Grain quality characteristics of imported rice in Ghana: Implications for breeding for consumer-preferred varieties

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ABSTRACT

Rice is the fastest growing food source in Ghana. The country, however, imports about 70 per cent of its rice requirement due to low volumes of production and poor grain quality of domestic rice compared to imported ones. In the study, 10 popular imported rice brands on the Ghanaian market were characterised for grain quality traits including grain dimensions, apparent amylose content, gelatinisation temperature, paste viscosity properties, and aroma using physiochemical properties and DNA markers. The rice brands came from Asia and the USA. The rice type from Asia was found to be Jasmine-styled aromatic long grained with low amylose content and gelatinisation temperature, whereas those of USA were conventional long grain with intermediate amylose content and gelatinisation temperature. These findings were confirmed through DNA marker analyses, with the rice types from Asia and the USA revealing the presence of the TAC and GCC of the waxy gene SNP haplotypes, respectively. The implication for rice breeding in Ghana is discussed.

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Introduction

Rice is the fastest growing food staple in Ghana. The consumption of rice keeps increasing at a very fast pace as a result of population growth, urbanisation and change in consumer habits. The current per capita consumption of approximately 40 kg/ person/year is expected to reach 63 kg/ person/ year by 2018 (MOFA, 2009). This implies an increase from the current annual demand of 500,000 – 600,000 tons to about 1,680,000 tons within the period (MOFA, 2009). However, 60 per cent to 70 per cent of the current demand is met by imports. Imported rice has taken over the markets in Ghana and West Africa because of low production levels, and failure of the rice industry to meet the grain quality standards demanded by consumers (Nwanze *et al*., 2006; Manful, 2010; Demont & Rizzotto, 2012). In Ghana, most rice brands on the market are imported from Thailand, Vietnam and the USA. Even where locally produced rice is available, most consumers prefer imported

ones due to better appearance, cooking and eating qualities (Diako *et al*., 2010; Tomlins *et al*., 2005).

The use of rice worldwide is mainly as whole milled kernels, and most rice is consumed in cooked form (Bergman, Bergman, Bhattcharya & Ohtsubo, 2004). The physical appearance of the grain (grain length, grain length to width ratio and chalkiness/ translucence) as well as cooking and eating qualities are, therefore, very important characteristics for rice consumers.

Cooking and eating quality is mainly controlled by apparent amylose content (AAC) and other starch properties including, gelatinisation temperature (GT), gel consistency (GC) and paste viscosity properties (also called RVA, because it is commonly measured by Rapid Visco Analyzer). AAC and most RVA pasting properties are mainly controlled by the waxy gene (WX), which encodes the granule bound synthase (GBSS) enzyme (Chen *et al*., 2008b). GT is also mainly controlled by the alkaline degeneration gene (ALK), which encodes the soluble synthase II (SSIIa) enzyme. Mutations in the WX gene has particularly proven useful in selecting for specific cooking and eating qualities. Three single nucleotide polymorphism (SNP) mutations; a guanine to thymine (G→T) SNP at the 5' splice site of the first intron, an adenine to cytosine (A→C) transversion in exon 6 and a cytosine to thymine (C→T) transition in exon 10 have been used in predicting AAC and RVA properties in rice (Larkin & Park, 2003). Using these three SNPs across intron 1, exon 6 and exon 10 (In1Ex6Ex10) of the WX gene, a total of four haplotypes TAC, GCC, GAC and GAT have been found in diverse rice germplasm (Chen *et al*., 2008b; Asante *et al*., 2013b). The WX SNP haplotypes, TAC, GCC, GAC and GAT have been found in rice with low AAC (10 -18%), medium AAC (19-23%), high AAC (>23%) and high AAC plus high RVA pasting properties, respectively, in germplasm (Chen *et al*., 2008b; Asante *et al*., 2013b).

The use of conventional methods to select for the desired rice grain quality can be very difficult due to the lack of discrete phenotypic classes in the progeny. In addition, tedious subjective testing methods requiring taste panels, and biochemical evaluation procedures can be costly. Thus, WX, ALK as well as the Betaine aldehyde dehydrogenase 2 (BADH2) gene for aroma are presently routinely used as markers by breeders to facilitate selecting for the desired grain quality of rice.

In Ghana, even though imported rice has become the standard by which consumers measure the quality of rice, studies on popular imported brands aimed at informing breeders on the quality traits to select for have not been reported. The objective of the study was to characterise a set of popular imported rice brands for their grain qualities based on physicochemical attributes and DNA markers found in the WX, ALK and BADH2 genes. Insights from the study will be essential in helping breeders to develop rice varieties with grain qualities that are preferred by consumers.

Materials and methods

Rice samples

A total of 10 popular imported rice brands (Table 1) were used for the study. The rice brands were selected based on informal interviews with 20 rice sellers from Accra and Kumasi. The rice sellers were randomly in-

terviewed about the most popular imported rice brands based on patronage. The milled rice samples of the most popular brands were then collected from the Kejetia market in Kumasi in June, 2011, and shipped to the USDA, ARS-Dale Bumpers National Rice Research Center (DBNRRC) in Stuttgart, Arkansas, USA for physicochemical analysis.

TABLE 1

Rice Brands Used for the Study

Rice brand	*Country of origin*
Ohemma Jasmine	Thailand
Royal Stallion	Thailand
Sultana	Thailand
Uncle Sam	Thailand
Chicago Stars	USA
First Choice	USA
Texas Star	USA
Golden Perfume	Vietnam
Red Eagle	Vietnam
Special	Vietnam

Measurement of grain dimensions

Ten milled grain representatives of each rice brand were used to measure the grain length and width using a WinSeedle Pro 2007aTM image analysis system (Regent Instruments Inc.; Sainte-Foy Quebec, Canada). The grain length to width ratio also referred to as the grain shape was calculated using MS Excel.

Scoring for aroma

The aroma status of the rice brands were obtained from the rice sellers and confirmed by chewing five grains of each sample. Aroma was scored as "present" or "absent".

Alkali spreading value

GT was indirectly measured by evaluating the alkali spreading value (ASV) using the method of Little, Hilder & Daveson (1958). Briefly, six intact milled grains were put in a plastic container and 10 ml of 1.7 per cent KOH added to it. The grains were then carefully separated from each other using forceps and incubated in a growth chamber at 30 °C for 23 h to allow spreading of the grains. The spreading value of the grains was scored by visual assessment on a scale of 2 – 7 as follows: A rating of 2 is for no reaction (kernel firm); 3 for whole kernel with slight to moderate collar dispersion; 4 for slightly split or whole kernel with collar surrounding the kernel; 5 for severely split kernel with extreme collar; 6 for kernel with cotton center and 7 for clear center (Little *et al*., 1958). A low ASV corresponds to a high GT, and conversely, a high ASV indicates a low GT. ASV scores of 2 (low), 3 - 5 (intermediate) and 6 - 7 (high) corresponds to high (> 74 °C), intermediate (70 - 74 °C) and low (< 70 °C) GT, respectively (Bergman, Bhattharya & Ohtsubo, 2004).

Measurement of AAC and RVA pasting properties

The milled rice grains were pulverised using an Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO, USA) and sieved through a 100-mesh sieve. AAC was measured using an auto-analyzer 3 (model AA3; Bran and Luebbe, Roselle, Ill., U.S.A.) along with the evaluation software AACE ver. 5.24. Fifty milligrams of powdered samples were weighed into 50 ml centrifuge tubes, wetted with 0.5 ml of 95 per cent ethanol and the tubes swirled for thorough mixing. Aliquots of 4.5 ml of 1*M* sodium hydrox-

ide with Triton X-100 were added down the sides of the tube to wash down samples that may be adhering on the sides of the tubes. Tubes were covered and samples allowed to stand overnight at room temperature. On the 2nd day, 45 ml of deionized water was added to each tube, the mixture was vortexed for 10 – 15 s and then held overnight at room temperature. On the 3rd day, samples and standards were vortexed at the maximum speed, and the supernatant collected to determine the percentage amylose content.

The RVA profiles were measured on a rapid visco analyzer (RVA) (Model No. RVA-3D, Newport Scientific, Sydney, Australia), according to the standard method AACC 61- 02 given by the American Association of Cereal Chemists (AACC, 2000). Three grammes of rice flour was mixed with 25 ml of water; a paddle was placed in the canister and rotated at 960 r.p.m. for 10 s to disperse the rice sample. The viscosity was evaluated using a constant paddle rotation of 160 r.p.m. The sequential temperature curve for a 12.5 min test was as follows: (1) incubate at 50 °C for 1.0 min, (2) increase to 95 °C in 3.8 min, (3) kept at 95 °C for 2.5 min, (4) cooled down to 50 °C in 3.8 min, and (5) held at 50 °C for 1.4 min. Viscosity values were recorded in rapid visco units (RVU). The following paste viscosity characteristics were measured: Peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown (BD = PV - HPV), and setback (SB = CPV - PV).

Genotyping

Genomic DNA of each rice brand was extracted from 20 mg of milled rice using the modified CTAB method (Fjellstrom *et al*., 2004). The materials were evaluated with gene markers associated with major genes for the grain quality traits including AAC, GT, and aroma. Three SNPs in the WX gene including W×In1 (Ayres *et al*., 1997), W×E×6, and W×E×10 (Chen *et al*., 2008b; Chen *et al.*, 2010) were used to screen for AAC. Specific markers developed to detect the 8 bp deletion (also called the badh2.1 mutation) (Kovach *et al*., 2009) and the GC/TT SNP (Bao, Cork & Sun, 2006) for aroma and GTs, respectively, were used to screen for the two traits. For each marker, controls were included for each known allele.

PCR reactions were performed in 25µ*l* reaction volumes consisting of 20 ng of DNA, 10 m*M* Tris–HCl *p*H 8.3, 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 300 n*M* of each primer, 1 U of Taq DNA polymerase (Promega, Madison, WI, USA). Reverse primers were unlabeled in order to reduce the cost, and the forward primers were labeled with either 6FAM, Tamra, or Hex (Integrated DNA Technologies, Coralville, IA, USA). DNA was amplified with MJ Research Tetrad PCR machines (Waltham, MA, USA) under the following conditions: Initial denaturation at 94 °C for 5 min, then 30 cycles at 94 °C for 30 s, 55 – 67 °C (dependent on the marker) for 30 s, 72 °C for 1 min and 5 min final extension at 72 °C.

PCR products were pooled based on sizes of amplified fragments (typically three markers per run along with ROX-labeled size standard) to reduce the cost, and the DNA was denatured by heating samples at 94 °C for 5 min. The samples were separated on an ABI Prism 3100 DNA analyser using methods as described by the manufacturer (Applied Biosystems, Foster City, CA, USA). The sizes of SSR fragments were estimated using the GeneMapper software,

version 3.7 (Applied Biosystems, Foster City, CA, USA).

Data analysis

Descriptive statics comprising means, ranges and standard deviations (SD) for all the traits measured were calculated using GenStat Discovery Edition 4.

Results

Classification of imported rice types by grain dimensions

Grain length and shape (length to width ratio) is an important criteria for determining the appearance and, hence, consumer preference and the marketability of rice. Consequently, grain length and shape are used to classify rice for different markets. Grain length and shape ranged from 6.92 to 7.18 mm and from 3.11 to 3.48 mm, respectively (Table 2). All 10 brands are, thus, long slender grain rice.

Physicochemical analyses of imported rice brands

Rice imported to the Ghanaian market has become the benchmark by which consumers measure the quality of domestic rice. The rice brands analysed had low to intermediate AAC ranging from 15.9 per cent to 21.4 per cent with a mean of 18.3 per cent (Table 3). ASV ranged from three to seven, with rice brands from the USA at the lower end, and those from Asia at the higher end.

RVA profiles were moderately strong with generally low SB averaging around 17 RVU. SB for Ohemma Jasmine, Sultana and Red Eagle had negative values. All the rice brands from Asia were aromatic, but the types from the USA had no aroma (Table 3).

Functional marker analyses of rice brands

All the rice brands from Thailand and Vietnam had the 243 bp allele (Table 4), which indicates the presence of the badh 2.1 mutation on chromosome 8 of the rice

TABLE 2

Grain Dimensions of Imported Rice Brands on the Ghanaian Market

Rice brand	*Source*	*Grain length (L) (mm)*	*Grain Width (W) (mm)*	*L/W ratio (mm)*
Ohemma Jasmine	Thailand	7.01	2.15	3.26
Royal Stallion	Thailand	7.16	2.06	3.48
Sultana	Thailand	7.15	2.10	3.40
Uncle Sam	Thailand	7.13	2.06	3.47
Chicago Stars	USA	7.05	2.05	3.42
First Choice	USA	6.95	2.23	3.11
Texas Star	USA	7.16	2.18	3.28
Golden Perfume	Vietnam	7.18	2.28	3.14
Red Eagle	Vietnam	7.12	2.23	3.19
Special	Vietnam	6.92	2.17	3.19
Mean		7.08	2.15	3.29
SD		0.09	0.08	0.14
Range		6.92-7.18	2.05-2.28	3.11-3.48

TABLE 3

Physicochemical Properties of Popular Imported Rice Brands on the Ghanaian Market

Rice brand	*Source*	*Aroma**	*ASV*	*AAC (%)*	*PV (RVU)*	*HPV (RVU)*	*BD (RVU)*	*CPV (RVU)*	*SB (RVU)*
Ohemma Jasmine	Thailand	P	6.3	15.9	328	183	145	313	-15
Uncle Sam	Thailand	P	7.0	17.9	310	209	101	360	50
Royal Stallion	Thailand	P	6.8	17.2	314	196	118	344	30
Sultana	Thailand	P	6.8	16.2	321	179	142	308	-13
Texas Star	USA	A	3.5	21.4	283	171	112	348	65
First Choice	USA	A	3.0	19.9	306	170	136	327	21
Chicago Stars	USA	A	3.3	20.4	309	170	138	329	20
Golden Perfume	Vietnam	P	7.0	18.1	327	186	141	335	7
Red Eagle	Vietnam	P	6.5	18.4	344	184	160	323	-21
Special	Vietnam	P	5.0	17.4	330	197	133	355	26
Mean			5.5	18.3	317.2	184.5	132.6	334.2	17.0
SD			1.7	1.8	16.8	13.0	17.5	17.4	28.2
Range			3.0 - 7.0	15.9 - 21.4	283 - 344	170 - 209	101 - 160	308 - 360	-21 to 65

* P and A indicate presence and absence of aroma, respectively.

gene. "Special" was heterozygous at the BADH2 locus. On the other hand, the USA brands had the 251 bp allele indicating the absence of the badh 2.1 mutation. Similarly, the USA brands had the 90 bp allele of the ALK marker, whilst the brands from Thailand and Vietnam had the 92 bp allele. The rice brands from Thailand and Vietnam had the TAC, WX and SNP haplotype, whereas the USA types had GCC haplotypes, even though First Choice and Texas Star brands were heterozygous at exon 6, and "Special" was heterozygous at the intron 1 and exon 6 markers.

Discussion

Grain quality improvement in rice is extremely important because unlike other

TABLE 4

Alleles of Functional Markers for Aroma, ASV and AAC

Rice brand	*Source*	*Aroma marker*	*ALK marker*	*WX SNP haplotype*		
				Intron 1	*Exon 6*	*Exon 10*
Ohemma Jasmine	Thailand	243	92	T	A	C
Royal Stallion	Thailand	243	92	T	A	C
Sultana	Thailand	243	92	T	A	C
Uncle Sam	Thailand	243	92	T	A	C
Chicago Stars	USA	251	90	G	C	C
First Choice	USA	251	90	G	A/C	C
Texas Star	USA	251	90	G	A/C	C
Golden Perfume	Vietnam	243	92	T	A	C
Red Eagle	Vietnam	243	92	T	A	C
Special	Vietnam	243:251	92	G/T	A/C	C

cereals, rice is consumed mainly as whole grain. Consumers, therefore, pay much more attention to grain quality. The grain quality of rice is complex and the attributes include nutritional, cooking and eating, and appearance. The appearance of the whole grain is influenced by the translucence of the endosperm, grain length and grain shape.

In the study, all the rice brands were classified as having long and slender grain. This confirms earlier reports that most consumers in Ghana prefer rice with long and slender grains (Asante *et al*., 2013a; Bam *et al*., 1998). Although the preference for rice grain dimensions vary from country to country, most consumer groups prefer long and slender grains (Juliano & Villareal, 1993; Unnevehr, Duff & Juliano, 1992). Rice with long and slender grain is preferred by many consumers in USA, China, India, Pakistan and Thailand. However, in Japan, Sri Lanka and South Korea, consumers prefer short grain varieties (Wan *et al*., 2006). Short- and medium-grain varieties tend to produce clumped, moist and chewy grains after cooking, whereas long-grain varieties tend to produce dry, fluffy and separated cooked grains (Juliano, 1985).

The cooking and eating quality of rice is mainly controlled by the amylose content of the grains. Amylose content in rice can be classified as follows: Waxy rice (0 - 2% amylose), very low amylose (3 - 9%), low amylose (10 - 18%), intermediate amylose (19 - 23%) and high amylose (>23%) (Fitzgerald, 2004; Bergman, Bhuttcharya & Ohtsubo, 2004). In addition to amylose content, GT (usually measured as ASV), gel consistency and paste viscosity are used to predict cooking and eating qualities of rice.

In the study, rice brands with low AAC had the TAC SNP-haplotype, whereas those with intermediate AAC had the GCC haplotype, even though some rice brands were heterozygous at some of the SNPs. Heterozygosity at some SNPs as indicated in the results could most likely be due to grain mixtures from other rice varieties as DNA was extracted from grain but not from leaf tissues. Consequently, WX SNP haplotype for First Choice (AAC = 19.9%) and Texas Star (AAC = 21.4%) are most likely GCC and for Special Rice (AAC = 17.4%) being TAC. This is in agreement with the reports (Chen *et al*., 2008b; Asante *et al*., 2013b) that the WX SNP haplotypes, GCC and TAC are only found in rice with intermediate and low AAC, respectively.

ASV ranged from three to seven with rice brands from the USA at the lower end and those from Asia at the higher end. This perfectly correlated with the ALK marker; brands with ASV lower than five had the wild allele (90bp) and those with ASV of five to seven had the 92 bp allele. ASV scores of two (low), three to five (intermediate) and six to seven (high) corresponding to high (>74 °C), intermediate (70-74 °C) and low (<70 °C) GT, respectively (Bergman Bhuttcharya & Ohtsubo, 2004). The rice brands from the USA and Asia can, thus, be classified as having intermediate and low GTs, respectively.

For paste viscosities, all the rice brands studied had moderately high RVA profiles with the highest SD being approximately 28 RVU for SB. SB is a measure of retrograding, i.e. the hardness of cooked rice upon cooling. Rice with SB of less than 25 RVU and BD greater than 100 RVU is reported to have acceptable eating quality (Shu *et al*., 1998). BD for the rice brands studied

ranged from 101 to 160 RVU and, thus, fell into the acceptable eating quality. SB ranged from -21 to 65 RVU. Since Texas Star with SB of 65 RVU is a very popular brand in Ghana, rice varieties with SB value less than 70 RVU would be acceptable to Ghanaian consumers. Rice breeders in Ghana could, thus, select for rice with BD greater than 100 RVU, and SB less than 70 RVU

In addition to starch physicochemical properties, aroma is an important quality trait for rice consumers in many parts of the world. In Ghana, aroma is one of the major trait for adoptability of rice varieties by farmers, because it is easier to sell locally produced aromatic rice to consumers (Asante *et al*., 2013a). All the rice brands from Asia were aromatic, whilst those from the USA were non-aromatic. The BADH2 gene codes for the synthesis of 2-acetyl-1-pyrroline (2-AP), the volatile compound depicting aroma in rice. The badh 2.1 mutation is the cause of aroma in a majority of rice varieties including Basmati and Jasmine rice (Bradbury *et al*., 2005). This essential quality attribute was probed with a functional marker for aroma, which clearly distinguished aromatic rice from non-aromatic ones. All the aromatic rice brands had the 243 bp allele indicating the presence of the badh2.1 mutation in exon 6 of chromosome 8. The wild-type allele (251 bp) lacks aroma and was characteristic of all the American brands used in the study. It must be emphasised, however, that eight other polymorphisms (badh2.2 — badh2.10) in the BADH2 gene resulting in elevated levels of 2-AP have been found in some rice varieties (Kovach *et al*., 2009).

Conclusion

In conclusion, the popular rice brands on the Ghanaian market can be classified into two main groups: Jasmine-styled aromatic long grain rice with low amylose content and GT (high ASV) from Asia and conventional US long grain rice with intermediate amylose content and GT. The attention of breeders must be focused on breeding for varieties with the following characteristics: Long slender grains, 15 per cent to 22 per cent amylose content, three to seven ASV (intermediate to low GT) and less than 70 RVU setback viscosity. These varieties should ideally have aroma, but non-aromatic ones similar to the conventional US long grained brands are also acceptable to most consumers. The selection of these traits can be facilitated my using molecular markers.

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