice (*Zizania palustris*) and African Rice Species (*Oryza sativa*, *Oryza glaberrima*, and Interspecific Hybrids)

By

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(Under the Direction of Stanley J. Kays)

ABSTRACT

Wild rice (*Zizania palustris* L.) is an aquatic cereal grain that is gaining in popularity among consumers due to its unique nutty, roasted flavor. Rice production in West Africa, in contrast, is comprised of species of Asian (Oryza sativa L.) and African (O. glaberrima Steud.) origin. The aroma chemistry of wild rice and selected cultivars of African O. sativa ssp. japonica and *indica*, O. glaberrima, and interspecific hybrids was analyzed and characterized using gas chromatography-mass spectrometry, gas chromatography-olfactometry, and descriptive sensory analysis. Seventy-three volatile compounds were identified and quantified in cooked wild rice, 58 of which had not been previously reported. Thirty-four odor-active compounds were identified, with the dominant nutty and roasted aroma. Descriptive sensory panelists described wild rice as having high intensities of 'nutty', 'smoky', 'hay-like', 'earthy', and 'green' aroma attributes. The fermentation and parching steps in wild rice processing are believed to play an important role in creating the unique nutty, roasted aroma based on the fact that a number of pyrazines conferring these aromas, typically formed via thermal reactions, were present in the processed grain prior to cooking. Of forty-two volatiles identified across seven representative rice cultivars grown in West Africa, 3,5,5-trimethyl-2-cyclopenten-1-one, styrene, eucalyptol,

linalool, myrtenal, and L- α -terpineol had not been previously reported in rice. Thirty-three odoractive compounds were characterized. 4-Ethylphenol ('musty') and *(E,E)*-2,4-heptadienal ('fruity') were unique to *O. glaberrima* and pyridine ('cheese'), styrene ('pungent'), eucalyptol ('clove', 'sweet'), and myrtenal ('spicy') were described only in the interspecific hybrid. Descriptive sensory analysis indicated 'cooked grain', 'barny', and 'earthy' attributes were statistically different among African *O. sativa* ssp. *japonica* and *indica*, *O. glaberrima*, and the interspecific hybrid. While based on a very limited selection of germplasm, the aroma chemistry data suggests that the general flavor of African rice is distinct from typical Asian rice and that there appears to be sufficient variation in flavor within the African germplasm to allow separating it into distinct flavor types. The aroma chemistry of wild rice and the selected African rice information provides a foundation upon which product chemistry and consumer preference can be integrated to ascertain the aroma traits conferring superior flavor.

INDEX WORDS: Flavor chemistry; Rice flavor; Odor-active compounds; Volatile compounds; GC-MS; GC-O; Descriptive sensory analysis; Wild rice; *Zizania palustris*; African rice; *Oryza sativa*; *Oryza glaberrima*; Interspecific hybrids

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DEDICATION

To my beloved father, Hyungu Cho, mother, Hyangja Shin who offered me unconditional love and support, and to my beloved brother's family, Hangwoo Cho, Yunjeong Choi, and Youngki Cho

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CHAPTER 1

INTRODUCTION

Rice (Oryza sativa L.) is a staple food in many countries and is consumed by over half of the world's population as a food crop. Rice ranks second in the world in production after maize (FAOSTAT 2006) and its production is forecasted to be a record 466.4 million tons (milled basis) in 2012/13, an increase of less than 1 percent from the previous year due to an increase in production area (USDA, 2012). Rice production is geographically concentrated in Asia. Based on a number of morphological, physiological, biochemical and molecular traits, Asian cultivated rice can be organized into two major subspecies, i.e. O. sativa ssp. japonica and O. sativa ssp. indica (Glaszmann 1987). Asia accounts for about 95% of the world's production and consumption of rice (Bhattacharjee et al., 2002); India, China, Japan, Indonesia, Thailand, Burma and Bangladesh are the major countries for rice production, with China and India supplying over half of the world's production (USDA, 2003). In Asia, the average per capita consumption of rice is over 80 kg/person/year, though rice consumption per capita in many Asian countries is declining. In contrast, less than 10 kg/person/year are consumed in Europe and the U.S. where consumption is increasing sharply (Suwannaporn and Linnemann, 2008; UNCTAD, 2008).

The sensory properties (e.g., texture, stickiness, flavor/taste, aroma, grain size/shape) are crucial to rice quality and small changes in aroma/flavor can make rice highly favored or unacceptable to consumers. Superior flavor increases consumer satisfaction, acceptance and the probability of repeated purchase (Champagne, 2008; Del Mundo and Juliano 1981; Suwansri *et al.*, 2002; Yau and Liu 1999). Due to the importance of aroma in the flavor of rice, a number of studies have focused on identifying the volatile compounds emanating from rice and rice products to understand the characteristic aroma. This involves using gas chromatography-mass spectrometry, gas chromatography-olfactometry, or descriptive sensory analysis.

Wild rice (*Zizania palustris* L.), indigenous to the northern United States and southern Canada, has been consumed as a staple food by native North Americans such as the Ojibway, Menomi, and Cree tribes, since prehistoric time (Johnson, 1969; Lorenz 1981a; Lorenz 1981b). The utilization of wild rice has been gaining in popularity among consumers due to its unique 'nutty' flavor (Johnson, 1969). In addition, wild rice has been recognized as a whole grain by the U.S. Food and Drug Administration (FDA), an agency of the U.S. Department of Health and Human Services. Since whole grains are known to reduce the incidence of chronic disease, wild rice consumption can be beneficial to human health. As a consequence, there has been a significant increase in production volume (i.e., 220%) between 1997 and 2007 to meet the increased demand. Withycombe *et al.* (1978) isolated and identified 112 volatile compounds from wild rice (*Z. palustris*) using gas chromatography-mass spectrometry, however, to the best of my knowledge, wild rice aroma chemicals have not been subsequently studied in spite of the increased interest in the crop.

Oryza glaberrima Steud., domesticated in West Africa more than 3500 years ago, is a second cultivated Oryza species grown there (Jones *et al.*, 1997a; Singh *et al.*, 1997). African O. *sativa* cultivars have high yields but are less adapted to the various abiotic and biotic stresses in African environments. Compared to O. sativa, African O. glaberrima is genetically less diverse and has a much narrower distribution; however, it possesses a cross-section of genes that allow it

to thrive in the harsh environments of West Africa (Jones *et al.*, 1997a; Shim *et al.*, 2010). As a consequence, *O. glaberrima* is considered a source of genes for improving *O. sativa*. Due to its local ancestry and numerous generations of selection, *O. glaberrima* has superior weed competitiveness, drought tolerance, ability to respond to low agronomic inputs and wide adaptability to adverse ecosystems (Baggie *et al.*, 2002; Dingkuhn *et al.*, 1996; Fofana and Rauber 2000; Johnson *et al.*, 1998; Jones *et al.*, 1997a; Khush 1989; Maji *et al.*, 2001; Sahrawat and Sitka 2002). *O. glaberrima* is also resistant to several biotic stresses including rice yellow mottle virus (Abo *et al.*, 1998; Attere *et al.*, 1983; Ndjiondjop *et al.*, 2001), blast (Elizenga *et al.*, 2006; Jones *et al.*, 1997a; Silue and Notteghem, 1991), sheath blight (Elizenga *et al.*, 2006; Wasano and Hirota 1986), African gall midge (Linares 2002), *Heterodera sacchari* (Reversat and Destombes 1995), and leaf hopper (Khush 1989).

The African Rice Center has developed interspecific hybrids between *O. sativa* and the locally adapted and multiple-stress resistant *O. glaberrima* (Jones, *et al.*, 1997b) which appear to increase upland rice production in Western Africa (FAO, 2007). Hybridization between the *O. sativa* and *O. glaberrima* also occurs naturally on farms and the progeny in some cases represent new cultivars (Jusu, 1999; Nuijten and van Treuren, 2007; Nuijten *et al.*, 2009).

In rice breeding programs, researchers have focused mainly on increasing the yield potential and introducing tolerance to environmental stresses, such that rice flavor has been relegated to a very low selection priority in breeding programs (Khush, 2005). Flavor is far more difficult to select for than many other traits and accurate selection decisions are difficult to make. To assist rice breeders, a rapid, accurate screening method is needed for flavor. Such a method would greatly increase the rate of flavor improvement and allow selecting specific and/or unique flavor types.

The objectives of the following research are to: 1) identify the volatile compounds emanating from cooked wild rice, identify the odor-active compounds which are responsible for the unique roasted, nutty flavor, and contrast the volatile composition with traditional brown rice; and 2) characterize and compare the aroma chemistry, odor-active compounds, and aroma attributes of *O. glaberrima*, *O. sativa*, and their interspecific hybrids grown in West Africa, and contrast their volatile composition with a typical Asian *O. sativa*.

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CHAPTER 2

LITERATURE REVIEW

Wild Rice

Wild rice is an aquatic cereal grain belonging to the genus *Zizania* that grows in shallow and sluggish water (Prybylski *et al.*, 2009). Figure 2.1 shows the grain of brown (*Oryza sativa* L.) and wild (*Zizania palustris* L.) rice.



Figure 2.1. Brown (left) and Wild Rice (right) Grains

Wild rice, known also as Canadian rice, Indian rice, and water oats, grows from 0.5 to 2 m high. There are four species, *Zizania palustris, Z. aquatica, Z. texana*, and *Z. latifolia*; the first three of which are native to North America and the fourth to Asia. *Z. palustris* and *Z. aquatica* are annual plants while *Z. texana* and *Z. latifolia* are perennials (Aiken *et al.*, 1998; Catling and Small., 1989; Oelke, 1993; Zhai *et al.*, 2001). Wild rice (*Z. palustris* and *Z. aquatica*), indigenous to the northern United States and southern Canada, has been consumed as a staple food by the native North Americans such as the Ojibway, Menomini, and Cree tribes, since prehistoric time (Johnson, 1969; Lorenz 1981a; Lorenz 1981b). *Z palustris*, found in the temperate and boreal areas of eastern and midwestern North America, is commercially important and is most commonly harvested from lakes, rivers, and streams in the Great Lakes region using boats (Catling and Small 2001; Hoover *et al.*, 1996; Oelke, 1993; Stevenson, 1988). Southern wild rice (*Z aquatica*), found in temperate eastern North America has a short, thin seed and is less used. Texas wild rice (*Z texana*) is endemic to the San Marcos River in Texas and Manchurian wild rice (*Z latifolia*) occurs in eastern Asia and is used as a vegetable and for forage production (Catling and Small 2001).

The wild rice (*Z. palustris*) plant is 1.5 to 1.8 m tall with five or six leaves on the main tiller, can develop 20-30 tillers, is cross-pollinated, matures in about 120 days, and requires 2600 growing degree days (40 °F base temperature) (Oelke and Boedicker, 1991). The wild rice kernel, usually used dehulled but non-polished, has a long and narrow cylindrical shape (Figure 2.1), with a length from 7.5 to 18.0 mm and a width from 1.5 to 4.0 mm (Hoover *et al.*, 1996). Compared with common rice (*O. sativa*) which has shorter and thicker white grains, wild rice grains are longer and brown to black (Catling and Small, 2001).

Wild Rice Production and Utilization

The utilization of wild rice is increased due to its unique flavor (Johnson, 1969). To meet the increased demand, wild rice is being cultivated commercially and is commonly available in supermarkets and restaurants (Oelke *et al.*, 2000). In the United States, part of the wild rice crop is produced as a "domesticated" field crop in diked, flooded fields (Oelke *et al.*, 1982). Minnesota and California accounted for 98.1 % of the U.S. field crop acreage (35,221 and 20,873 acres, respectively) in 2007. With the change a largely cottage industry to a more traditional agribusiness, there has been a significant increase in production volume [i.e., ~ 220% between 1997 (1.2×10^7 kg) and 2007 (3.3×10^7 kg)] (USDA/NASS, 2009).

Most wild rice is sold as a homogenous dried grain, however, mixed wild rice (e.g., with *O. sativa* or other materials) and processed wild rice such as "quick cooking" are also available in markets (Qiu *et al.*, 2009). In addition, it is used as an ingredient in a wide range of gourmet food products such as soups, breakfast cereals, meat dishes, pancakes, muffins, desserts, and others (Oelke, 1993; Qiu *et al.*, 2009; Zhai *et al.*, 2001).

Wild Rice Nutrition

Wild rice (*Z palustris*) contains 74% starch, 14% protein, 6.8% dietary fiber, 1.7% lipids, and 1.8% ash, while white rice (*O. sativa*) contains 82% carbohydrates, 7% protein, 2.8% dietary fiber, 0.6% lipids, and 0.5% ash (USDA Nutrient database, 2008; Hoover *et al.*, 1996). The relatively high level of ash in wild rice suggests that the grain may serve as a good source of minerals such as potassium and phosphorus (Oelke, 1976; Oelke, 1993). It is also rich in essential amino acids, especially lysine and methionine (Wang *et al.*, 1978). The U.S. Food and Drug Administration (FDA), an agency of the U.S. Department of Health and Human Services, has recognized wild rice as a whole grain. The regular consumption of whole grains is known to be beneficial to human health, reducing the incidence of chronic diseases. Phytochemicals, potentially beneficial compounds in whole grains, present in the rice are thought to be key contributors to the health benefits due to their antioxidant properties (Liu, 2007).

Wild Rice Processing

Wild rice undergoes a series of postharvest processing steps: 1) separating immature kernels, 2) curing or fermentation, 3) parching (drying), 4) dehulling, and 5) scarification. The

processing steps contribute to the development of a commercially acceptable product and confer a pleasant, roasted, nutty flavor that distinguishes wild rice from other cereals (Withycombe *et al.*, 1978; Oelke *et al.*, 1997; Bunzel *et al.*, 2002).

Separation of Immature Kernels

Immature wild rice kernels are lighter in weight, have a higher moisture content, and yield less finished product than mature grain. As a consequence, it is advantageous to separate and discard the light-weight kernels which reduces the volume that has to be processed by 20-30 percent (USDA, ERS, 1996).

Fermentation or Curing

Fermentation or curing of wild rice, a complex process involving numerous microorganisms, helps breakdown the tough hulls to facilitate the dehulling process and alters the flavor and the color from green to brown (Oelke and Boedicker, 1991; USDA, ERS, 1996). The typical fermentation process may range from 4 to 7 days. The grain is formed into windrows (0.9 to 1.8 m wide and 0.2 to 0.3 m deep) on an impervious surface outdoors and periodically stirred and watered for several days. Watering keeps the moisture content high and, along with stirring, helps limit heat buildup to minimize dry matter loss and the development of undesirable molds (Oelke and Boedicker, 1991).

Parching (Drying)

Parching (drying) reduces the moisture content from 40 - 50% to approximately 7% and produces a finished product with a slightly roasted flavor and a glassy, translucent appearance of the interior of the kernel (Anderson *et al.*, 1976; Oelke and Boeidicker, 1991; USDA, ERS, 1996). Parching at most processing plants is performed in batch-type, rotary drum dryers that are

typically about 4 feet in diameter and 6 - 8 feet in length. Parching usually takes 2 hours per batch at less than 275 °F (Oelke and Boeidicker, 1991).

Dehulling & Scarification

Dehulling removes the fibrous hull surrounding the kernel while scarification removes part of the outer impermeable layer of the kernel after dehulling, which reduces cooking time (USDA, ERS, 1996).

African Rice

Rice in Africa currently consists of two cultivated species, *O. sativa* L. and *O. glaberrima* Steud. The two subspecies (*japonica* and *indica*) of *O. sativa* were domesticated independently, both in Asia. In ecogeographical terms, the *japonica* variety, a sticky, short grain, is typically found in temperate East Asia, upland areas of Southeast Asia and high elevations in South Asia, while the *indica* subspecies is mainly lowland rice, grown mostly submerged, throughout tropical Asia (Oka, 1988). Asian rice cultivars, especially the Green Revolution semi-dwarf cultivars, have been bred for intensive production and high yield, largely outside of Africa. *O. glaberrima* was probably domesticated from the wild annual rice *O. barthii* A.Chev. (formerly known as *O. breviligulata* A.Chev. et Roehr.) by people living in the floodplains at the bend of the Niger River long before the introduction of *O. sativa* to Africa (~1500 AD) (Linares, 2002; Porters, 1950). *O. glaberrima* is well adapted to the African environment due to its local ancestry and numerous generations of selection *in situ*.

O. glaberrima grows generally between 66 and 120 cm tall, but it is highly variable. The dryland types have smooth, simple culms that can form roots at the lower nodes and are simply branched up to the panicle; the floating types can form branches and even roots at the upper nodes (NRC, 1996). From a distance, *O. glaberrima* and *O. sativa* have similar morphological

characteristics and, as a consequence, it is difficult to tell them apart in the field. Generally *O. glaberrima* has small, pear-shaped grains, a red bran and an olive-to-black seedcoat. The panicles are straight, short and with rounded ligules. However, some of *O. sativa* cultivars have the pear-shaped grains and some *O. glaberrima* cultivars have pointed ligules (Richards, 1996). From the point of selection, the ecological characteristics of *O. glaberrima* and *O. sativa* are more important than their morphological characteristics. *O. glaberrima* cultivars have certain negative features where contrasted with *O. sativa* such as grain shattering, brittle grains, and relatively low yield. However, the *O. glaberrima* types also have distinct advantages; 1) wide leaves that shade out weeds, 2) resistance to diseases and pests, 3) tolerance of fluctuations in water depth, iron toxicity, infertile soils, severe climates, and human neglect, and 4) faster maturity in some of the O. *glaberrima* types, making them important as an emergency food (NRC, 1996; Linares, 2002).

In Africa both the area under cultivation and grain yield per unit of land have not increased significantly even though the human population continues to grow rapidly (Otsuka and Yamano 2005). Thus, it is important to develop new rice cultivars to avoid widespread hunger in the region. There has been considerable effort directed toward increasing productivity and overall production (WARDA 2001). To this end, interest has been focused on creating interspecific hybrids between *O. glaberrima* and *O. sativa* in an attempt to obtain the positive attributes of each such as high yield and the ability to survive in the African environment. An estimated 3000 lines were created in breeding programs in the 1990s and some are now being grown by West African farmers (Sarla and Swamy, 2005).

New Rice for Africa

Interspecific hybridization between the African *O. sativa* and *O. glaberrima* species has produced new cultivars possessing superior traits of both parents: high yields from *O. sativa* and genetic resistance to drought, weed competition, blast, virus diseases, soil iron toxicity, and acidity from *O. glaberrima* (Audebert *et al.*, 1998; Dingkuhn *et al.*, 1998; Dingkuhn *et al.*, 1999; Jones *et al.*, 1997; Johnson *et al.*, 1998). In 2000, the first 7 interspecific hybrid cultivars were released and an additional 11 were released in 2005. The hybrids are now cultivated in 13 Africa countries (Rodenburg *et al.*, 2006) and have enhanced upland rice production (FAO, 2007).

Recent studies have focused on the yield performance and the impact of the new hybrids on income and poverty in Africa. In Uganda, where a program was initiated to measure of poverty eradication, they found that the interspecific hybrid cultivars decreased poverty without an adverse effect on income distribution (Kijima *et al.*, 2008). The hybrids have a greater ability to absorb N under upland conditions and increased grain yield (Matsunami *et al.*, 2009). Salinity tolerance of *O. glaberrima*, *O. sativa* and their hybrids has shown that the latter are fairly tolerant during the seedling stage, while *O. glaberrima* is more sensitive to salinity (Awala *et al.*, 2010). Saito *et al.* (2010) suggested that specific adaptations of the hybrids are likely to provide a significant yield advantage in certain production environments since some lowland genotypes appear to have higher yields under both unfertilized and fertilized conditions in West Africa. Surprisingly the flavor and flavor preference among the interspecific hybrids has not been assessed. To date, I am unaware of any published studies on the aroma chemistry of the interspecific hybrids though the taste and aroma of some progenies are said to be favored by farmers (WARDA, 2001).

Farmer Interspecific Hybrids

To increase local and global food security, the genetic diversity of rice is a critical factor and West Africa is an important source for genetic diversity due to the coexistence of the two species. Recent studies have demonstrated hybridization between the *O. sativa* and *O. glaberrima* occurs in farmers' fields even though it was originally thought that this was not possible without using biotechnological methods (Barry *et al.*, 2007; Nuijten and van Treuren, 2007; Nuijten *et al.*, 2009; Semon *et al.*, 2005). The genotypes of the hybrids may have potential relevance for exploitation by plant breeders.

In a recent study, Nuijten *et al.* (2009) utilized amplified fragment length polymorphisms (AFLP) and morphological analyses to assess 315 cultivars collected in several West African countries. The two subspecies of *O. sativa* (*indica* and *japonica*), *O. glaberrima* and interspecific hybrids were discerned. They found a cluster which appeared to indicate a group of farmer-selected interspecific hybrids that were *O. sativa* with some introgression of *O. glaberrima*. A possible mechanism for field hybridization is spontaneous back-crossing (Nuijten *et al.*, 2009). Field hybridization is a recurrent and continuing process, resulting in different groups of genetic diversity in different rice growing environments. Their findings support the hypothesis that hybridization followed by backcrossing between *O. sativa* and *O. glaberrima* might lead to the development of "new variants not belonging to either of the two species" (Sano *et al.*, 1980). A subsequent study found that hybrid-derived interspecific rice types may be able to perform better than improved types in highly variable and very low input farming conditions (Offei *et al.*, 2010).

Importance of Rice Flavor

The sensory properties of rice (e.g., texture, stickiness, flavor, aroma, grain size/shape, etc.) are very important since it is usually eaten in Asia without seasoning (Champagne 2008).

The physicochemical properties (e.g., quality, pasting properties, texture) are also crucial to eating quality (Okadome et al., 2002; Srisawas and Jindal 2006). Among these properties, the texture of cooked rice is very important (Champagne et al., 1998; Del Mundo et al., 1989; Lyon et al., 2000; Naito and Ogawa 1998; Ohino and Ohisa 2005; Ohino et al., 2007; Ohtsubo et al., 1990; Otobe et al., 1995; Rousset et al., 1995; Shimize et al., 2000; Tsuji 1981). Consumers from different rice-eating countries prefer rice with distinctly different sensory characteristics and tend to prefer their own country's rice types (Kaosa-ard and Juliano 1991; Lyon et al., 1999; Suwannaporn and Linnemann 2008). Juliano (1990) investigated the preference of cooked rice among Asian consumers living in Asian countries and found that the consumers preferred rice with high milling and cooking quality (e.g., few broken grains, polished, intermediate amylose content). For example, Japanese consumers prefer sticky rice containing low amylose and protein contents while aroma, taste and grain length are the most critical quality traits for Indian consumers (Bergmann et al., 2000; Isono et al., 1994). Asian consumers living in U.S., however, considered appearance and aroma to be the most important factors in determining the acceptance of cooked non-aromatic and aromatic cultivars (Meullenet et al., 2001). Suwansri and Meullenet (2001) also indicated that the acceptance by Asian consumers living in the U.S was impacted not only by appearance and aroma but also by flavor and texture of Jasmine rice. In a recent study, Suwannanporn and Linnemann (2008) assessed rice-eating quality among consumers of different nationalities for 5 categories of rice: 1) long grain preference - Southeast Asian and South Chinese; 2) short grain preference - Japanese/Korean, North Chinese/Taiwanese; 3) Basmati preference – South Asian/Middle East; 4) Jasmine preference – Thailand; and 5) nonspecific preference – American/Canadian, European, Australian/New Zealander and a number of other nationalities. They also confirmed that eating qualities (e.g., hardness, stickiness and aroma) were the best discriminators for a country's preference.

With sensory properties such as texture, taste, aroma, and appearance, a small change in aroma/flavor can make rice highly favored or unacceptable to consumers. Superior flavor is known to increase consumer satisfaction and acceptance, and the probability of repeated purchase (Champagne 2008; Del Mundo and Juliano 1981; Suwansri *et al.*, 2002; Yau and Liu 1999). Therefore, a number of studies have focused on identifying the volatile compounds emanating from rice and rice products to understand the characteristic aroma. Instrumental analysis such as gas chromatography-mass spectrometry has identified over 200 volatile compounds present in rice and over a dozen different flavor types of rice have been identified through descriptive sensory analysis (Champagne 2008). The number and concentration of volatile compounds isolated from rice are highly dependent on which method is applied and how the sample is prepared.

Sensory analysis involving measuring human response has also been conducted for identification of aroma/flavor in rice. After the volatiles from rice enter the nasal passages, they bind to receptors on the millions of tiny, hair-like cilia that cover the olfactory epithelium located in the roof of the nasal cavity, from which the aroma is delineated (Meilgaard *et al.*, 2007). To assess rice aroma/flavor and consumer preference, descriptive sensory analysis and quantitative consumer analysis have been studied (e.g., Paule and Powers, 1989; Qingyun *et al.*, 2007; Srisawas and Jindal, 2007; Suwannaporn and Linnemann, 2008; Widjaja *et al.*, 1996). In descriptive sensory analysis, rice aroma/flavor is characterized and assessed by panelists using a lexicon with 10 - 12 descriptors. In quantitative consumer tests, rice preference and acceptance are determined (Lawless and Hyemann, 1999; Meilgard *et al.*, 2007). The combined use of

descriptive and preference sensory panels can identify quality characteristics desired by various markets (Champagne 2008).

Odor-Active Compounds in Rice

A large number of volatile compounds (> 200) emanate from cooked rice, however, only a relatively small number appear to affect the aroma. A total of 36 odorants from six distinctly different rice flavor types (basmati, jasmine, two Korean japonica cultivars, black rice, and a non-aromatic rice) have been characterized [i.e., 2-AP (popcorn), hexanal (green tomato, green), (E)-2-nonenal (beany, cucumber), octanal (citrus), heptanal (floral), nonanal (citrus, fatty), 1octen-3-ol (mushroom), (E)-2-octenal (nutty, cooked flour), (E,E)-2,4-nonadienal (nutty, fatty), 2-heptanone (fruity, sweet), (E,E)-2,4-decadienal (fatty), decanal (citrus), and guaiacol (black rice-like, smoke)] and are the primary volatiles thought to delineate differences in aroma (Yang et al., 2008a). Yang et al. (2010) also found that hexanal was the main odorant in premiumquality and waxy cultivars. Jezzussek et al. (2002) found 41 odor-active compounds in cooked brown rice. 2-Aminoacetophenone had the highest dilution value (DV) and was considered to be an important odorant. A total of 25 odor-active compounds were identified in black rice, an aromatic specialty rice popular in Asia. 2-Acetyl-1-pyrroline (popcorn) and guaiacol (black ricelike, smoky) were the major contributors to the unique aroma, based on their odor thresholds, relative concentrations, and olfactometry (Yang et al., 2008b).

2-Acetyl-1-Pyrroline

Buttery and Ling (1982) reported 2-acetyl-1-pyrroline (2-AP) as a main active flavor component of aromatic rice at a concentration of <100 ppb. Subsequent studies have shown 2AP concentrations range from several hundred ppb to several thousand (Buttery and Ling 1982; Grimm *et al.*, 2011). 2-AP has a lower odor threshold than the other volatile rice odorants and is

described as "popcorn" and/or "nutty-like" (Buttery *et al.*, 1983; Buttery *et al.*, 1986; De Kimpe *et al.*, 1993; Lin *et al.*, 1990; Paule and Poswer, 1989; Petrov *et al.*, 1996). 2-AP has also been reported in non-aromatic rice at a concentration of only a few ppb (Buttery *et al.*, 1988; Grimm *et al.*, 2001). During the growing season 2-AP is synthesized in the plant and can be found in the plant (leaves, stems, and grain), though not in the roots. In aromatic rice, 2-AP is synthesized in its aerial plant parts from L-proline and L-ornithine enzymatically while growing in the field and not during cooking or postharvest processing (Yoshihashi 2002). Maillard reaction products based on L-proline generate roasted notes that contribute to the aroma of many thermally-treated food products such as aromatic rice and bread crust (Blank *et al.*, 2003). Yoshihashi *et al.* (1999) found that accumulation of proline had a positive correlation with 2-AP formation and strong aroma emission in Thailand aromatic rice cultivars (Khao Dawk Mali 105). The biochemical pathway leading to the aroma is largely unknown, though L-proline is believed to be the probable precursor of 2-AP rice (Yoshihashi 2002).

Lipid Oxidation Products

Aldehydes are secondary breakdown products of unsaturated lipids which are highly susceptible to oxidation and play an important role in the aroma of rice; most have low odor thresholds (e.g., 0.07 ppb for (E,E)-2,4-decadienal, 0.7 ppb for octanal, 5 ppb for hexanal) (Buttery *et al.*, 1988). These lipid oxidation products formed during degradation of oleic, linoleic and linolenic acids are those expected from the cleavage of alkoxyl radicals formed from the hydroperoxides of autoxidized and/or photosensitized oxidation of fatty esters (Frankel, 1998). Free fatty acids released by lipase hydrolysis in the residual bran on the surface of milled rice oxidize readily and contribute to off-flavors (Juliano 1985; Jelen *et al.*, 2000). The development of off-flavor depends on temperature, time, and exposure to air or oxygen, and the degree of

milling (Ohta *et al.*, 1990). Octanal, heptanal, nonanal, (E)-2-nonenal, decanal, and 2-heptanone are formed from oleic acid; hexanal, pentanol, pentanal, (E)-2-octenal, (E,E)-2,4-decadienal, and 2-pentylfuran are formed from linoleic acid; and (E,E)-2,4-heptadienal is formed from linolenic acid (Monsoor and Proctor, 2004; Belitz *et al.*, 2004). Lam and Proctor (2004) found that linoleic acid contributed more than oleic and linolenic acids to the total volatile components from milled rice, and therefore to the overall milled rice flavor. During storage changes in linoleic and oleic acids were important indicators of milled rice quality. In broken rice, the concentration of hexanal was higher in head rice due to greater surface lipids and free fatty acids (Monsoor and Proctor, 2004). During storage, the concentration of hexanal, which has been associated with rancidity and consumer rejection of rice, increased significantly, resulting in the development of an off-flavor (Bergman *et al.*, 2000; Monsoor and Proctor, 2004).

Maillard Reaction Products

The Maillard reaction, between the primary amino group of an amino acid and the carbonyl group of the open-chain form of a reducing sugar, is a major route for flavor development in cooked food. The reaction is complex and involves many different pathways leading to a wide range of products (Vernin and Parkanyi, 1982). 2-Aminoacetophenone was found in cooked brown rice and it is formed from tryptophan via Strecker degradation, which is considered as a part of the overall Maillard reaction (Christoph *et al.*, 1999; Jezussek *et al.*, 2002). 2-Phenylethanol and phenylacetic acid were reported in cooked rice and are Strecker degradation products of L-phenylalanine (Etschmann *et al.*, 2005; Hofmann and Schieberle, 2000; Jezussek *et al.*, 2002; Widjaja *et al.*, 1996).

Rice Breeding Programs

Rice breeding programs throughout the world have focused mainly on increasing the yield potential of rice, introducing tolerance to several stresses (biotic and abiotic) and improving grain quality (Khush 2005). For example, rice breeders have been trying to develop cultivars that require less water and agricultural chemicals; breeders have also been working on identifying desirable alleles from new sources of germplasm and combining the information available from the rice genome sequence data and functional genomics to develop superior new cultivars (Negrao *et al.*, 2008). However, the success of incorporating desirable genetic traits into superior new cultivars has been limited due sterility, poor plant type and linkage drag (Jeung *et al.*, 2005; Negrao *et al.*, 2008).

Aroma has been relegated to a very low selection priority in rice breeding programs since it is thought to be too complex and the difficulty of accurately screening large numbers of progeny. Therefore, a better understanding of rice aroma chemistry with the identification of the critical odorants and the determination of their contribution to the overall aroma is needed, along with rapid methods for assessing aroma that will allow rice breeders to make accurate progeny selection decisions. This approach will allow integrating flavor chemistry and consumer preference to develop superior new cultivars.

Flavor Analysis by Gas Chromatography

Determinating of volatile compounds through instrumental analyses requires collection, concentration, separation, identification, and quantification. Static headspace, purge and trap (dynamic headspace), distillation, solvent extraction, and solid-phase micro-extraction (SPME) have been used for collecting and concentrating volatile flavor compounds. One of the most popular collection/concentration methods is dynamic headpace. The dynamic headspace method

removes large amounts of volatiles utilizing a constant flow of a carrier gas across the matrix being assessed via cryogenic, Tenax (or alternative polymer), charcoal or other suitable trapping technique (d'Acampora Zellner et al., 2008; Reineccius 2010). Tenax has a high affinity for nonpolar compounds and has the advantage that water adsorption is very inefficient which allows trapping volatile compounds from vapor-saturated headspace samples (Lindinger et al., 2005). A static headspace method is characterized by sampling the atmosphere in the headspace over the food matrix, after an equilibrium has been reached (d'Acampora Zellner et al., 2008; Reineccius 2010). Direct headspace injections into a GC require relatively high concentrations since the volume injected is such a small part of the total headspace (Reineccius 2010). Distillation methods include high-vacuum molecular distillation, steam distillation, and simply heating of the food and sweeping of the 'distilled' aroma constituents into a GC. One of the potential concerns of the distillation methods is the formation of artifacts due to heating of the sample. Solvent extraction is simple, however, a major limitation is that it is most useful for foods that do not contain lipids, since lipids are extracted along with the aroma constituents. In addition, acids and non-volatile compounds can easily partition into extraction solvent and require extensive cleaning up prior to analysis (Plutowska and Wardencki, 2008). SPME is a technique for the isolation of food aromas from the headspace around a sample. It is a widely applied solvent-free method that utilizes the high adsorption ability of a fused silica fiber coated with a specific extraction phase, the latter of which is selected depending upon the type of sample. A deficiency, however, is the possibility of non-representative extracts. SPME is an equilibrium technique and, as a consequence, the chemical profile of the collected volatiles strongly depends upon the type, thickness and length of the fiber, as well as on the sampling time and temperature (d'Acampora Zellner et al., 2008; Reineccius 2010).

Gas chromatography is the single most widely used method for the separation and quantification of the volatiles collected. Quantification typically utilizes flame ionization or mass spectrometry (MS). Over 7000 volatile compounds have been identified to date (Reineccius 2010).

Gas Chromatography-Olfactometry (GC-O)

The perceived odor of a food product is the composite of one or more volatile compounds that are present in concentrations above the volatile's sensitivity threshold in humans. In order to understand the contribution of a specific volatile compound to the odor of a sample, knowledge of how it is perceived at a given concentration must be understood (Ruth and O'Conner, 2001).

GC-O was proposed by Fuller and co-workers in the 1960's and uses human assessors to detect and evaluate volatile compounds eluting from the GC after separation. Its introduction was a major breakthrough in analytical aroma research, enabling the differentiation of a multitude of volatiles into odor-active and non-odor-active categories and relating their concentrations in the matrix under investigation to the overall aroma (Ruth and O'Conner, 2001; d'Acampora Zellner *et al.*, 2008). The human assessors act as a detector, using their selective olfactory sensitivity. A properly educated individual or a team of evaluating personnel detect the odor of the eluate from chromatographic column, identifying the odor-active volatiles that are responsible for the perceived odors of a specific food product (Perez-Cacho and Rouseff, 2008; Plutowska and Wardencki, 2008). GC-O, therefore, provides not only an instrumental, but also sensorial analysis.

Various methods of GC-O analysis have been developed to determine the relative importance of the odorants in a sample extract: 1) detection frequency, 2) dilution to threshold

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(CharmAnalysisTM) and aroma extraction dilution analysis (AEDA), and 3) direct intensity (posterior intensity method and Osme [the Greek word for smell]).

Detection Frequency

The detection frequency method employs a group of assessors (6 – 12 participants) that detect the odors. The proportion of the group that is able to detect an odorant at a particular retention time is used as an estimate of the odor's intensity (Linssen *et al.*, 1993). The main advantage is simplicity, and as a result assessors do not require much training; however, when a compound at a particular concentration and odor intensity is perceived by all assessors (100% above threshold), its detection frequency cannot increase when the concentration or the odor intensity continues to increase (Delahunty *et al.*, 2006).

Dilution to Threshold

Dilution analysis is based on stepwise dilution to the perception threshold to quantify the odor potency of a compound (van Ruth, 2001). A dilution series of an extract is prepared (usually as a series of 1:2 or 1:3 dilutions) and each dilution (usually between 8 to 12 in total) is assessed by GC-O. Assessors record when they detect an odor and usually characterize the odors with the odor potency being equivalent to the concept of 'aroma values', 'odor units', 'odor values', 'flavor units' and 'odor activity values' (OAVs). AEDA measures the maximum dilution of an extract in which an odor is found and gives the flavor dilution (FD) factor which corresponds to the maximum dilution value in which the compound was detected via GC-O (Lee 2003; Delahunty *et al.*, 2006). CharmAnalysisTM, is based on the detection of decreasing serial dilutions of volatiles with the duration of the odors (start and end) noted similar to a chromatographic peak (Delahunty *et al.*, 2006; van Ruth, 2001).

Direct Intensity

In direct intensity measurements, assessors are required to use a scale to indicate the perceived intensity of the compound as it elutes which can be expressed as a posterior intensity (a single time-averaged measure) and/or time intensity (continuous record of the odor intensity, Osme) (Delahunty *et al.*, 2006). The posterior intensity method involves recording the odor intensity on a scale after a peak has eluted from the column. Osme uses trained assessor(s) directly recording the intensity and duration of each odor-active compound detected at the sniff port using a variable resistor with a pointer moving along a 16-point category scale and describing the odor perceived (van Ruth, 2001). The plot of retention index versus odor intensity, called an Osmegram, represents the significance of each compound in the food aroma, with a higher peak and a larger area under the peak suggesting greater importance (Garruti *et al.*, 2003).

Descriptive Sensory Analysis

Sensory analysis uses a variety of sensitive methods to measure human responses (sight, smell, taste, touch, and hearing) to foods and other products (Drake, 2008). It is important to select the appropriate test, conditions, and data analysis for reproducible and relevant results. Appropriate sensory techniques can be used to enhance product understanding, establish relationships between sensory and instrumental measurements, and enhance consumer insights (Drake, 2008). Descriptive sensory tests are among the most sophisticated descriptive tools utilized by a sensory scientist (Lawless and Hyemann, 1998) in that they allow profiling a product on all of its sensory characteristics (Murray *et al.*, 2001). All descriptive analysis methods involve the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects of a product by trained panels (5 to 100 individuals) (Meilgaard *et al.*, 1991). The qualitative aspects of a product (e.g., aroma, appearance, flavor, texture, after-
taste, sound properties) are quantified using trained panels to describe the perceived product attributes, and are expressed using assigned values on an appropriate scale (e.g., category scales, line scales, magnitude estimation) (Meilgaard *et al.*, 2007; Murray *et al.*, 2001). Descriptive sensory analysis uses a group of individuals, generally 6 to 12, to identify and quantify sensory attributes of a food. It requires training the panelists; the extent of the training depends on the complexity of the sensory attributes that are to be profiled and may range from a few hours to several hundred hours (Drake, 2008). The power of this sensory tool is that the panel and its training can be adjusted to meet specific goals.

There are several different methods of descriptive analysis [e.g., the Flavor Profile Method (FPM), Texture Profile Method (TPM), Quantitative Descriptive Analyis[™] (QDA), Quantitative Flavor Profiling (QFA), the Spectrum[™] method, Free-choice Profiling (FCP)]. In FPM, panelists estimate the overall intensity of a sensation (amplitude) with the intensity of individual descriptors, the order of their perception, and the aftertaste and then during a discussion session directed by the leader, they reach an agreement with regard to the evaluated product (Zawirska-Wojtasiak, 2011). FPM uses panels (generally 4 to 6 individuals) that are trained to precisely define the attributes of the product category (Murray et al., 2001). TPM is based on the FTM but improves the interpretability of the relationship between rheology and its nomenclature (Piggott et al., 1998). QDA, the primary version of descriptive analysis, was developed with the intention of including strategies to account for behavioral aspects of perception. The estimation to be performed requires special preparation with the development of descriptors using standardized verbal descriptions for flavor sensations, an interval scale to measure their intensity, a trained group of the panelists, and appropriate statistical interpretation (Piggott et al., 1998; Zawirska-Wojtasiak, 2011). QFP, a modification of QDA, is used to assess

flavor sensations created by food aromas, food products, and even cosmetics such as perfume (Zawirska-Wojtasiak, 2011). The Spectrum method utilizes principles drawn from FPM and QDA, providing detailed sensory categories and, as with FPM, reference products are chosen to provide attribute-intensity references (Piggott *et al.*, 1998). In FCP, the data cannot be averaged across a panel since each descriptor has a unique meaning to a particular assessor and as a consequence, a special statistical procedure is needed for this method (Piggott *et al.*, 1998).

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CHAPTER 3

Aroma Chemistry of Wild Rice (Zizania palustris)¹

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ABSTRACT

Wild rice (Zizania palustris L.), an aquatic cereal grain, is gaining in popularity among consumers due to its unique nutty, roasted flavor. This study assessed the volatile chemistry of cooked wild rice to determine the odor-active compounds using a dynamic headspace system with Tenax trap, gas chromatography-mass spectrometry (GC-MS), GC-olfactometry (GC-O), and descriptive sensory analysis. Traditional brown rice (Oryza sativa L.) was used to contrast with wild rice. Seventy-three volatile compounds were identified by GC-MS; seven accounted for 52.6% of the total relative concentration of volatiles. A complex mixture of 34 odor-active compounds was identified by GC-O with nutty and roasted the dominant aroma notes. Primary contributors to the unique nutty, roasted aroma were 2-ethylfuran, hexanal, 2-methylpyrazine, 2methyl-2-pentenal, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, benzaldehyde, 2-ethyl-6methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine. Descriptive sensory analysis illustrated the distinct aroma differences between brown and wild rice in that brown rice was primarily described as having 'cooked-rice' and 'buttery' aroma attributes and wild rice as 'nutty', 'smoky', 'hay-like', 'earthy', and 'green'. The fermentation and parching steps in the wild rice processing are believed to play an important role in creating the unique nutty, roasted aroma based on the fact that a number of pyrazines conferring these aromas, typically formed via thermal reactions, were present in the processed grain prior to cooking. The data provide the foundation upon which wild rice product chemistry and consumer preference can be integrated and used to optimize critical processing steps and select for progeny with superior flavor in wild rice breeding programs.

KEYWORDS: Wild rice; *Zizania*; GC-MS; GC-O; descriptive sensory analysis; pyrazines; alkylpyrazines; nutty; roasted; wild rice parching.

INTRODUCTION

Wild rice, known also as Canadian rice, Indian rice, and water oats, is an aquatic cereal grain belonging to the genus *Zizania* that grows in shallow and sluggish fresh water. There are four species (*Z. palustris* L., *Z. aquatica* L., *Z. texana* Hitchc., and *Z. latifolia* Griseb.); the first three are native to North America and the fourth to Asia (1-4). *Z. palustris* and *Z. aquatica*, indigenous to the northern United States and southern Canada, have been consumed as a staple food by native North Americans, especially the Ojibway, Menomini, and Cree tribes, since prehistoric times (5-8). *Z. palustris* is the most commonly harvested as a grain. It grows naturally in lakes, rivers, and streams in the Great Lakes region where it is harvested from boats (2, 9, 10). It is also grown as a domesticated crop in flooded fields primarily in California and Minnesota (11).

Wild rice (*Z. palustris*) has been commercially marketed since the latter half of the 20th century to meet increasing consumer demand due to its unique texture, nutty flavor, and nutritional benefits. It is widely available in grocery and specialty stores as a dried grain or mixed with other grains (e.g., *Oryza sativa* L.) (11, 12) and as an ingredient in a diverse range of gourmet foods such as soups, breakfast cereals, meat dishes, and desserts (2, 4, 12).

Nutritionally the grain is high in protein (14%) and carbohydrate (74%) and very low in fat (1.7%) (9, 13) and contains dietary fiber (6.8%) and sufficient ash (1.8%) to make it a good source of minerals such as potassium and phosphorus (2, 9, 14). It is also rich in essential amino acids, especially lysine and methionine (15). The U.S. Food and Drug Administration (FDA) recognizes wild rice as a whole grain, which is believed to confer a number of human health benefits such as reduced risk of major chronic diseases (e.g., cardiovascular disease, type II diabetes, and some cancers) due to the unique phytochemicals present (16).

Unlike other cereal grains, wild rice undergoes a series of postharvest processing steps: 1) separating immature kernels, 2) curing or fermentation which helps breakdown the tough hulls and alters the grain color and flavor, 3) parching to lower its moisture content from approximately 40-45% to 7% and to impart a slightly roasted flavor to the grain, 4) dehulling which removes the fibrous hull surrounding the kernels, and 5) scarification which removes a portion of the outer layer of the kernel after dehulling (17). The processing steps contribute to the development of a commercially acceptable commodity and confer a pleasant, nutty flavor that distinguishes wild rice from other cereals (18-20).

With the change from a largely cottage industry to a more traditional agribusiness, there has been a significant increase in production volume [i.e., ~ 220% between 1997 and 2007 (21)]. Agronomic changes that have contributed to increased production include the use of commercial rice culture methods and new higher yielding, non-shattering cultivars/lines. The increased volume of product has necessitated significant changes in processing methods (e.g., the use of parboiling, commercial natural gas forced air dryers instead of wood fires). In some instances, traditional processors consider their processing methods proprietary to perpetuate a small scale image. While many of the changes in production and processing methods are believed to improve product quality and consistency, some can alter the quality (e.g., loss of the smoky aroma). The effect of new production and processing methods on the flavor of the final product and consumer acceptance is not known.

Flavor is the major criteria for preference among consumers of traditional rice (*Oryza sativa*) (22). As a consequence, a number of studies have focused on identification of the volatile compounds emanating from cooked rice and rice products to understand their characteristic aroma. In wild rice, based on sensory assessment of the general aroma after fermentation and

parching, these processing steps were considered critical for developing the unique, characteristic flavor of wild rice (18). Few studies, however, have focused on the aroma chemistry of wild rice and there is no data available demonstrating consumer preference nor flavor volatiles emanating from cooked wild rice. Withycombe *et al.* (18) first isolated and identified a number of the volatile constituents of wild rice^{*} using vacuum steam distillation. The roasted aroma was conferred by pyrazines and the smoky aroma appeared to be associated with phenols. Since this initial study, there have been significant analytical advances in the collection, separation, detection and identification of aroma volatiles, increasing the qualitative and quantitative precision of characterizing the flavor chemistry of foods. The objectives of this study were to: describe the aroma of cooked wild rice aroma; to quantify the volatile compounds; and identify the odor-active compounds that are responsible for the unique flavor. The volatile composition was also contrasted with traditional brown rice to illustrate the unique composition of wild rice.

MATERIALS AND METHODS

Wild Rice. Minnesota wild rice (*Z. palustris*) was purchased from www.Nativeharvest.com (2010 harvest) and Mahatma brown rice (*O. sativa*) from a local supermarket (long grain rice grown in the U. S. with only the outer hull removed). Wild rice and brown rice are both whole grains and often cooked together. Samples were sealed in glass and held at -20 °C until analysis.

^{*}Listed as *Z. aquatica*, however, more recent clarification of the taxonomy (23) indicates that the grain was most likely *Z. palustris*.

Chemicals. Analytical standards utilized were: 2-acetylthiazole, 2-acetylpyrazine, benzeneacetaldehyde, benzothiazole, 2-n-butylfuran, &-carvone, (E,E)-2,4-decadieanl, (E)-2decenal, dimethyl disulfide, 2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-6-methylpyrazine, 1-(2-furanylmethyl)-1H-pyrrole, furfural, (E,E)-2,4-heptadienal, heptanal, hexanal, (E)-2-hexenal, 2-hydroxybenzaldehyde, indene, 2methyl-2-pentenal, 1-(2-methylphenyl)-ethanone, 2-methylpyrazine, 2-methylpyridine, 3methylpyridine, 3-nonanone, (E)-2-nonenal, octanal, (E)-2-octenal, 1-octen-3-ol, 3-octen-2-one, pentanal, (E)-2-pentanal, (Z)-2-penten-1-ol, 2-pentylfuran, pyrrole, safranal, and 2-undecanal from Aldrich Chem. Co., Milwaukee, WI; acetophenone, benzaldehyde, biphenyl, dimethyl trisulfide, 2-heptanone, 1-hexanol, D-limonene, 1-methylnaphthalene, naphthalene, 2-nonanone, styrene, and *p*-xylene, from Fluka Chem. Co., Milwaukee, WI; decanal, 2-methoxyphenol (guaiacol), phenol, and pyridine from Sigma-Aldrich, Milwaukee, WI; C7 - C30 saturated alkanes and 2-methylphenol from Suspelco Inc., Bellefote, PA; 2,3-dimethyl-5-ethylpyrazine, 2ethylhexenal, methyl ester benzoic acid, 5-methyl-2-furancarboxaldehyde, 3-nonen-2-one, and 1-(2-pyridinyl)-ethanone from TCI America, Portland, OR.

Sample Preparation and Dynamic Headspace Sampling. Wild rice samples (60 g for GC-MS analysis and 100 g for GC-Olfactometry analysis) were cooked for 40 minutes using a 2:1 water to rice ratio (by weight) on a hotplate (100 °C) in an all-glass system (24). Water to rice ratio was established on the basis of the texture after cooking during preliminary tests. Immediately after cooking, headspace volatiles from the sample were collected for 1 hr while being kept warm in a hot water bath (70 °C). Air introduced into the container was purified by passing it through a Pyrex glass tube containing 2.5 g of activated charcoal (Alltech Assoc. Inc., Deerfield, IL) at 150 mL/min using a vacuum sampling pump (Aircheck Sampler, model 224-

44XR, Eighty-Four, PA). The volatiles were trapped using 150 mg of 60/80 mesh Tenax-TA (Alltech Assoc. Inc., Deerfield, IL). A 50 mL glass flask containing 1 g of CaSO₄ drying agent was placed between the container with cooked rice and the Tenax trap to collect any condensation. After sampling, the trap was thermally desorbed at 250 °C for 5 min using He at a flow rate of 10mL/min via an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, Ringoes, NJ) onto the gas chromatograph fused capillary column (30 m × 0.25 mm i.d., a 0.25 μ m film (5% phenyl-methylpolysiloxane) thickness, HP-5MS, Agilent, Palto Alto, CA) in a GC-MS (model 6890N/5973, Agilent, Palo Alto, CA). Analytes were retrapped on the first 3 cm of the column using a CO₂ cooled cryofocus trap (-40 °C) (SIS 2 inch Cryo-Trap, Scientific Instrument Services, Ringoes, NJ) and subsequently rapidly heated to 200 °C. The desorbed volatiles were separated on the column using temperature programming.

GC-MS Analysis. The volatile compounds were separated, identified and quantified by GC-MS using an injector temperature of 225 °C, split ratio of 0.5:1 and helium carrier gas at a flow rate of 1.0 mL/min. The column temperature was held at 40 °C for 1 min, then programmed at 1.5 °C/min to 65 °C, held for 1 min, increased at 2 °C/min to 120 °C, held for 1 min, and finally increased at 15 °C/min to 280 °C and held for 5 min. Mass spectrometry conditions were ion source at 230 °C, electron energy at 70 eV, multiplier voltage at 1247 V, GC-MS interface zone at 280 °C, and a scan range of 35 – 350 mass units.

Identification and Quantification of Volatile Compounds. The mass spectra and relative abundance of the volatiles were compared with NIST 02 and Wiley 7 spectral libraries for the identification of each volatile. The Kovats retention index (RI) and mass spectra of available authentic standards were also used to confirm identities. The concentrations of the volatile compounds were expressed as δ -carvone equivalents, treating all response factors as 1.0

(24). The concentrations are considered relative since recovery and calibration factors related to the standard were not determined. Five mL of δ -carvone, the internal standard, was placed in a sealed 1 L Erlenmyer flask at room temperature (21 °C). After 24 hr, 10 mL of air saturated with δ -carvone from the flask was injected into the glass beaker containing cooked rice at the beginning of volatile collection. The concentration of the internal standard in the cooked rice sample was calculated by direct GC injection of a range of δ -carvone concentrations in hexane.

GC-Olfactometry (GC-O) Panel Training. A panel of 7 participants, 2 men and 5 women, was trained using a 10-volatile mix (1-hexanol, 2-heptanone, heptanal, 2,5-dimethylpyrazine, 1-octen-3-ol, octanal, 2-acetylpyrazine, *(E)*-2-octenal, 2-nonanone, and *(E)*-2-nonenal). The trained assessors were familiarized with odor description terms and had experience with GC-O from preliminary tests. Odorants from wild rice perceived by at least three assessors were classified as odor-active compounds. The respective odor intensities were scored as: 1 = very mild; 2 = mild; 3 = intermediate; 4 = strong; and 5 = very strong.

GC-O Analysis. An Olfactory Detector Outlet (ODO II, SGE Intl., Austin, TX) was attached to the Agilent 6890N GC. The conditions for the GC-MS and the temperature programming were the same as described above except the flow rate of the carrier gas 2.0 mL/min. The volatile compounds from cooked wild rice were described and the odor intensities rated from 1 to 5 by the evaluators. Each cooked sample was considered as a unique experimental replicate from a homogenous bulk sample of wild rice.

Descriptive Sensory Analysis. Brown rice was used as a reference standard for the descriptive sensory analysis since wild rice is often cooked with brown rice. Microcomputerized rice cookers (Zojirushi model NP-HBC 18, Zojirushi, Japan) were used with a 1:2 water:rice ratio (by weight). The cooking time varied between 28 to 35 min depending upon the type of rice.

Cooked samples were immediately transferred to 92mL standard Pyrex® weighing bottles with short length external 45/12 standard taper joint (Corning #1686-40100, Corning Incorporated, Corning, NY), placed in an insulated container and maintained slightly above 70 °C. The sensory panel consisted of 11 members (3 men and 8 women) drawn from graduate students and staff of the Food Science and Technology department at the University of Georgia. The principles and concepts for the descriptive analysis of rice aroma using the SpectrumTM method (25) were introduced to the panel and a modified 150-mm unstructured line scale (0 = none and 150 = veryhigh) was used instead of a 15-point scale in the original protocol (26). A flavor lexicon for sensory descriptive profiling of traditional rice (27) was used to select the aroma attributes for the study. Three trained panelists with significant experience in rice descriptive sensory analysis initially deleted five of the rice descriptors (sulfury, dairy, rancid, sweet-aromatic, and smoky) due to their absence in wild rice. During training sessions, panelists selected which of the remaining 19 rice aroma descriptors should be included and identified reference intensities. Popcorn, cooked grain, nutty, hay-like, buttery, smoky, floral, earthy, and green attributes were selected as the most important aroma descriptors during the training sessions (4) after panel agreement. Panelists chose cooked-grain over grainy and hay-like over woody. The reference material for the green attribute was changed from alfalfa sprouts (26) to green beans with the same intensity (80) and smoked gouda cheese (King's Choice brand) was used for the smoky attribute (intensity of 90). The wild and brown rice samples were given a 3-digit code and were evaluated by 11 panelists at each session.

Data Analysis. Data from the descriptive sensory analysis was evaluated by analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC.). ANOVA with Duncan's multiple

pairwise comparisons were performed to determine the difference among individual samples for each sensory attribute.

RESULTS AND DISCUSSION

Volatiles from Cooked Wild Rice. A total of seventy-three volatile compounds emanating from cooked wild rice were collected using a dynamic headspace system with a Tenax trap and identified and quantified by GC-MS (Table 3.1). The largest chemical class was pyrazines which included 12 compounds. Seventeen of the volatiles, including 6 pyrazines, had been previously reported (Table 3.1). The 6 most abundant compounds were 2,5dimethylpyrazine (10.8%), (*E,E*)-2,4-heptadienal (10.6%), 2-methylpyrazine (9.8%), (*E,E*)-2,4decadienal (5.4%), benzaldehyde (4.3%), and hexanal (4.2%); the remaining 67 volatiles comprised essentially half of the total (i.e., 47%).

Twelve pyrazines (2-methylpyrazine, 2,5-dimethylpyrazine, ethylpyrazine, 2,3dimethylpyrazine, ethenylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-dimethyl-5ethylpyrzine, and 2-isoamyl-6-methylpyraizne) accounted for 30.3% of the total relative concentration of volatiles present. The alkylpyrazines which are widely found in wild rice are not present in traditional rice (*O. sativa*) types (e.g., aromatic rice, non-aromatic rice, black rice and brown rice) (24, 28-30).

Pyrazines have been recognized as important food flavor components for at least seventyfive years and alkylpyrazines have long been associated not only with heat-treated and fermented foods but also emanate from living organisms (31). Pyrazines are present in cocoa, coffee, roasted peanut and a wide range of other similar products in which they confer a "roasted," "roasted nutty" or "cooked" aroma (32). They had been reported previously as being formed in wild rice during the parching step (18). To test this, the presence of pyrazines in hydrated uncooked grain at 20 °C was investigated (2 hr collection, data not presented). 2-Methylpyrazine, ethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, ethenylpyrazine, 2-ethyl-6methylpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-3-methylpyrazine were detected indicating their presence prior to cooking and supporting their previously (18) proposed synthesis during the parching step. Pyrazines are known to commonly be formed via thermal reactions.

Comparing the previous (18) and current studies, there were major differences in the volatile profiles due to the different isolation methods (vacuum distillation for 4.5 hrs in a saturated NaCl solution versus headspace trapping for 1 hour from rice cooked using traditional methods), time interval between processing at the source and isolation from the sample, and other unknown variables (e.g., differences in fermentation and roasting methods). In this test, qualitatively and quantitatively greater differences in aliphatic aldehydes, such as hexanal, nonanal, and heptanal, were found which are commonly formed via lipid oxidation. They were considered to be important contributors to the overall aroma of wild rice due to their low odor thresholds. Such lipid oxidation products have been identified as key aroma compounds in barley, traditional rice, and whole-meal and white wheat flour (33, 34) and in some instances, are thought to represent off-flavors.

The lipid content of wild rice varies from 0.7 to 1.1% and is comprised primarily of linoleic (35-37%), linolenic (20-31%) and oleic (13-16%) acids (35). Wild rice has a higher percentage of linoleic and linolenic acids compared to brown and white rice which have 36% and 22% linoleic and 1.7% and 4.6% linolenic, respectively (35, 36). Lipid derived volatile

compounds formed during degradation of oleic, linoleic and linolenic acids are those expected from the cleavage of alkoxyl radicals formed from the hydroperoxides of autoxidized and/or photosensitized oxidation of fatty esters (37). (*E*,*E*)-2,4-heptadienal, the second most abundant volatile emanating from cooked wild rice, was most likely derived from linolenic acid and the formation of (*E*,*E*)-2,4-decadienal and hexanal, the fifth and the seventh most abundant volatiles, respectively, from linoleic acid (38, 39).

Table 3.1 shows that there are only 22 volatiles commonly identified and quantified in both wild rice and brown rice. In cooked brown rice, hexanal (18.5%), nonanal (14.9%), 2-pentylfuran (10.5%), octanal (8.4%), 1-octanol (5.9%), and benzaldehyde (5.6%) were the five most abundant volatiles, accounting for 67.6% of the total relative concentration. The most recent study (40) on brown rice found that hexanal, heptanal, nonanal, octanal, pentanal, 2-pentylfuran, and benzaldehyde were the most abundant volatiles in three different rice cultivars. Fifteen volatiles present in brown rice (e.g., 2-acetyl-1-pyrroline) were not found in wild rice. 2-Acetyl-1-pyrroline has long been associated with the characteristic aroma of aromatic rice conferring a popcorn-like aroma when present at concentrations of several hundred ppb (29, 41). It has been previously reported in cooked brown rice (42).

Odor-Active Compounds. In wild rice, 34 odor-active compounds, including 16 aldehydes, 5 pyrazines, 4 ketones, and 4 furans, were identified and characterized by GC-O (Table 3.2). Of the most abundant volatiles (2,5-dimethylpyrazine, (*E,E*)-2,4-heptadienal, 2-methylpyrazine, 1,2-dimethoxybenzene, (*E,E*)-2,4-decadienal, benzaldehyde and hexanal), all but 1,2-dimethoxybenzene were identified as odor-active. Most of the odor-active compounds (i.e., 30 of 34) had odor intensities ≥ 2 (mild); 16 were classified as intermediate (≥ 3) and 2 (hexanal and 2-ethyl-6-methylpyrazine) as strong (≥ 4). Due to the low odor thresholds and

relatively high concentrations, 13 of the 14 aliphatic aldehydes identified (Table 3.2) were odoractive and appear to play a key role in the overall aroma of cooked wild rice.

Benzaldehyde was also considered an important contributor to wild rice aroma. Benzaldehyde, the sixth most abundant compound, is commonly found in a number of distinctly different traditional rice (*O. sativa*) flavor types, including aromatic, non-aromatic, black and glutinous (24, 28, 43) where it is an important odor-active compound with odor descriptors of 'almond' and 'nutty'. Benzaldehyde is also considered a flavor-impact compound in cherry and almond (44, 45). Wild rice is known for its nutty aroma and benzaldehyde, with an odor descriptor of 'nutty' and an intensity of 2.5, would appear to be a significant contributor to this component of the aroma.

The composition of odor-active compounds, with the exception of the aldehydes, was quite different from that of aromatic, non-aromatic, brown and black rice, the latter of which has the same characteristic color as wild rice. In black rice, 2-acetyl-1-pyrroline (2-AP) and guaiacol were the major contributors to the unique aroma (24). The concentration of 2-AP is closely tied to the overall aroma of a number of rice types (24, 28, 41, 43, 46, 47); however, it was not found in wild rice.

Several volatiles described as 'cooked rice' by panelists (e.g., 2-methylpyrazine, 2methyl-2-pentenal, furfural, 2-pentylfuran, 3-ethyl-2,5-dimethylpyrazine, and one unknown) were found (Table 3.2). Wild rice had a relatively high concentration of 2-pentylfuran which was described as beany', 'green', and 'almond'; in traditional rice types, the odor of 2-pentylfuran was described as bean and nutty (43, 48). In wild rice, it was an important odor-active compound (Table 3.2). Zeng *et al.* (43) found 2-butylfuran in Japanese glutinous rice though it was not considered to be odor-active. The 4 furans identified in wild rice (e.g., 2-ethylfuran, furfural, 2-*n*- butylfuran, 2-pentylfuran) were weak [2] to intermediate [3] in their odor intensities and were described as 'nutty'. Furans are produced by both lipid oxidation and the Maillard reaction. They impart a sweet or caramel-like aroma to a number of foods [e.g., coffee (48-50)]. Parching during wild rice processing is the most probable step in which the Maillard reaction products such as pyrazines and furans were formed. Wild rice contains a relatively high level of glutamic acid (2.7 g/100 g), in contrast to brown and traditional white rice (i.e., 1.5 and 1.3 g per 100 g, respectively) (36), which with glutamine would favor furan and pyrazine synthesis (50).

Pyrazines confer 'roasted', 'toasted', and 'nutty' aromas to foods (50) and the alkylpyrazines, found in wild rice, contributed to the unique wild rice aroma. 2-Methylpyrazine [odor intensity: 3.5], 2,5-dimethylpyrazine [3.3], 2,3-dimethylpyrazine [3.5], 2-ethyl-6methylpyrazine [4.0], and 3-ethyl-2,5-dimethylpyrazine [3.0] were identified as odor-active compounds with 'nutty', 'roasted', 'almond', or 'cooked rice' descriptors. The odor-active pyrazines identified by the sensory panel had intermediate or greater odor intensities and appeared to contribute to the unique 'nutty' aroma to wild rice. Yang et al. (28) identified the odor-active compounds in basmati, jasmine, Korean aromatic, black pigmented and nonaromatic rice; 8 of the 34 odorants had a 'nutty' or 'almond' odor (e.g., pentanal, chlorobenzene, benzaldehyde, 3-ethyl-2-methyl-1,3-hexadiene, (E)-2-octenal, (E,E)-2,4-nonadienal, (E)-2decenal, and 4-vinylguaiacol) though none were pyrazines. Jezussek et al. (29) however, identified two odor-active pyrazines (2-methoxy-3,5-dimethylpyrazine and 2-isobutyl-3methoxypyrazine) in cooked brown rice. Methoxypyrazines rarely occur as heat-induced volatiles and are more likely natural secondary metabolites (31) which conferred an 'earthy' note. The unique processing steps used for wild rice could be one of the reasons for the presence of the alkylpyrazines. Among the five pyrazines, 2-ethyl-6-methylpyrazine had the highest odor

intensity [4.0]. Several studies have confirmed the nutty and roasted odor of 2-ethyl-6methylpyrazine [e.g., roasted hazelnut (51) and buckwheat tea (52)] though other studies have characterized its odor as 'fruity' and 'flowery' in coffee (53) and 'fruity' in roasted sesame (54). In wild rice, the panelists described 2-ethyl-6-methylpyrazine as having an 'almond' odor indicating that the 'nutty' aroma is due mainly to pyrazines and furans.

Jezussek *et al.* (29) reported the 10 most important odorants in cooked brown rice (2acetyl-1-pyrroline, (E,E)-2,4-decadieanl, (E)-4,5-epoxy-2-decenal, unknown compound, bis-(2methyl3-furyl)-disulfide, 3-hydroxy-4,5-deimthyl-2(5H)-furanone, 2-methoxy-4-vinylphenol, 2amino acetophenone, phenylacetic acid, and vanillin); 2-aminoacetophenone with the highest dilution value was considered to be an important odorant. Of the ten odorants 2-acetyl-1pyrroline and (E,E)-2,4-decadienal were also found in this study, though their concentrations were low (4.0 ng and 50.7 ng/100 g, respectively). The presence and concentration of 2-acetyl-1pyrroline (popcorn aroma) varies with analytical technique and the origin of the rice. For example, in Korea elevated 2-acetyl-1-pyrroline is considered highly desirable and pragmatically selected in breeding programs.

Descriptive Sensory Analysis. Differences in the aroma of brown and wild rice were characterized using descriptive sensory analysis. The aroma intensity of 9 aroma attributes ('popcorn', 'cooked grain', 'nutty', 'hay-like', 'buttery', 'smoky', 'floral', 'earthy', and 'green') evaluated by 11 trained panelists are displayed in Figure 3.1. Seven of the 9 attributes (i.e., 'cooked grain', 'nutty', 'hay-like', 'buttery', 'smoky', 'earthy', and 'green') were statistically different (p < 0.05) between the two species such that the panelists could readily distinguish between the aroma of brown and wild rice. In wild and brown rice, the values for the 'popcorn' attribute were not statistically different, though brown rice was slightly higher. Even though the

concentration of 2-acetyl-1-pyrroline in the brown rice was low (4 ng/ 100 g) (Table 3.1), it appeared to have a role in the overall aroma. In contrast, it was not identified in wild rice and the origin of the 'popcorn' descriptor is not clear. The 'floral' attribute was low in both wild (7.3 intensity) and brown rice (8.4) and even though in wild rice several odor-active compounds (i.e., octanal, 3-octen-2-one, benzeneacetaldehyde, nonanal) were described as 'floral' in GC-O analysis, these compounds did not appear to play a primary role in the aroma.

The 'cooked-grain' and 'buttery' attributes (42.1 and 36.8, respectively) of brown rice had significantly higher intensities than in wild rice and appeared to be important aroma attributes in brown rice. In contrast, the 'nutty', 'smoky', 'hay-like', 'earthy', and 'green' attributes were much higher in wild rice than in brown rice. The intensity of the 'nutty' and 'smoky' attributes were 18.8 and 20.7, respectively and have previously been considered important characteristics in wild rice (18). Two additional aroma attributes in wild rice were 'hay-like' (31.1) and 'earthy' (38.1).

In conclusion, 73 volatile compounds were identified, 58 of which had not been previously reported in cooked wild rice. Thirty-four were identified as odor-active compounds which were characterized by a cross-section of descriptors, among which 'nutty' and 'roasted' odors predominated. The overall aroma was derived primarily from pyrazines, furans, and aliphatic aldehydes. The fermentation and parching steps in wild rice processing are believed to play an important role in creating the unique nutty, roasted aroma based on the fact that a number of pyrazines conferring these aromas, typically formed via thermal reactions, were present in the processed grain prior to cooking. Descriptive sensory panelists described brown rice mostly with 'cooked-rice' and 'buttery' attributes and wild rice with 'nutty', 'smoky', 'hay-like', 'earthy', and 'green' attributes, confirming that aromas of brown and wild rice were distinctly different.

The information on the aroma chemistry of wild rice provides foundation upon which product chemistry and consumer preference can subsequently be integrated to ascertain the aroma traits that confer superior flavor. In addition the effect of processing (fermentation and parching conditions), storage (conditions and duration), and agronomic variables (e.g., wild versus cultivated, harvest date, weather) on product quality can be objectively assessed. As wild rice moves progressively further from its use solely as a staple, the role of its flavor when combined with white rice or as an ingredient of soups, frozen dinners and mixed vegetables remains to be ascertained. Clearly a better understanding of the factors modulating the flavor chemistry and consumer acceptance of wild rice are needed.

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RI ^a compound		relative concentration (ng/100 g) ^b
679	pentanal	29.2 ± 2.6
730	dimethyl disulfide	27.3 ± 3.2
741	pyridine ^c	312.3 ± 10.2
750	(<i>E</i>)-2-pentenal ^c	316.7 ± 23.3
752	pyrrole ^c	131.0 ± 9.3
773	(Z)-2-penten-1-ol	185.5 ± 10.5
807	hexanal ^d	1433.5 ± 8.5
820	2-methylpyridine ^c	51.8 ± 1.8
832	2-methylpyrazine ^c	3365.0 ± 144.2
837	2-methyl-2-pentenal	133.8 ± 7.2
840	furfural ^d	879.0 ± 12.0
852	(<i>E</i>)-2-hexenal ^d	215.7 ± 7.2
859	3-methylpyridine	39.8 ± 0.7
867	<i>p</i> -xylene ^d	39.0 ± 2.0
884	styrene ^c	218.5 ± 12.2
887	2-n-butylfuran	65.5 ± 2.2
907	heptanal ^d	346.5 ± 12.2
911	2,5-dimethylpyrazine ^c	3709.8 ± 153.2
916	ethylpyrazine	73.8 ± 5.2
918	2,3-dimethylpyrazine ^c	173.0 ± 8.3
924	ethenylpyrazine	222.0 ± 6.5

Table 3.1. Concentrations of Volatiles Identified in Cooked Wild	Rice
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932	3-ethyl-1,5-octadiene	150.3 ± 3.7
959	benzaldehyde ^{c,d}	1464.3 ± 32.8
961	dimethyl trisulfide	95.0 ± 6.0
965	5-methyl-2-furancarboxaldehyde	200.0 ± 4.2
980	5-methyl-2-furanmethanol	41.2 ± 2.5
997	2-pentylfuran ^d	1095.7 ± 13.5
998	2-ethyl-6-methylpyrazine ^c	1027.8 ± 61.8
999	2-ethyl-5-methylpyrazine ^c	689.0 ± 61.8
1000	2-ethyl-3-methylpyrazine ^c	638.8 ± 17.7
1002	(<i>E,Z</i>)-2,4-heptadienal	150.7 ± 7.2
1003	phenol ^c	111.0 ± 2.2
1009	octanal ^d	400.3 ± 9.0
1016	(E,E)-2,4-heptadieanl ^d	3655.2 ± 52.0
1017	2-acetylthizole ^d	60.8 ± 0.2
1029	D-limonene ^d	119.8 ± 4.0
1037	1-(2-pyridinyl)-ethanone	135.3 ± 7.0
1041	indene	97.7 ± 2.2
1042	2-hydroxybenzaldehyde	85.5 ± 3.2
1043	3-octen-2-one	260.5 ± 5.8
1045	bezeneacetaldehyde ^d	395.0 ± 13.3
1070	acetophenone ^{c, d}	219.0 ± 8.7
1073	2-ethylhexenal	54.8 ± 2.3
1078	3,5-octadien-2-one	128.8 ± 7.0

1080	2-methylphenol	104.3 ± 5.7
1085	3-ethyl-2,5-dimethylpyrazine	509.2 ± 16.5
1087	2-ethyl-3,5-dimethylpyrazine	80.7 ± 4.8
1088	2,3-dimethyl-5-ethylpyrazine	124.5 ± 7.3
1089	2-methoxyphenol (guaiacol)	295.3 ± 20.8
1090	3-nonanone	269.8 ± 7.5
1092	methyl ester benzoic acid	60.2 ± 2.0
1098	(<i>E,E</i>)-3,5-octadien-2-one	305.7 ± 7.3
1112	nonanal ^d	452.2 ± 10.2
1139	2-ethylbenzaldehyde	33.7 ± 3.0
1140	3-nonen-2-one	108.3 ± 6.5
1164	(<i>E</i>)-2-nonenal ^d	330.3 ± 6.3
1172	naphthalene ^d	832.5 ± 16.3
1184	1-(2-methylphenyl)-ethanone	75.3 ± 5.2
1193	1-(2-furanylmethyl)-1H-pyrrole	31.2 ± 1.7
1194	safranal	133.7 ± 9.8
1206	decanal ^d	96.7 ± 1.7
1216	benzothiazole ^{c, d}	36.7 ± 1.8
1245	2-isoamyl-6-methylpyrazine	99.8 ± 5.7
1260	(E)-2-decenal ^d	109.3 ± 7.5
1294	1-methylnaphthalene ^c	314.2 ± 8.8
1298	(E,Z)-2,4-decadienal ^d	1168.8 ± 20.3
1317	(E,E)-2,4-decadienal ^d	1868.7 ± 51.2

1358	2-undecenal ^d	52.0 ± 3.3
1362	biphenyl ^c	75.0 ± 4.5
1387	(E)-2-tetradecene	27.3 ± 2.3
1415	thujopsene	33.3 ± 3.2

^a Retention index based on HP-5MS column using a series of n-hydrocarbons. ^b Values expressed as δ -carvone equivalent (ng/ 100g). ^c Compounds previously reported by Withycombe *et al.* (18). ^d Compounds identified in brown rice.

RI ^a	odorant	odor description ^b	intensity ^c	reported odor
				threshold (ppb) ^d
700	2-ethylfuran	burnt, nutty, almond	3.4	N/A
730	dimethyl sulfide	sulfury	2.8	3100
807	hexanal	grass, green tomato, green, fruity	4.1	5
824	2-methylpyrazine	nutty, roasted, cooked rice	3.5	60000
839	furfural	sweet, roasted, nutty, cooked rice	2.8	23000
841	2-methyl-2-pentenal	fruity, sweet, cooked rice, roasted	3.3	290
862	(E)-2-hexenal	sweet, fruity, fresh	2.7	17
876	unknown ^d	roasted	3.5	N/A
887	2-n-butylfuran	roasted, nutty	3.0	N/A
907	heptanal	grass, fresh	3.5	5
911	2,5-dimethylpyrazine	nutty, roasted	3.3	1500
920	2,3-dimethylpyrazine	nutty, almond	3.5	2500
959	benzaldehyde	nutty	2.5	350

Table 3.2. Odor Intensity and Description of Odor-Active Compounds in Cooked Wild Rice

975	unknown	cooked rice, waxy	1.0	N/A
997	2-pentylfuran	beany, green, almond	2.6	6
998	2-ethyl-6-methylpyrazine	almond	4.0	N/A
1001	(<i>E,Z</i>)-2,4-heptadienal	citrus, grass	3.0	N/A
1009	octanal	floral, citrus	3.2	0.7
1016	(<i>E,E</i>)-2,4-heptadienal	green, fruity, citrus	3.2	560
1029	D-limonene	fresh, sweet	2.3	10
1043	3-octen-2-one	citrus, herbal, floral	3.2	N/A
1045	benzeneacetaldehyde	herbal, floral	3.5	N/A
1073	2-ethylhexenal	beany	1.7	N/A
1078	3,5-octadien-2-one	fruity	1.5	N/A
1085	3-ethyl-2,5-dimethylpyrazine	cooked rice, nutty	3.0	8.6
1090	3-nonanone	spicy, caramel, sweet	2.7	17-33
1098	(<i>E,E</i>)-3,5-octadien-2-one	fruity, medicinal, green	3.0	150
1112	nonanal	fresh, grass, cucumber, citrus, floral	2.8	1
1139	2-ethylbenzaldehyde	roasted, nutty	2.7	N/A

1164	(<i>E</i>)-2-nonenal	cucumber	2.3	0.08
1172	naphthalene	naphthalene	1.7	5
1206	decanal	citrus	2.0	2
1260	(E)-2-decenal	fatty	3.0	0.4
1317	(<i>E,E</i>)-2,4-decadienal	fatty, chicken	3.5	0.07

^a Retention index based on HP-5MS column using a series of n-hydrocarbons. ^b Odorants were described by at least three panelists during GC-O. ^c Average intensity of compounds that were detected by at least three panelists. ^d Odor thresholds acquired from compilations of threshold values in water by van Gemert (55). ^e Values expressed as δ -carvone equivalent (ng/ 100g).





^a Attributes with an * indicates a significant difference in intensity (p < 0.05) between wild and brown rice.

CHAPTER 4

Aroma Chemistry of African Oryza glaberrima and Oryza sativa Rice and Their

Interspecific Hybrids¹

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ABSTRACT

Rice production in West Africa is comprised of species of Asian (Oryza sativa L.) and African (O. glaberrima Steud.) origin. O. glaberrima is thought to have been cultivated in Africa for approximately 3500 years while O. sativa was introduced into West Africa by the Portuguese around 1500 AD. To increase rice production in Africa, considerable interest has been focused on creating interspecific hybrids between O. glaberrima and O. sativa in an attempt to obtain the positive attributes of each in new cultivars. Since flavor is a key criterion in consumer acceptance of rice, as an initial inquiry we characterized and compared the aroma chemistry of selected cultivars of African O. sativa ssp. japonica and indica, O. glaberrima, and their interspecific hybrids grown in West Africa using gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), and descriptive sensory analysis. Using a dynamic headspace system with Tenax trap, a total of 42 volatile compounds emanating from cooked samples were identified and quantified; six had not been previously reported in rice (e.g., 3,5,5-trimethyl-2-cyclopenten-1-one, styrene, eucalyptol, linalool, myrtenal, L-α-terpineol). The most abundant volatile compounds across all species were lipid oxidation products (e.g., hexanal, nonanal, 1-hexanol, 2-pentylfuran, benzaldehyde, (E,E)-2,4-decadienal, octanal). Eucalyptol and myrtenal were only found in an interspecific hybrid, L- α -terpineol was unique to both African O. sativa subspecies and 2,6-dimethylpyridine and 2-methylpyridine were quantitatively significant volatiles emanating from O. glaberrima. Thirty-three odor-active compounds were identified in African O. sativa subspecies (japonica and indica), O. glaberrima, and an interspecific hybrid. 4-Ethylphenol ('musty') and (E,E)-2,4-heptadienal ('fruity') were unique to O. glaberrima. Pyridine ('cheese'), styrene ('pungent'), eucalyptol ('clove', 'sweet'), and myrtenal ('spicy') were identified as odor-active compounds only in the interspecific hybrid. Principal component

analysis of the odor-active compounds demonstrated a distinct separation of African *O. sativa* subspecies (*japonica* and *indica*), *O. glaberrima*, and an interspecific hybrid. Descriptive sensory analysis indicated distinct differences in aroma among the four rice types with 'cooked grain', 'barny', and 'earthy' aroma attributes being significantly different. Principal component analysis of the descriptive sensory data also indicated separation of the four rice types. While based on a very limited selection of germplasm, the aroma chemistry data suggests that the general flavor of African rice is distinct from rice grown in Asia, suggesting that the Asian and African *O. sativa* germplasm pools began to diverge many years ago in response to differing selection criteria. There appears to be sufficient variation in flavor within the African germplasm to allow separating cultivars into distinct flavor types as we have previously demonstrated with Asian rice cultivars (Yang et al., 2008). Further analysis of the aroma chemistry using a much wider range of germplasm will establish the validity of this possibility and the subsequent inclusion of consumer sensory analysis will facilitate the selection of progeny with superior flavor in African rice breeding programs.

KEYWORDS: Aroma chemistry; odor-active compounds; GC-MS; GC-O; descriptive sensory analysis; *Oryza; Oryza glaberrima; Oryza sativa*; interspecific hybrid

INTRODUCTION

The genus Oryza is comprised of 24 species that are separated into 6 diploid and 4 tetraploid groups (1); only 2 species are cultivated. O. sativa L., the leading human food crop worldwide, is grown over an exceedingly wide geographic area. The species is broadly separated into two subspecies, *indica* and *japonica*. Rice grown in Africa is comprised of both O. sativa subspecies and an indigenous species, O. glaberrima Steud., believed to have originated in the Niger area around 3500 years ago (2-3). While the precise time O. sativa arrived in Africa is not known, appears to have been introduced into West Africa around 1,500 AD by the Portuguese (3). O. sativa subsequently spread and was adopted by peoples living along the Upper New Guinea coast and a number of local cultivars were selected by farmers in the region that reflected local agronomic considerations and other preferences (e.g., flavor, ease of milling and cooking). In 1965 two thirds of the cultivars used by the Jola in Senegal were *O. sativa* with the remainder O. glaberrina (4). The grain of both species in Africa typically has red bran though a small number of cultivars with white grain are found (5). The flavor differs markedly from that of rice imported from Southeast Asia and the United States (food aid to Senegal), neither of which are preferred (4).

O. glaberrima and *O. sativa* differ widely in physical traits, yield and resistance to biotic and abiotic stress (6). The grain of *O. glaberrima* readily shatters, is brittle, and has poor milling quality and a flavor that is distinct from cultivars of *O. sativa* grown in Asia. It also, however, has distinct advantages; 1) wide leaves that shade out weeds, 2) resistance to diseases and pests, tolerance of fluctuations in water depth, ion toxicity, infertile soils, severe climates, and human neglect, and 3) more rapid maturity in some of the *O. glaberrima* clutivars (4, 7). The combined yield of both species in Africa is sufficiently low that close to 10 million tons of milled rice are

imported into sub-Saharan Africa every year (8). As a consequence, considerable interest has been focused on creating interspecific hybrids between *O. glaberrima* and *O. sativa* in an attempt to obtain the positive attributes of each species. An estimated 3000 lines were created in breeding programs in the 1990s, some of which are now being grown by West African farmers (6). Recent evidence indicates that interspecific hybridization also occurs naturally in farmer's fields resulting in new lines and in some instances, cultivars (9-11). While the new hybrids developed in breeding programs are closely screened for agronomic traits impacting productivity, little is known about the chemical properties governing their flavor and consumer preference, as is also the case with farm selections.

Flavor, the composite of aroma (odor) and taste, is one of the most important factors in defining the character and quality of rice and in determining consumer preference (12). In rice, aroma is generally the primary contributor to the overall flavor. As a consequence, the identification and quantification of the volatile compounds emanating from cooked rice has been a major research objective. Over 300 volatiles have been reported using a cross-section of isolation techniques (13) though typically only a small number (< 40) are considered critical odor-active compounds that account for differences in the aroma among and within rice flavor types (14-18).

From a preliminary test with five panelists with considerable sensory panel experience, it was found that the aroma of African *O. glaberrima*, *O. sativa* and their interspecific hybrids were similar but could be discerned. The objective of this study was to characterize and compare the aroma chemistry of *O. glaberrima*, *O. sativa*, and their interspecific hybrids grown in West Africa, using gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), and descriptive sensory analysis.

MATERIALS AND METHODS

Rice Samples and Chemicals. The rice cultivars were grown in West Africa and samples of the grain shipped by airmail to the University of Georgia where it was stored in sealed glass jars until analysis. There were two cultivars of *Oryza sativa* ssp. *japonica* ('Gbengbeng' and 'Jebbehkomie'), two of *O. sativa* ssp. *indica* cultivars ('Buttercup' and 'Pla Camp'), one of *O. glaberrima* ('Maalay') and two interspecific hybrids ('Binta Sambou' and 'Painy Painy') (Table 4.1) which were designated in the text as Sj1, Sj2, Si1, Si2, G1, SG1, and SG2, respectively. The interspecific nature of the hybrids was substantiated by AFLP (amplified fragment length polymorphism) analysis which rapidly generates hundreds of highly replicable markers from DNA of any organism and allows high-resolution genotyping of fingerprinting quality (19-20) for screening genetic diversity. AFLPs are polymerase chain reaction (PCR)-based markers that are highly replicable and easy to use (20). A non-aromatic Asian rice (*O. sativa* ssp. *japonica* designated as SjAsian), purchased in a local supermarket, was used for comparison.

Analytical standards utilized were: 2-acetylpyrazine, benzeneacetaldehyde, δ -carvone, (E,E)-2,4-decadienal, (E)-2-decenal, 2,5-dimethylpyrazine, 2,6-dimethylpyridine, eucalyptol, furfural, (E,E)-2,4-heptadienal, heptanal, 1-heptanol, 2-heptanone, hexanal, (E)-2-hexenal, linalool, 2-methylpyridine, myrtenal, (E)-2-nonenal, 3-octen-2-one, octanal, (E)-2-octenal, 1-octen-3-ol, and 2-pentylfuran from Aldrich Chem. Co., Milwaukee, WI; acetophenone, benzaldehyde, 2-decanone, 2-ethyl-1-hexanol, 2-heptanone, 1-hexanol, limonene, naphthalene, 2-nonanone, 1-octanol, 1-pentanol, styrene, and *p*-xylene from Fluka Chem. Co., Milwaukee, WI; C7 –

C30 saturated alkanes and nonanal from Suspelco Inc., Bellefote, PA; and 3-furaldehyde, 3nonen-2-one, and tetradecanal from TCI America, Portland, OR.

Sample Preparation. Rice samples (100g) were cooked in a glass container using an 1.8:1 water to rice ratio (by weight) on a hotplate (100 °C). The water to rice ratio was based on the texture of the rice after cooking during preliminary tests. Immediately after cooking, headspace volatiles were collected while holding the container of rice in a hot water bath (70 °C) by passing air purified using a charcoal filter [Pyrex glass tube with 2.5 g of charcoal (Alltech Assoc. Inc., Deerfield, IL)] through the chamber at 150 mL/min for 2 hr using a vacuum sampling pump (Aircheck Sampler, model 224-44XR, Eighty-Four, PA) (for details see Yang *et al.*, 2008 (17)). The volatiles exiting the container were collected using a trap with 150 mg of 60/80 mesh Tenax-TA (Alltech Assoc. Inc., Deerfield, IL). A 50 mL glass flask containing 1 g of CaSO₄ (drying agent) was placed between the container with the cooked rice sample and the Tenax trap to collect any condensation.

GC-MS Analysis. After sampling, the Tenax trap was thermally desorbed at 250 °C for 5 min with He at a flow rate of 10mL/min via an automated short path thermal desorption system (Model TD-5, Scientific Instrument Services, Ringoes, NJ) onto the gas chromatograph column (GC-MS, model 6890N/5973, Agilent, Palo Alto, CA). Analytes were retrapped on the first 3 cm of the column using a CO₂ cooled cryofocus trap (-40 °C) (SIS 2 in. Cryo-Trap, Scientific Instrument Services, Ringoes, NJ) and subsequently heated up to 200 °C. The desorbed volatiles were separated on a 30m length, 0.25 mm i.d., 0.25 μ m film thickness (5% phenylmethylpolysiloxane), fused silica capillary column (HP-5MS, Agilent, Palto Alto, CA). The injector temperature was 225 °C, the split ratio of 0.5:1 and the carrier gas helium at a flow rate of 1.0 mL/min. The column temperature was held at 40 °C for 1 min and then programmed to

increase at 1.5 °C/min to 65 °C, held for 1 min, increased at 2 °C/min to 120 °C, held for 1 min, and finally increased at 15 °C/min to 280 °C for 5 min. Mass spectrometry conditions were ion source at 230 °C, electron energy at 70 eV, multiplier voltage at 1247 V, GC-MS interface zone at 280 °C, and a scan range of 35 – 350 mass units.

Identification and Quantification of Volatiles. The mass spectra and relative abundances of the volatiles were compared with NIST 02 and Wiley 7 spectral libraries for identification. The Kovats retention index (RI) and mass spectra of available authentic standards were also used to confirm identities. The concentrations of the volatile compounds were considered as relative and were expressed as δ -carvone equivalents, assuming that all of the response factors were 1 (17). The volatiles were analyzed in triplicates.

Gas Chromatography-Olfactometry. An olfactory detector outlet (ODO II, SGE Intl., Austin, TX) was attached to the Agilent 6890N GC and the compounds emanating identified by GC-MS using the same fused silica capillary column. At the end of the capillary column, the effluent was split between the mass spectrometer and the sniffing port of the olfactory detector outlet, where panelists assessed the volatiles. The instrumental conditions were same as the GC-MS analysis with the exception of the carrier gas flow rate which was 2.0 mL/ min.

Panel Training. A panel of 7 participants, 2 men and 5 women, was trained in GC-O using a 10-volatile mix (1-hexanol, 2-heptanone, heptanal, 2,5-dimethylpyrazine, 1-octen-3-ol, octanal, 2-acetylpyrazine, (E)-2-octenal, 2-nonanone, and (E)-2-nonenal). The trained assessors were familiarized with odor description terms and had experience with GC-O from preliminary tests.

Panel Analysis. Volatiles emanating from the sniffing port perceived by at least three assessors were classified as odor-active compounds. The respective odor intensities were scored

as: 1 = very mild; 2 = mild; 3 = intermediate; 4 = strong; and 5 = very strong. Individual cooked rice volatile samples for each panelist were considered a unique experimental replicate prepared from a rice sample.

Descriptive Sensory Analysis.

Sample Preparation and Presentation. Microcomputerized rice cookers (Zojirushi model NP-HBC 18, Zojirushi, Japan) were utilized with a 1:1.8 water to rice ratio (by weight). Cooking time varied between 38 to 40 min based on the type of rice and the programming of the cookers. Cooked samples (~70 g) were placed in 92 mL standard Pyrex® weighing bottles (~ ³/₄ full) with an external 45/12 standard taper joint to prevent the loss of volatiles (Corning #1686-40100, Corning Incorporated, Corning, NY) and held in an insulated container containing hot water to maintain the rice temperature slightly above 70 °C.

Panelists. The panel consisted of 11 members (3 men and 8 women) recruited from graduate students and staff of the Food Science and Technology department. The principles and concepts of descriptive analysis of rice aroma using the SpectrumTM method (21) were introduced to the panel. A 150-mm unstructured line scale (0 = none and 150 = very high) was utilized instead of the 15-point scale in the original protocol (22). A flavor lexicon for sensory descriptive profiling of rice (23) was used to decide the aroma attributes of the rice samples in this study. Before the panel training sessions, three trained panelists with considerable rice descriptive sensory panel experience, excluded from the training sessions the sulfury, dairy, rancid, sweet-aromatics, and smoky attributes in that they were not detected in the cooked rice samples. During four subsequent training sessions, panelists decided which attributes should be kept from the 19 rice aroma descriptors and were familiarized with the intensities using reference materials. After panel agreement, starch, barny, cooked-grain, woody, green, and earthy

attributes were selected to describe the important aroma properties of the samples. From the panel discussion, panelists decided not to keep the popcorn attribute since they could not perceive a popcorn aroma in the samples. They also chose cooked-grain over grainy and woody over hay-like. The reference material for the green attribute was changed from Sunny Creek organic alfalfa sprouts (22) to green beans with same intensity (i.e., 80).

Panel Analysis. Eleven panelists evaluated four rice samples, Sj2, Si2, G1, and SG2 (Table 4.1) with 6 descriptors chosen during the training sessions. There were two sessions and four coded samples presented at each session.

Data Analysis.

Data from the descriptive sensory analysis was evaluated by analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC.). ANOVA with Duncan's multiple pairwise comparisons was performed to determine differences among individual samples for each sensory attribute. Principal component analysis (PCA) was also performed using SAS (SAS Institute Inc., Cary, NC.) to explain GC-O and descriptive sensory data across the rice samples.

RESULTS AND DISCUSSION

Volatiles from Cooked *O. glaberrima*, African *O. sativa* ssp. *japonica* and *indica*, and Interspecific Hybrids. Forty-two volatile compounds were identified and quantified by GC-MS across the Africa rice samples (Table 4.2) with 34, 35, 27, 28, 34, 32, and 30 volatiles isolated from Sj1, Sj2, Si1, Si2, G1, SG1, and SG2, respectively. Seventeen volatile compounds were present in all seven cultivars (hexanal, 3-furaldehyde, furfural, *(E)*-2-hexenal, *p*-xylene, styrene, heptanal, benzaldehyde, 1-octen-3-ol, 2-pentylfuran, octanal, limonene, 3-ethyl-2-methyl-1,3hexadiene, acetophenone, nonanal, *(E)*-2-nonenal, and decanal). Included were aliphatic alcohols, aliphatic aldehydes, aromatics, ether, furans, hydrocarbons, ketones, and pyridines. Aliphatic aldehydes were the largest chemical class with 10 to 13 compounds, followed by 4 to 8 alcohols and 3 to 5 ketones. Only twenty-four out of the 42 compounds were identified in a typical Asian rice (SjAsian) (see footnote in Table 4.2). Several African rice volatiles had not been previously identified in traditionally prepared rice (boiled in water) (e.g., 3,5,5-trimethyl-2-cyclopenten-1one, styrene, eucalyptol, linalool, L- α -terpineol, myrtenal), but have been found in other food products. Styrene is an intermediate product in the reaction between amino acids and alkadienals via the Maillard reaction under anaerobic conditions and has been identified in flash-fried rice (similar to stir-fried rice, but with direct fire contact cooking in Thailand) (24-25). 3,5,5-Trimethyl-2-cyclopenten-1-one has been identified in sesame seed oil (26). Eucalyptol (1,8cineole) is known to play a significant role in the 'eucalyptus' character in wine (27) and linalool is one of the most important volatiles in tomato flavor, imparting a sweet, floral, and alcoholic note and is also a major component of the scent of flowers in a number of species (28-30). L- α terpineol which has a delicately floral, lilac-like aroma occurs in many essential oils, juice, and wine and it has been proposed as an indicator of orange juice storage duration (31-34). Myrtenal was one of the important volatiles identified in green walnut husks (35) and ginger (36).

The most abundant volatiles emanating from the African cultivars, regardless of species, were similar, a number of which represent lipid oxidation products (e.g., aliphatic aldehydes and alcohols). Hexanal was the most abundant volatile in all cultivars followed by nonanal, 1-hexanol, 2-pentylfuran, benzaldehyde, (E,E)-2,4-decadienal, and octanal. The ten most abundant volatiles in each cultivar quantitatively accounted for 77.7, 79.5, 86.8, 74.1, 76.9, 70.3, and 71.8% of the total volatiles in Sj1, Sj2, Si1, Si2, SG1, SG2 and G1, respectively. Aliphatic aldehydes such as hexanal, nonanal, decanal, octanal, and heptanal were abundant in all of the

cultivars. Aliphatic aldehydes are lipid derived volatiles that are formed during the degradation of fatty acids such as oleic, linoleic, and linolenic acids (37). The most abundant volatile, hexanal, was most likely derived from linoleic acid (38). Lipid-derived alcohols (e.g., 1-pentanol, 1-hexanol, 1-octen-3-ol) were also among the most abundant volatiles.

Myrtenal and eucalyptol were unique to the interspecific hybrids (SG) and L- α -terpineol was found only in *O. sativa* ssp. *japonica*. Pyridine was a quantitatively significant volatile in G1. 2-Methylpyridine and 2,6-dimethylpyridine also displayed higher relative concentrations in G1 compared to the other species in which they were present. Both had been previously reported in rice (39).

Odor-Active Compounds. Odor-active compounds were determined in two *O. sativa* cultivars (Sj1 and Si1), one *O. glaberrima* (G1), and one interspecific hybrid (SG1) due to the limited amount of grain available. Thirty-three odor-active compounds were identified and characterized; there were 21, 18, 21, and 21 volatiles in Sj1, Si1, G1, and SG1, respectively (Table 4.3). Ten odor-active compounds commonly found in cooked rice were present in the four rice types (i.e., 1-pentanol, hexanal, 3-furaldehyde, heptanal, 1-octen-3-ol, 2-pentylfuran, octanal, benzeneacetaldehyde, nonanal, and *(E)*-2-nonenal). Five of the volatiles (i.e., 1-pentanol, hexanal, heptanal, 2-pentylfuran, and nonanal) were considered quantitatively abundant based on their relative concentrations. The intensities of these compounds were equal to or greater than intermediate [\geq 3]. The intensity of hexanal, the most abundant volatile compound in each of the four cultivars (Sj1, Si1, G1, and SG1), was very strong [5] with its odor descriptions varying from 'green', 'grassy (leafy)', 'green tomato', and 'waxy' (Table 4.3). 1-Pentanol, heptanal, octanal, nonanal, and *(E)*-2-nonenal were classified mostly as intermediate [3], strong [4], or very strong [5] in odor intensity. 2-Pentylfuran, a significant volatile in each cultivar had been

previously reported as an odor-active compound in aromatic, non-aromatic, and black rice with a 'floral', 'fruity', 'nutty', and 'beany' aroma (14, 17-18, 40-43). 2,6-Dimethylpyridine, present in *O. glaberrima* (G1), displayed a 'nutty' or 'roasted' aroma. It was an odor-active compound even though the concentration was relatively low (8.4 ng/100 g) in Sj1 compared to G1 (91.0 ng/100 g) due to its very low odor threshold (0.003 in air). 2,6-Dimethylpyridine has been described as having a 'roasty', 'green', or 'milk' aroma (44).

Among the 33 odor-active compounds, benzaldehyde, limonene, (*E*)-2-octenal, and acetophenone were found in the two African *O. sativa* subspecies (Sj1 and Si1), but not in *O. glaberrima*; furfural, 4-ethylphenol, and (*E*,*E*)-2,4-heptadienal were identified only in *O. glaberrima* (Table 4.3). Benzaldehyde, limonene, (*E*)-2-octenal, acetophenone, and furfural, however, were identified in both *O. glaberrima* (G1) and each of the *O. sativa* cultivars (Sj1, Sj2, Si1 and Si 2) (Table 4.2), though 4-ethylphenol and (*E*,*E*)-2,4-heptadienal were not present. Therefore, two odor-active volatiles were unique to *O. glaberrima* (G1), 4-ethylphenol ('musty') and (*E*,*E*)-2,4-heptadienal ('fruity'). 4-Ethylphenol, an important volatile phenol in wine, is responsible for a phenolic off-flavor (45-46) and (*E*,*E*)-2,4-heptadienal is a major volatile emanating from rice foliage that has an 'orange oil' or 'oily' aroma in kiwi fruit essence and puree (47-48).

Pyridine, styrene, eucalyptol, and myrtenal were described only in the interspecific hybrid (SG1) and their odor thresholds are very low (i.e., 0.01 to 7 ppb in water) (Table 4.3). Pyridine is usually considered an unpleasant odor and has been characterized as 'spoiled milk' (49) and 'sour', 'fishy', and 'amine' in Thai fragrant rice (50). Seven panelists in this study characterized pyridine as a 'cheese' odor. Styrene was described as having a 'pungent' odor while eucalyptol had a 'clove' or 'sweet' odor and myrtenal a 'spicy' odor. Myrtenal, an oxygen-

containing monoterpene derivative of the pinane series, is known as a 'spicy' flavor compound (51-52) while eucalyptol imparts 'eucalyptus-like' aroma in wine (27).

Several odor-active compounds (i.e., 3-(methylthio)-propanal, eucalyptol, and myrtenal) had not been previously reported in rice. For example, 3-(methylthio)-propanal was found only in Sj1 and has been characterized in dried hop cones as having a 'cooked pototo-like' odor (53). Benzeneacetaldehyde, characterized as 'floral' and 'jasmine' in this study, has been reported in a *O. sativa* subsp. *japonica* cultivar (54) and in Thai fragrant rice (50) though in neither study was it considered odor-active. In Riesling wine, benzeneacetaldehyde was considered as 'flowery' and was one of the 10 volatiles that characterized the aroma of the Rhine Riesling must (55).

Limonene and acetophenone were previously reported in cooked rice (56). The two enantiomers of limonene have different odors: (-)-S enantiomer (limonene) has a turpentine (mint) like odor; and the (+)-R enantiomer (d-limonene) is associated with an orange-like smell (57). In this study, limonene was identified in each of the rice samples, however, only in Sj1 and Si1 was it considered odor-active (Table 4.3). Panelists described limonene as 'minty' and 'floral' and the intensity was weak in Sj1 and intermediate in Si1. Acetophenone was also identified in each of the rice samples (Table 4.2) but exhibited a 'floral' or 'almond' odor only in Sj1 and Si1. In peanut, acetophenone was described as 'fruity' and 'sweet' (58).

To initially characterize differences in aroma among four of the cultivars representing each of the species (Sj1, Si1, G1, SG1), principal component analysis (PCA) of the intensities of the odor-active compounds was utilized. The PCA biplot accounted for 73.1% of the total variance, with PC1 and PC2 explaining 42.5% and 30.6%, respectively. The four rice types were distinctly separated from each other, indicating the probability of distinct differences in aroma (Figure 4.1). G1 was largely explained by *(E,E)*-2,4-heptadienal, 4-ethylphenol, dimethyl trisulfide, and benzeneacetaldehyde. 3-Furaldehyde and 3-octen-2-one were the major odoractive compounds separating Si1 and heptanal, 3-(methylthio)-propanal, 1-nonanol, and 3nonen-2-one were the compounds to most closely associated with Sj1. Nonanal, myrtenal, eucalyptol, styrene, and pyridine mostly contributed to the separation of SG1 from the other cultivars. The critical odor-active compounds for each rice type were either the volatile(s) only found in that cultivar or those with the highest intensity among the cultivars.

Descriptive Sensory Analysis. Six aroma attributes ('starch', 'woody', 'cooked grain', 'barny', 'green', and 'earthy') were assessed in Sj2, Si2, G1, and SG2 by eleven trained panelists (Figure 4.2 and Table 4.4). The mean intensity for the 'starch' aroma was similar across the four rice types; the intensity of the 'woody' aroma was similar in Sj2, Si2, and G1 (Figure 4.2). The mean intensities for 'cooked grain', 'barny', and 'earthy' attributes were significantly different across the four rice types, however, 'starch', 'woody', and 'green' were not significantly different (Table 4.4). 'Cooked grain' and 'earthy' in G1 and Sj2 and 'barny' in SG2, G1 and Sj2 were significantly different and appeared to differentiate the *O. glaberrima* and *O. sativa* ssp. japonica cultivars. 'Barny' was the only odor that differentiated the interspecific hybrid (SG2) and Sj2. Descriptive sensory analysis of 36 Asian rice types (e.g., Basmati, Jasmine, sweet rice, pigmented rice, brown rice, parboiled rice) for 'cooked grain', 'barny', 'green', and 'earthy' attributes had intensities of 17.1, 6.3, 4.3, and 4.9, respectively (23). In this study, the African rice cultivars had much higher average intensities for the same attributes (34.8, 31.5, 22.5, and 37.6, respectively) which indicate distinct differences between African and Asian rice in overall aroma. The distinction was further substantiated via a preliminary sensory analysis comparing the African cultivars with a typical, non-aromatic Asian rice. While the intensity of the sensory attributes did not differ as greatly among the African rice species as between the African and

Asian cultivars, the overall flavors of the African cultivars were sufficiently different to allow distinguishing each.

Correlation analysis (Table 4.5) demonstrated that none of the attributes were highly correlated (i.e., > 0.80) with any other. 'Cooked grain' and 'barny' were positively correlated with 'starch' whereas 'earthy' was negatively correlated. 'Woody' was negatively correlated with 'cooked grain' and 'cooked grain' is negatively correlated with 'earthy'. These sensory attributes were presented independently from other attributes since the correlations were very weak (Figure 4.3). PCA analysis of the six sensory odor attributes indicated that nearly 82% of the total variation in the data set was explained (Figure 4.3), PC1 and PC2 accounted for 56.5% and 25.4%. Sj2 was primarily characterized as 'barny' and the 'green' attribute was the most important in separating SG2 and Si2. 'Cooked grain' and 'woody' most closely described G1. SG2 and Si2 had similar intensities for the aroma attributes (Figure 4.3); SG2 and Si2 were not significantly different (Table 4.4). 'Cooked grain' and 'earthy' were significantly different between G1 and Sj2 (Table 4.4).

In conclusion, a total of 42 volatile compounds were identified across all African rice samples (*O. sativa* ssp. *japonica* [Sj1 and Sj2] and *indica* [Si1 and Si2], *O. glaberrima* [G1], and interspecific hybrids [SG1 and SG2]), several of which had not been previously reported in traditionally cooked rice (e.g., 3,5,5-trimethyl-2-cyclopenten-1-one, styrene, eucalyptol, linalool, myrtenal, L- α -terpineol). Eucalyptol and myrtenal were unique to the interspecific hybrid (SG1) while L- α -terpineol was unique to *O. sativa* ssp. *japonica* (Sj1&2). Pyridine, 2-methylpyridine and 2,6-dimethylpyridine were quantitatively significant volatiles in *O. glaberrima* (G1). Thirty-three odor-active compounds were identified and characterized in Sj1, Si1, G1, and SG1. 4-Ethylphenol ('musty') and *(E,E)*-2,4-heptadienal ('fruity') were present only in G1. Pyridine

('cheese'), styrene ('pungent'), eucalyptol ('clove', 'sweet'), and myrtenal ('spicy') were described only in the interspecific hybrid SG1. Descriptive sensory analysis indicated 'cooked grain', 'barny', and 'earthy' were statistically different while 'starch', 'woody', and 'green' were not among African *O. sativa* ssp. *japonica* and *indica* (Sj2, Si2), *O. glaberrima* (G1), and the interspecific hybrid (SG2). While based on only a very limited selection of germplasm, the aroma chemistry data from GC-MS, GC-O, and descriptive sensory analysis indicates that the flavor of representative cultivars of African *O. sativa* subspecies, *O. glaberrima*, and interspecific hybrids differed considerably. In addition, the general flavor of rice grown in West Africa appears to differ distinctly from typical Asian rice (*O. sativa*) suggesting that the Asian and African *O. sativa* germplasm pools began to diverge many years ago in response to differing selection criteria. Further studies of the aroma chemistry on a wider range of cultivars and subsequent consumer sensory analysis will faciliate the selection of progeny with superior flavor in African rice breeding programs.

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cultivar	botanical group	code	country	pericarp color
Gbengbeng	<i>O. sativa</i> ssp <i>. japonica</i>	Sj1	Sierra Leone	red
Jebbehkomie	<i>O. sativa</i> ssp <i>. japonica</i>	Sj2	Sierra Leone	red
Buttercup	<i>O. sativa</i> ssp <i>. indica</i>	Si1	Sierra Leone	white
Pla Camp	<i>O. sativa</i> ssp. <i>indica</i>	Si2	Sierra Leone	red
Maalay	O. glaberrima	G1	Sierra Leone	red
Binta Sambou	Cluster 4	SG1	Gambia	whitish
Painy Painy	Cluster 4	SG2	Sierra Leone	red

 Table 4.1. African Rice Samples

Table 4.2. Concentrations of Volatiles Identified in Cooked African Rice (*Oryza sativa* ssp. *japonica* and *indica* [S_j1, S_j2, S_i1 and S_i2],*O. glaberrima* [G1], and Interspecific Hybrids [SG1 and SG2]).

		relative concentration (ng/100 g) ^b								
RI ^a	volatile	Sj1°	Sj2	Si1 ^d	Si2	G1	SG1	SG2		
741	pyridine	f	_	_	_	91.6±4.1	48.44±3.6	-		
780	1-pentanol ^e	201.8±10.4	181.4±17.3	82.3	_	149.3±4.6	86.7±3.1	48.1±10.6		
807	hexanal ^e	896.5±62.3	780.9±19.7	463.0	562.7±19.1	1004.2±14.9	446.8±35.3	321.0±11.3		
813	3-furaldehyde ^e	4.8±0.4	7.2±1.4	4.5	8.7±0.3	6.9±1.8	4.7±0.4	3.3±0.8		
820	2-methylpyridine	_	8.1±0.4	_	12.1±4.4	23.1±1.9	_	1.5±1.2		
840	furfural	9.6±2.8	9.8±6.5	2.4	6.5±1.6	6.3±0.5	3.9±1.0	2.8±0.3		
852	(E)-2-hexenal ^e	17.8±2.2	12.9±1.4	11.7	11.0±1.0	17.0±4.9	9.9±1.2	4.3±1.4		
860	3,5,5-trimethyl-2-cyclopenten-1	4.3±0.9	3.3±0.5	6.6	_	4.3±1.0	7.3±1.1	3.6±0.8		
	-one									
867	<i>p</i> -xylene ^e	7.2±2.4	7.9±0.7	9.2	10.6±2.1	20.0±4.3	6.0±0.9	5.1±0.9		
872	1-hexanol ^e	191.8±17.6	275.6±29.3	_	115.6±4.4	102.4±18.1	34.2±1.4	71.4±29.5		
879	2,6-dimethylpyridine	8.4 ±0.5	9.8±1.3	_	_	91.0±4.5	_	_		

884	styrene	7.7±3.7	10.5±1.5	7.6	11.1±0.8	16.0±2.4	8.2±0.4	4.0±1.5
890	2-heptanone ^e	75.2±10.0	_	29.5	36.0±4.7	84.6±5.4	29.4±2.0	22.3±2.8
907	heptanal ^e	83.4±6.0	78.2±3.5	82.6	91.5±3.7	107.3±5.9	71.8±6.9	43.9±1.6
959	benzaldehyde ^e	186.6±10.4	177.8±22.4	96.1	142.1±9.5	190.0±17.6	130.0±10.3	87.4±2.9
964	1-heptanol ^e	30.2±5.9	44.8±2.5	19.3	19.1±1.4	8.5±1.2	_	11.9±0.3
970	1-octen-3-ol ^e	55.2±5.3	46.6±2.8	47.2	37.1±4.7	58.8±1.9	77.9±4.1	30.1±10.8
985	6-methyl-5-hepten-2-one	_	_	26.1	28.9±1.4	_	_	_
997	2-pentylfuran ^e	206.1±24.1	263.4±9.6	120.4	135.3±7.8	207.5±15.2	116.2±8.1	104.8±6.0
1009	octanal ^e	98.5±8.2	106.6±3.4	81.2	123.1±9.7	240.3±19.0	110.5±5.7	66.2±7.9
1016	(E,E)-2,4-heptadienal	_	2.4±0.6	_	11.2±15.7	8.1±1.8	_	_
1025	limonene	50.2±5.9	45.0±2.4	47.5	67.5±11.3	77.2±9.8	23.5±1.0	45.4±6.0
1030	eucalyptol	_	_	_	_	_	13.9±1.7	_
1036	3-ethyl-2-methyl-1,3-hexadiene ^e	21.6±2.4	30.3±2.5	7.9	21.7±1.2	33.7±9.7	17.1±0.8	11.6±1.2
1040	2-ethyl-1-hexanol ^e	15.7±1.2	13.1±4.6	9.2	_	19.9±0.8	13.8±0.09	5.2±3.2
1043	3-octen-2-one ^e	_	_	8.7	_	39.4±4.0	_	4.2±0.5
1045	benzeneacetaldehyde ^e	152.4±5.2	134.0±13.9	8.2	_	32.8±0.9	13.0±1.6	30.7±16.6

1060	(E)-2-octenal ^e	31.8±3.4	47.7±5.2	23.9	40.4±0.9	69.4±5.5	22.1±1.4	_
1070	acetophenone ^e	12.0±0.7	10.7±1.2	7.2	15.5±1.8	17.0±0.4	8.2±1.0	9.1±0.6
1085	1-octanol ^e	43.6±5.2	40.7±0.9	_	45.4±4.5	56.2±8.2	52.6±5.6	27.2±10.6
1095	linalool	54.6±1.9	5.3±0.5	6.3	_	4.5±0.9	_	5.2±0.1
1112	nonanal ^e	226.1±7.8	200.4±17.4	84.4	400.2±14.5	253.1±8.0	439.8±17.3	160.5±1.6
1140	3-nonen-2-one	10.2±1.0	8.4±0.6	_	_	15.5±2.8	_	-
1164	(E)-2-nonenal ^e	23.8±0.01	24.7±2.1	3.0	24.9±1.3	17.9±5.8	49.6±2.3	11.3±0.4
1172	naphthalene	21.3±0.6	26.4±2.6	_	27.7±3.1	27.3±1.5	6.7±0.06	16.9±2.0
1180	myrtenal	_	_	_	_	_	67.7±7.8	_
1185	L-a-terpineol	10.7±1.1	4.1±1.2	_	_	_	_	_
1190	2-decanone	11.9±3.5	10.2±1.3	_	_	_	5.6±0.8	_
1206	decanal ^e	36.8±0.5	35.8±3.6	4.7	51.9±0.03	28.2±1.8	31.5±2.1	29.8±1.6
1260	(E)-2-decenal ^e	7.3±0.7	10.4±0.6	_	13.4±0.9	-	5.2±1.2	-
1205	tetradecanal	6.5±1.0	_	_	_	-	22.9±3.0	20.1±1.4
1317	(E,E)-2,4-decadienal	_	4.4±0.5	_	181.8±2.7	_	_	_

^a Retention index based on HP-5MS column using a series of n-hydrocarbons. ^b Values expressed as δ-carvone equivalents (ng/100g). ^c Each sample in triplicate. ^d Duplicate only due to an insufficient quantity of Si1. ^e Compounds found in SjAsian (purchased in a local supermarket). ^f Not detected.

odor intensity^d reported odor number odorant odor description^c Sj1 Si1 G1 SG1 threshold (ppb)^b pyridine 1 2 cheese nd nd nd 3 2 1-pentanol 4 sweet, fruity, grassy 3 3 2 4 3 hexanal 5 green, grass, green tomato, leafy, waxy 5 5 5 5 3-furaldehyde N/A skunk 3 5 4 3 4 1000^{e} furfural 5 sweet, nutty, floral, solvent nd nd 3 3 *p*-xylene 0.53-1 medicinal 4 4 3 6 nd unknown cooked rice 3 nd nd 7 nd 0.003 in air 8 2,6-dimethylpyridine nutty, roasted nd 4 nd 3 9 styrene 0.065 sweet, pungent nd nd 3 nd 10 heptanal 5 soapy, green, citrus, fruity, nutty, rancid 5 4 4 4 11 3-(methylthio)-0.06 in air cooked cabbage, savory 4 nd nd nd

Table 4.3. Odorant, Odor Threshold, Description, and Odor Intensity of Odor-Active Compounds in Cultivars of African Oryza sativa

ssp. *japonica* (Si1) and *indica* (Si1), *O. glaberrima* (G1) and their Interspecific Hybrid (SG1)

	propanal						
12	4-ethylphenol	0.6	musty	nd	nd	4	nd
13	(E)-2-heptenal	N/A	cooked flour, nutty, fatty	nd	3	nd	3
14	benzaldehyde	350	nutty, woody, cherry	2	2	nd	2
15	dimethyl trisulfide	N/A	spicy, pungent, unpleasant, garlic	4	nd	5	3
16	1-octen-3-ol	0.01	mushroom	4	4	5	3
17	2-pentyl furan	6	sesame leaf, fruity, grassy, herbal	4	3	5	5
18	octanal	0.0009-0.03	citrus, floral, fruity, sweet, pleasant	4	3	5	3
19	(E,E)-2,4-heptadienal	560	fruity	nd	nd	2	nd
20	limonene	10	minty, floral	2	3	nd	nd
21	eucalyptol	0.001-1	clove, sweet	nd	nd	nd	3
22	3-octen-2-one	20 in air	earthy	nd	4	nd	nd
23	benzeneacetaldehyde	N/A	floral, jasmine	4	3	5	4
24	(E)-2-octenal	0.0003	waxy, popcorn, green, cucumber, nutty	3	4	nd	4
25	acetophenone	0.065	floral, almond	3	1	nd	nd
26	1-octanol	0.1-1	burnt, waxy, unpleasant	2	nd	2	3

4	4	4
nd	nd	nd
3	5	2
nd	nd	nd
nd	3	nd
nd	nd	5
nd	3	4
	nd 3 nd nd nd nd nd	nd nd 3 5 nd nd nd 3 nd nd nd 3 nd nd nd 3

^a Retention index based on HP-5MS column using a series of n-hydrocarbons. ^b Odor thresholds values in water and air from van Gemert (59). ^c Odorants were described by at least three panelists during GC-O. ^d Average intensity of odorants by at least three panelists. ^e Odor threshold in water from Marsili *et al.*, 1994 (60). ^f Odor threshold in water from Burdock, 2005 (61).

Table 4.4. Mean Intensity of Aroma Attributes of African Oryza sativa ssp. japonica (Sj2) and*indica* (Si2), O. glaberrima (G1) and an Interspecific Hybrid (SG2)

	intensity								
	starch ^a	woody	cooked	barny	green	earthy			
		Weedy	grain	Juny	Broom				
Sj2	25.7a	22.1a	23.5b	47.5a	15.7a	47.4a			
Si2	20.5a	23.0a	33.9ab	32.3ab	29.7a	40.4ab			
G1	23.6a	23.2a	47.2a	24.2b	21.5a	26.0b			
SG2	21.3a	14.4a	34.5ab	22.0b	23.1a	36.4ab			

^a Intensity means with the same letter are not significantly different from each other (p > 0.05).

	starch	woody	cooked grain	barny	green	earthy
starch	1.00					
woody	0.01	1.00				
cooked grain	0.38 ^a	-0.40	1.00			
barny	0.21	0.17	-0.10	1.00		
Green	0.12	0.17	-0.06	0.678	1.00	
Earthy	-0.22	0.17	-0.36	0.11	0.10	1.00

 Table 4.5. Correlations Among the Six Dominant Aroma Attributes

^a Data in bold represent significant at p < 0.05 level.

Figure 4.1. Principal Component Analysis Biplot of the Mean Odor-Active Compound^a Intensity Ratings for Sj1 (*Oryza sativa* ssp. *japonica*), Si1 (*O. sativa* ssp. *indica*), G1 (*O. glaberrima*), and SG1 (Interspecific Hybrid).

Footnote: ^a The number assigned to each odor-active compound corresponds to those in Table 3. Numbers within each circle indicate important odor-active compounds for that cultivar; boxed numbers within the circles indicate compounds unique to that cultivar.



Component 1 (42.5%)

Figure 4.2. Aroma Intensities of Six Attributes in Cultivars of African *Oryza sativa* ssp. *japonica* (Sj2) and *indica* (Si2), *O. glaberrima* (G1) and an Interspecific Hybrid (SG2).



Figure 4.3. Principal Component Analysis of Mean Sensory Attribute Ratings for Sj2 (*Oryza sativa* ssp. *japonica*), Si2 (*O. sativa* ssp. *indica*), G1 (*O. glaberrima*), and SG2 (Interspecific Hybrid).



Component 1 (56.51%)

CHAPTER 5

CONCLUSIONS

Flavor, the composite of aroma (odor) and taste, is the major criteria defining the character, quality and consumer preference of rice. Unlike many foods, the aroma of rice is the primary contributor to the overall flavor. As a consequence, a number of studies have focused on identifying and quantifying the volatile compounds emanating from cooked rice to better understand its aroma.

The aroma chemistry of wild rice (*Zizania palustris*) and seven representative rice cultivars of considerable genetic diversity (i.e., *Oryza. sativa* ssp. *japonica* and *indica*, *O. glaberrima*, and their interspecific hybrids) grown in West Africa were assessed. Gas chromatography-mass spectrometry was used to identify and quantify the volatile compounds and gas chromatography-olfactometry to characterize the odor active compounds emanating from the cooked samples. Aroma attributes of rice cultivars were also evaluated by descriptive sensory analysis. A typical Asian *O. sativa* was used to contrast its volatile composition with wild rice and the Africa cultivars.

Seventy-three volatile compounds were identified and quantified including 58 newly reported in wild rice; 34 odor-active compounds were characterized. 2-Ethylfuran, hexanal, 2-methylpyrazine, 2-methyl-2-pentenal, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, benzaldehyde, 2-ethyl-6-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine were considered primary contributors to the unique nutty, roasted aroma. Descriptive sensory analysis illustrated the distinct aroma differences between wild and brown rice; wild rice was primarily described as

having 'nutty', 'smoky', 'hay-like', 'earthy', and 'green' aroma attributes and brown rice as 'cooked-rice' and 'buttery'. A number of pyrazine compounds conferring the unique nutty, roasted aroma, typically formed via thermal reactions, were present in the processed grain prior to cooking. As a consequence, the fermentation and parching steps in wild rice processing are believed to play a crucial role in creating the unique nutty, roasted aroma. Integrating information on the aroma chemistry with consumer preference will allow ascertaining which aroma traits confer superior flavor. In addition, factors affecting wild rice flavor quality, such as processing (fermentation and parching conditions), storage (conditions and duration), and agronomic variables (e.g., wild versus cultivated, harvest date, weather), can be objectively assessed.

Forty-two volatile compounds were identified and quantified across the seven African rice cultivars, six of which were newly reported in cooked rice (e.g., 3,5,5-trimethyl-2-cyclopenten-1-one, styrene, eucalyptol, linalool, myrtenal, L- α -terpineol). Eucalyptol and myrtenal were unique to the interspecific hybrid while L- α -terpineol was unique to *O. sativa* ssp. *japonica*. Pyridine, 2-methylpyridine, and 2,6-dimethylpyridine were quantitatively significant volatiles in *O. glaberrima*. Thirty-three odor-active compounds were characterized in *O. sativa* ssp. *japonica*, *O. sativa* ssp. *indica*, *O. glaberrima* and the interspecific hybrids. Several odor-active compounds were unique to specific cultivars. Ethylphenol ('musty') and (E,E)-2,4-heptadiental ('fruity') were present only in *O. galberrima*; pyridine ('cheese'), styrene ('pungent'), eucalyptol ('clove', 'sweet'), and myrtenal ('spicy') were described only in the interspecific hybrid. Descriptive sensory analysis found 'cooked-grain', 'barny', and 'earthy' attributes to be statistically different among African *O. sativa* ssp. *japonica* and *indica*, *O. glaberrima*, 'woody', 'green' were not. While based

on a very limited selection of germplasm, the aroma chemistry data suggests that the general flavor of African rice is distinct from typical Asian *O. sativa* and that there appears to be sufficient variation in flavor within the African germplasm to allow segregation of cultivars into distinct flavor types. The aroma chemistry data also suggests that the flavor of cultivated African *O. sativa* lines appears to have diverged from Asian *O. sativa* flavor types many years ago via progressive selection by West African farmers. Further analysis of the aroma chemistry using a much wider range of germplasm will establish the validity of this possibility and the subsequent inclusion of consumer preference will facilitate the selection of progeny with superior flavor in African rice breeding programs.