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Elucidating Basis of Rice Discoloration and Developing Prevention Strategies

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Elucidating Basis of Rice Discoloration and Developing Prevention Strategies

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Food Science

by

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Abstract

Economically, discolored rice kernels have less market value, which can seriously impact farm net profit. The reasons for rice discoloration during storage have not been studied extensively and many questions remain. Therefore, the primary goal of this study was to improve understanding of the role and contributions of storage practices on rice quality degradation and discoloration. In this study, three rice cultivars including XL753 (hybrid), Roy J (pureline), and Titan were assessed for changes in quality and microbial kinetics. The rice samples at a high moisture content (MC) of 21% w.b. and a low MC of 16% were stored at three temperature levels, 20°C, 30°C, and 40°C. Samples were analyzed every four weeks for up to 16 weeks of storage. Because fungi may be responsible for rice discoloration, the samples were also treated with antifungals (Sodium chloride and Natamycin) to compare rice discoloration differences between non-treated samples and the antifungal treated samples during storage. Electron beam (EB) irradiation was also used to silence microbes in high MC rice before they were stored at the same storage temperatures for up to eight weeks as indicated above. The discoloration development in rice, the fungal growth, and fungal diversity, chemical changes, quality attributes, and their associations were investigated.

Rice discoloration was highly dependent on storage MC, temperature, and duration, increasing significantly as MC, temperature, and duration increased. The highest rice discoloration occurred in all rice cultivars when stored at 21% MC and 40°C for 16 weeks. However, discoloration was also profoundly higher for Roy J than for other cultivars when stored at 21% MC and 30°C for 16 weeks. The discoloration was significantly lower for the sodium chloride treatment, while natamycin was not effective compared to control. Hybrid rice had significantly less discoloration compared to pureline and medium-grain. Fungal counts were similar across all

cultivars. Fungal growth followed a decreasing trend with higher storage temperature as discoloration increased. An inverse relation between fungi and discoloration suggests that discoloration particularly at higher temperatures was not explained by microbe activities. Metagenomics analysis confirmed that the rice had been infected before storage by a diverse group of fungi whose fungal abundance was found to vary considerably with storage conditions. Alternaria, Penicillium, Aspergillus, Nigrospora, and Fusarium were the five most abundant fungal genera among all identified fungi on initial pre-stored rice samples. Each of these fungi can produce pigment and change the color in rice samples depending on storage temperature and rice cultivars. In some cases, these fungi decreased more during storage with sodium chloride treatment. Rice discoloration was significantly induced in rice treated with EB irradiation. The induced discoloration in EB irradiated and non-irradiated samples was explained by chemical changes particularly at high storage temperature that discouraged fungal growth.

Rice discoloration is an issue that is developed by microbial and chemical reactions during storage. Microbial and chemical involvement could explain most of the discoloration in rice stored at low and high temperatures, respectively. The results of this study provide valuable information to growers, processors, and industries on conditions necessary to maintain rice quality.

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Mohammadi Shad, Z., & Atungulu, G. G. (**2019**). Post-harvest kernel discoloration and fungi activity in long-grain hybrid, pureline and medium-grain rice cultivars as influenced by storage environment and antifungal treatment. *Journal of stored products research*, 81, 91-99. (Chapter 2)

Mohammadi Shad, Z., & Atungulu, G. G. Physical Integrity of Long-grain Hybrid, Pureline, and Medium-Grain Rice Kernels as Affected by Storage Conditions. (**2020**). *Applied Engineering in Agriculture*, submitted for publication. (Chapter 3)

Mohammadi Shad, Z., & Atungulu, G. G. Identification of fungal population in stored rice and its relationship with rice discoloration using high throughput sequencing techniques. To be submitted. (Chapter 4)

Mohammadi Shad, Z., Steen, E., Devlieghere, F., Mauromoustakos, A., & Atungulu, G. G. (**2019**). Biochemical changes associated with electron beam irradiation of rice and links to kernel discoloration during storage. *Cereal Chemistry*, 96(5), 824-835. (Chapter 5)

Chapter 1: Introduction

Commercial rice drying facilities do not have the capacity for immediate drying of all the grain during the short harvesting season. However, prolonged storage of the rice at moisture contents (MCs) more than 14% leads to an overall reduction in rice quality - notably rice kernel discoloration (Atungulu et al., 2019).

At high MC, grain mass respires, releasing energy that heats the kernels above ambient temperatures, possibly leading to further kernel dry matter loss.

Rice downgrading due to discoloration represents a significant loss to growers, and especially the State of Arkansas, which produces nearly 50% of national rice volume per year (Mohammadi Shad and Atungulu, 2019).

The Federal Grain Inspection Service (FGIS) of the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration assigns grades to rice based on the number of discolored or otherwise unacceptable kernels in a sample. U.S. No. 1 grade milled rice may contain, at maximum, only one "heat-damaged" kernel per 500-g sample (USDA, 2009). This low threshold can have a tremendous impact on farmers' profits if their rice exceeds the number of "heat-damaged" kernels permitted. To minimize the issues of rice discoloration, the trend among many rice growers in Arkansas, in the last three years, has been the adoption of a recently-introduced technology for on-farm, in-bin rice drying and storage. The new technology allows controlling drying fan operation by the principle of Equilibrium Moisture Content, which is the MC that a specific grain will attain if exposed to air with a given relative humidity and temperature for a long enough duration. Thus, drying fans are operated only under set conditions to avoid over-drying of grain. The new technology comprises sensors to measure ambient air conditions, as well as cables to monitor grain MC and temperature throughout the grain bin mass,

and the data of rice drying characteristics can also be accessed anytime via the internet, which has revolutionized monitoring capabilities for the drying process. From an electronic monitor and fan control standpoint, this new technology appears very promising. However, the ultimate success hinges on effectively maintaining the quality of rice, especially those in the upper bin layers. The causes of rice discoloration are still debatable among researchers (Borlagdan, 2018). Researchers have reported that microbial involvement or chemical changes may cause the issue of rice discoloration (Haydon & Siebenmorgen, 2017; Kushiro, 2015); however, there is not a conclusive report on causes of rice discoloration. Besides, little research can be found trying to identify what specific storage condition makes rice kernels more susceptible to discolor. Overall, the impact of storage conditions on rice discoloration is not understood well.

1. Hypothesis

Rice discoloration is affected drastically by storage conditions. The causes of rice discoloration include the grain moisture content and temperature; however, the presence of microorganism and other chemical changes occurring during storage may also have a significant role in inducing rice discoloration.

2. Objectives

1. To evaluate the extent of rice discoloration and association to fungal growth
2. To assess the kinetics of physical and chemical changes associated with rice discoloration
3. To investigate the potential of physical treatments for prevention of rice discoloration

3. References

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Chapter 2: Post-harvest kernel discoloration and mold activity in long grain hybrid, pureline and medium grain rice cultivars as influenced by storage environment and antifungal treatment

Mohammadi Shad, Z., & Atungulu, G. G.

Abstract

Milled rice grade, and therefore its value, is diminished as the quantity of materially discolored and moldy kernels increases. This study evaluated the magnitudes of kernel discoloration and mold growth for three different rice cultivars (cv.) exposed to different storage moisture content (MC), temperature, and duration and also determined if postharvest treatment of rice kernels with antifungal agents, natamycin and sodium chloride, abate rice discoloration and mold growth. Samples of long-grain hybrid (cv. XL 753), long-grain pureline (cv. Roy J.), and medium-grain (cv. Titan) rough rice were evaluated. Antifungal-treated and untreated (control) samples were stored at two MC levels (16% and 21%, wet basis (w.b.)), and at three temperatures (20°C, 30°C, 40°C). Subsamples were taken, for all storage conditions, every four weeks for up to 16 weeks of storage. The samples were then analyzed to determine mold count, discoloration, and water activity (a_w). Discoloration was overwhelmingly dictated by temperature with rice stored at 40 °C having six- to eleven-times more discoloration than at 20 °C. Increased storage duration also significantly increased discoloration. However, discoloration was not different between MC levels. Discoloration was significantly lowered in rice treated with sodium chloride, while natamycin was not effective compared to control. Samples treated with sodium chloride had significantly less mean a_w (0.73) compared to samples treated with natamycin (0.84) and control samples (0.83). Hybrid rice was significantly less susceptible to discoloration compared to pureline and medium-grain, but had the same total mold count as other cultivars. Unlike discoloration, increasing

temperature and duration decreased mold count. Observed inverse relations between mold and discoloration suggests microbial activities did not explain discoloration. The findings from this study provide growers and processors important information on conditions necessary to maintain rice quality during storage.

Keywords. Rice storage; Kernel discoloration; Long-grain hybrid and pureline; Medium grain

1. Introduction

Cultivated rice (*Oryza sativa* L.) is one of the most important food crops in terms of economic and nutritional value (Li et al., 2011). Rice sustains over three billion people on the planet (Bouman, 2012). Despite significant agronomic improvements in rice cultivation, postharvest storage losses remain a vital threat to food supply. Rice production losses can be as high as 37% in Southeast Asia and average about 15-16% worldwide (Kumar and Kalita, 2017). Causes and effects of storage loss are complex and variable but mainly arise from interactions between abiotic and biotic factors, which may further interact with the rice physical or morphological characteristics (Atungulu et al., 2015 and 2016). The two main abiotic factors found to be related to quality deterioration during rice storage are temperature and moisture content (MC), as well as storage duration (Christensen and Sauer, 1982; Dillahunty et al., 2001). Microbes, inherent in harvested rice, have been implicated as leading biotic factors, of which fungi are perhaps the most severe spoilage agents (Phillips et al., 1989). Other biotic factors include insects and mites. Physical characteristics that may also interact with given abiotic and biotic factors to influence deterioration processes include the rice form (e.g., paddy, brown, or milled rice), and the rice cultivar (cv.).

Rough rice, also called paddy rice, is whole un-milled rice grain, which in practice is usually harvested when mature grains have MC of 22-28% (w.b.) (de Lucia and Assennato, 2006; Kumar

and Kalita, 2017). According to Atungulu et al., (2016), the ideal harvest MC to maintain milled rice quality is 19 to 21% (w.b.). After harvest, it is necessary to dry rice to a final MC of 13-14% w.b. (Espino et al., 2014; Savi et al., 2018; Atungulu et al., 2015), which is considered sufficiently low enough to inhibit mold growth and maintain the rice quality during storage (Atungulu et al., 2015).

One widely used method to achieve rice drying on-farm is with natural (20-30 °C) or slightly heated (40 °C) air in bin drying systems (Espino et al., 2014). In these systems, the drying air is forced through the grain column using a fan; as the air moves through the moist rice, from the bottom to the top, the air picks up moisture from the grain, thereby drying it. The top layers of rice in the bin drying systems can remain moist for an extended period depending on airflow rate and ambient air conditions (Atungulu and Zhong, 2016). During prolonged or delayed drying, fungi may grow in moist layers of rice resulting in significant rice deterioration. The fungi grown during rice storage may produce toxigenic substances such as aflatoxin (Atungulu et al., 2018; Mohammadi Shad and Atungulu, 2017; Ismail et al., 2018).

One serious form of rice quality degradation, especially of milled rice dried using in-bin drying systems, is “rice discoloration”. The terms “discolored rice” and “rice discoloration” are used henceforth with reference to rice deemed yellow, stained and of any color (black, brown, pink, opaque or such like) deviating from U.S. standard, white milled rice. The color of milled rice affects consumer preferences and price. In the U.S., rice standards set by the Department of Agriculture (USDA, 2009), downgrade rice as the proportion of heat damaged (materially discolored rice) increases. Hence, growers run the risk of significant loss of revenues due to discoloration. Several factors have been connected to rice discoloration including elevated storage temperature and water activity (a_w) (Bason et al., 1990), rice storage duration (Haydon and

Siebenmorgen, 2017), and biochemical reactions within rice kernels stimulated by high moisture content at elevated temperature (Trigo-Stockli and Pedersen, 1994). Some researchers, such as Bason et al. (1990) and Belefant-Miller (2009), have found little evidence of fungal growth as an explanation for rice yellowing. However, it was reported that fungal respiration at very high humidity can lead to discoloration of stored un-threshed grain (Phillips et al., 1988). Also, Trigo-Stockli and Pedersen (1994) found paddy rice stored at MCs greater than 20% (at a fixed temperature of 25 °C) discolored completely and associated this to high mold growth and grain heating.

The population of storage fungi on rice, depending on the storage environment, generally increases with storage duration, although the fungi have also been reported to decline under hermetic storage conditions (De Dios et al., 2004). Rice discoloration has been reported to be due to certain species of fungi on rough rice. Black to brownish rice kernels have been associated with Curvularia spp. (Phillips et al., 1988) and Fusarium chlamydosporium (Schroeder, 1963 and 1965). Several Aspergillus spp. and Penicillium spp. have been reported present in yellowish kernels (Phillips et al., 1988; Atungulu and Shad, 2017; Atungulu and Mohammadi-Shad, 2019).

Rice decontamination methods may also affect rice color. Sterilizing 23% MC rough rice with steam increased rice discoloration. However, rice sterilization using sodium chloride inhibited the kernel discoloration (Quitco and Ilag, 1982). Another mechanism attributed to rice discoloration is non-enzymatic browning, which influences yellowing rate (Gras et al., 1989). For example, Maillard reaction, a chemical reaction that occurs between certain amino acids and reducing sugars produces brown pigment (Troller and Christian, 1978). Non-enzymatic browning is affected by a_W, temperature, and storage atmosphere, where the influences of temperature and a_W are considered the most predominant (Bason et al., 1990). The a_w is often used to predict the

growth of bacteria, yeasts and molds (Troller and Christian, 1978). The microbial prevalence may also be cultivar dependent. For example, Atungulu et al. (2015) reported that long-grain hybrid cultivars had significantly lower surface aerobic plate counts, and mold count levels compared to long-grain pure line and medium grains.

Presently, there has not been a solid consensus within the research community on the linkage between microbes and rice discoloration under storage conditions. In this study, we simulated postharvest storage and drying environment conditions that are typical of those used today in commercial rice farms. The objectives were to evaluate the extent of kernel discoloration and mold growth for three different rice cultivars exposed to different storage MCs, temperatures, and storage durations. We also evaluated the hypothesis that microbes are directly related to rice discoloration, and that kernel discoloration can be suppressed by postharvest application of antifungal agents such as sodium chloride and/or natamycin on stored grain.

2. Material and Methods

2.1 Rice sampling

Two lots of long-grain rice, *cv.* Roy J (Pureline) and *cv.* XL753 (Hybrid), and one lot of medium-grain rice, *cv.* Titan, were procured for this study. The Roy J and Titan cultivars were grown at the University of Arkansas System Division of Agriculture, Pine Tree Research Station, in Colt, Arkansas. The *cv.* XL753 was grown on a commercial field of Florenden Farms in Burdette, Eastern Arkansas. All the rice samples were harvested at optimal harvest MC, 21-22% w.b. in August of 2017 (moisture content, henceforth is in wet basis terms, unless stated otherwise). Immediately following harvest, the samples were placed in Styrofoam containers with dry ice to prevent deterioration and to maintain the ambient MC during transport to University of Arkansas in Fayetteville. The MCs of the samples reported in this study were determined using an AM 5200

Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden), which was calibrated using method by (Jindal and Siebenmorgen, 1987). Determination methods for total mold count and discoloration of rice are detailed below.

2.2 Experimental conditions

The experiment evaluated mold and discoloration responses from sampled rice kernels of the three rice cultivars (Roy J, XL753, and Titan). The experimental design included effects of two storage MCs (16 and 21%) and three storage temperatures (20, 30, and 40 °C). Additionally, antifungal treatments were included in which cultivar samples were either treated with natamycin, treated with sodium chloride, or untreated (control). All sample combinations of cultivar/treatment/MC/temperature were stored until they were taken for measurements of mold and discoloration at storage durations of four, eight, 12, and 16 weeks.

Before conditioning to target MCs, each lot of rice was passed through a cleaner (MCi Kicker Laboratory Grain Cleaner, Mid-Continent Industries, Inc., Newton, KS) to remove materials other than rice. Preliminary MC of the rough rice was determined. Based on these initial MCs, rice was then conditioned to target MCs. To condition the samples to 16 and 21% MC, rough rice samples were air dried at 25±3 °C and 60±5% relative humidity (RH). Once samples were at the two target MCs, separate rice samples for each cultivar were mixed at the ratio of 30% w/w with sodium chloride and at the ratio of 0.46% w/w with natamycin. Subsequently, 600-g samples of each rice cultivar at each MC and antifungal treatment (including control) were filled into glass jars, which were then sealed airtight. Three separate 600-g jars of the cultivar by antifungal by MC combinations were then stored in incubators set at either 20, 30 or 40 °C. After each four-week storage period, subsamples from jars for determination of a_w and total mold count were taken and stored at 5 °C until analyzed. The reminder of the jar sample was split into two 150-g subsamples,

which were then dried at 25±3 °C and 60±5% RH to attain 12.5% MC for milling.

2.3 Discoloration analysis

For kernel-to-kernel rice color variability, an image analysis system (WinSEEDLE Pro 2005a, Regent Instruments, Sainte-Foy, Quebec, Canada) was used to quantify the relative amounts of specific kernel colors. The software was calibrated with discolored kernels of interest from previous studies (Haydon and Siebenmorgen, 2017). For each of the two discoloration subsamples, 100 head rice kernels were arranged on the acrylic tray and scanned against a blue plastic background that was used to create the analysis profile. The software analyzed the area of each of the 100 kernels in the resulting image and determined, by a pixel-by-pixel assessment, the percent of the 100-kernel projected area that was occupied by specific colors from the profile. The sum of the areas of each of the seven discoloration descriptors (translucent white, opaque white, three shades of yellow, red/brown, black/brown, pink/red, and light pink) was considered the total projected discolored kernel area for the subsample. The white or non-discolored areas of the 100 kernels were represented by the sum of the translucent white and opaque white areas.

2.4 Microbial analysis

Microbial analysis was conducted using the Association of Official Analytical Chemists International (AOAC Int.) Official Method 997.02, Yeast and Mold Counts in Food Petrifilm™ Method (AOAC Int., 2012). 3M™ Petrifilm™ Mold Count Plates (3M Microbiology Product, St, Paul, MN) were used to determine the total microbial load on stored rice. Phosphate-buffered dilution water (0.5 M, pH = 7.2) was used and sterilized by autoclaving at 121 °C. Three, 10-g samples of rice were then mixed with 90 mL phosphate-buffered dilution water in a sterile stomacher bag. Then, samples were homogenized through masticating using a lab masticator (Silver Panoramic, iUL, S.A., Barcelona, Spain) to dislodge the microorganisms. The masticator

was set at 240 s and 0.7 stroke/s. This process resulted in rice samples that were pulverized into powder for total microbial load analysis. Sequential dilutions were made by mixing 1 mL of the original mixture with 9 mL of phosphate-buffered dilution water. The sequential dilutions were continued until plates with countable mold loads were achieved. For total mold counts, the 10^{-4} to 10^{-7} times dilutions were plated. Total mold count was expressed in terms of base 10 log of CFU/g, where CFU is Colony Forming Units.

Starting at the eight-week sampling and continuing at weeks 12 and 16, the a_w of the samples was determined. The a_w was measured by a water activity meter (AquaLab 4TE, Dew Point, Pullman, WA).

2.5 Statistical analyses

For discoloration, total mold count, and a_w, measurements made for the individual subsamples from each jar (two for discoloration, three for mold count, and two for a_w) were averaged and the coefficient of variation (CV) was calculated. The purpose in averaging subsamples was to provide a reliable measurement for each case without artificially inflating sample size and statistical significance by using highly correlated subsamples as replicates (Lazic, 2010). Indeed, the variability for subsamples was small with mean CVs of 0.08 ±0.10 and 0.05 ±0.04 over all discoloration and total mold count data, respectively.

All statistical analyses for discoloration, total mold count, and a_w were performed using SAS 9.3 software (SAS, 2013). Statistical significance of the main effects, storage temperature, MC, duration, antifungal treatment, and cultivar, and their interactions were identified by the stepwise regression procedure (Neter et al., 1990) using the PHREG procedure in SAS with the 'stepwise' option. The initial stepwise regression models for each of the three response variables contained all possible 2nd to 5th order interaction terms. Then, selection of effects and interactions

to the final regression model are made in the procedure one-step at a time. During the procedure, individual chi-square scores determine which of the explanatory effects is first selected into the regression model. The chi-square statistic is a goodness-of-fit test and the score for each explanatory effect is the global test score for the regression model containing that effect as the only explanatory effect. For model entry of an effect, the chi-square statistic had to be significant at $p<0.05$. Based on the final stepwise regression results, further analyses of effects were made including first order inferences by partial correlation (CORR procedure), and when appropriate, analysis of variance (ANOVA procedure). For significant effects ($p<0.05$) found in the ANOVA, the Tukey option in SAS (honestly significant difference) was used to compare means. The Shapiro-Wilk univariate test (Neter et al., 1990) was performed with the MODEL procedure to test normality for response variables. Linear regression was performed using the REG procedure.

3. Results

3.1 Discoloration

3.1.1 Significant effects on discoloration

Percent rice discoloration is shown graphically for each cultivar by storage MC, storage temperature, storage duration, and antifungal treatment (Figure 1). The stepwise regression procedure for the discoloration data selected four significant main effects and four interaction effects, each significant at $p<0.05$. All other effects and interactions were deemed to be not significant by the procedure, i.e., at p values>0.05. Table 1 shows the effects ranked from highest to lowest chi-square score obtained in the final stepwise regression model. According to these results, the differences in discoloration was most-dominated by the effects of storage temperature followed in order by antifungal treatment, storage duration, and cultivar. Storage temperature by duration had the highest chi-square score of the four significant interactions. The other three

significant interactions in Table 1 each contained cultivar, temperature by cultivar, temperature by duration by cultivar, and duration by cultivar. The interaction effects are described in more detail below. By observation, there appeared to be differences in discoloration level related to storage MC (Figure 1), e.g., higher discoloration at 21% versus 16% moisture content at the 30 °C storage temperature, for natamycin and non-treated samples. However, the stepwise regression procedure revealed an overall effect due to MC was not significant (i.e., p=0.18), where the MC effect (and its interactions) were overwhelmingly obscured by temperature effects and the other effects and interactions shown in Table 1.

3.1.2 Storage temperature, duration, and interactions

Evidence of the significance of storage temperature on discoloration is described by partial correlations evaluated across the three antifungal treatments for each cultivar (Table 2), while controlling for the effects of storage duration and moisture content. As seen in Table 2, discoloration by temperature partial correlation was consistent and highly significant for a given antifungal treatment. Somewhat smaller correlation coefficients (r) were observed for sodium chloride ranging from 0.63 to 0.77 compared with 0.86 to 0.89 for the control treatment. The difference in r values between cultivars for a given antifungal treatment also indicate the interaction effect on discoloration that occurred between temperature by cultivar, as determined in the stepwise regression procedure (Table 1).

Partial correlations of discoloration with storage duration (Table 3) disclose the smaller isolated impact of storage duration on discoloration compared to temperature. Only for cultivar Roy J was partial correlation significant and the correlation coefficients were uniform across the three antifungal treatments for Roy J. The non-significant partial correlation across all antifungal treatments for Titan and XL753 suggests minimal effects of storage duration on the discoloration

for those cultivars. The highly variable partial correlation results between Roy J and the two other cultivars depict the effects of duration by cultivar interaction, as in Table 1.

The significant temperature by storage duration interaction can be observed by the marked differences across temperature regimes and resultant slopes obtained by regressing discoloration by week (Figure 1). At 20 °C, discoloration tendencies for the cultivars were either somewhat linear with duration (Roy J) or showed negligible change over the 16 weeks (Titan, XL753). Discoloration patterns with duration were notably different as storage temperature increased from 20 °C to 30 °C. At 30 °C, Roy J discoloration notably increased with storage week under 16% MC for all three antifungal treatments and for both natamycin and control under 21% MC. Less apparent compared with Roy J at 30 °C, discoloration for Titan and XL753 cultivars showed correlation with storage week for natamycin and control treatments, but less correlation with week for sodium chloride. Discoloration with storage duration was highly different at 40 °C than at 20 °C and 30 °C temperatures for all cultivars. At 40 °C, near 100% rice kernel discoloration occurred after four weeks of storage, except for samples treated with sodium chloride. Discoloration for the natamycin and control treatments exhibited no difference with week at 40 °C, thus showing no effect due to natamycin treatment on discoloration at that temperature. On the other hand, at 40 °C, kernel discoloration generally increased with week for cultivars Roy J and XL753 under sodium chloride, while it generally decreased with week for Titan at 40 °C. While discoloration across the experiment showed interactive effects associated with temperature, duration, and cultivar (Table 1), significant interactions between antifungal and other effects did not occur.

3.1.3 Antifungal treatment, cultivar, and water activity

Controlled partial correlations between discoloration and antifungal treatment were significant for each cultivar (Table 4). Though the correlation coefficient was highest for Roy J (r

=0.50) and lowest for XL753 (r =0.39), antifungal treatment impacted all cultivars to some extent.

Two factor analyses of variance (ANOVAs) were performed to determine the combined effects of antifungal treatment and cultivar on rice discoloration. However, the discoloration data were highly skewed to both the left and right due to temperature effects (Figure 1) and thus were not normally distributed. Several common transformations of the discoloration data were made to try and correct lack of normality including square root, cube root, and logarithmic transformations (Neter et al., 1990). Nevertheless, the data remained skewed even with transformation. However, it was revealed in the process that cube root transformation improved the normalcy when considering data separately at the 20 °C and 30 °C temperatures. After ascertaining that the data for these temperatures passed the Shapiro-Wilk normality test (Neter et al., 1990), ANOVAs were performed separately for the two temperatures. An ANOVA for data at 40 °C was not made since the transformed data at that temperature remained non-normally distributed.

Over the 20 °C temperature, results indicated both antifungal treatment ($p=0.001$) and cultivar ($p=<0.001$) effects were significant, but not the antifungal by cultivar interaction ($p=0.462$). Mean discoloration of rice treated with sodium chloride was nearly 50% lower than for control at 20 °C and the difference was significant (Table 5), whereas the 33% mean discoloration difference between sodium chloride and natamycin and the mean difference between natamycin and control were not statistically different at 20 °C.

Cultivar discoloration at 20 °C was highly and significantly different for XL753 than the other two cultivars (Table 5). At the 30 °C temperature, ANOVA results also indicated both antifungal treatment ($p=0.002$) and cultivar ($p=0.031$) effects were significant, while a significant interaction did not occur ($p=0.687$). The effect of sodium chloride treatment on discoloration was more pronounced at 30 °C than 20 °C, where mean discoloration was now about one-third that for

control and about 40% that for natamycin (Table 5). As found for the 20 °C temperature, the mean discoloration for XL753 was again significantly less than that for Roy J at 30 °C, though not significantly lower than that for Titan. While an ANOVA was not made for data at 40 °C, the means for antifungal treatments provided in Table 5 show similar trends found for the two lower temperatures. A reduction in the discoloration mean for sodium chloride treated rice of about 50% from that for both control and natamycin suggests an effect due to the treatment may have occurred at this extreme storage temperature. Less apparent was a strong difference in rice discoloration for XL753 than other cultivars at 40 °C, unlike the trends for the 20 °C and 30 °C storage temperatures. The discoloration means across the three temperatures in Table 5 indicate that percent discoloration at 40 °C was six- to eleven-times greater that at 20 °C for a given antifungal treatment or cultivar.

Water activity when evaluated by stepwise regression procedure was significantly affected by only MC ($p<0.001$) and antifungal ($p<0.001$) and their two-way interaction ($p=0.007$). ANOVA for a_w was not made since the data were not normally distributed and lacked measurements at the four-week duration. However, looking at the means for antifungals by moisture content (Table 6) we find that a_w was slightly lower for sodium chloride (mean = 0.726) than for both control and natamycin when rice MC was at 16%.

However, when moisture content was at 21%, the a_w for sodium chloride (mean = 0.74) rose just slightly, whereas the mean a_w for control and natamycin increased substantially. Combined over both MCs, mean a_w was 0.73, 0.84, and 0.83 for sodium chloride, natamycin, and control, respectively (Table 6). Thus, sodium chloride treatment maintained lower a_w at both moisture contents while exhibiting less variation across all conditions.

3.2 Microbial contamination

3.2.1 Significant effects on total mold count

Total mold count is shown graphically for each cultivar by storage MC, storage temperature, storage duration, and antifungal treatment (Figure 2). Viewed in its entirety, the distribution of the total mold count data appears to be less affected by the different experimental conditions imposed as compared to discoloration data (Figure 1). For total mold count, the stepwise regression procedure selected just three significant main effects and just one interaction, controlling the significance level for a selected effect to enter the model at $p<0.05$. All other effects and interactions were at higher p values and therefore were not entered in the stepwise regression model. Table 7 shows the effects ranked from highest to lowest chi-square scores and the partial correlation coefficients for storage effects, as well as those for treatment and cultivar, which were not significant. Evaluation of the storage effects based on the Chi-Square scores suggests the greatest effect on total mold count was storage temperature followed by moisture content, storage duration, and storage temperature by duration interaction. The linear regression lines of total mold count by week for the antifungal treatments for each cultivar are shown in Figure 2, do not depict clear differences or notable trends in mold activity due to the treatment effect or cultivar type. On the other hand, decreasing trends for mold count with increasing temperature, increasing MC, and increasing storage duration can be observed in Figure 2.

3.2.2 Analysis of effects on total mold count

Unlike the variable distributions noted for discoloration, the distribution of total mold count approximated a normal distribution (Figure 3). Primary reasons for more normally distributed data for mold count, as opposed to discoloration, include the far less extreme total mold count variation across the three temperatures and less variation with weeks in storage. For discoloration, antifungal treatment and cultivar had significant effects causing some additional distortion and skewness in the data distribution. Assuming negligible antifungal treatment and cultivar effects on mold count

total, further evaluations of storage temperature, MC, and duration effects on mold count were made. Evaluation was made utilizing a three factor ANOVA, in which all interaction terms including the three-way interaction of temperature by MC by duration were considered in the ANOVA model. Excluding treatment and cultivar as non-effects in the ANOVA permitted additional observations and degrees of freedom for the error-term. The data for the ANOVA passed the Shapiro-Wilk normality test at $p=0.12$ (i.e., the test passes if p is greater than 0.05).

Results of the ANOVA showed that total mold count was significantly affected by temperature ($p<0.001$), MC ($p=0.002$), duration ($p=0.002$), and temperature by duration ($p=0.001$). Like in the stepwise regression selection, the ANOVA also concluded that all other interactions were not significant. Mean tests of the data revealed that total mold count significantly decreased with each step increase in temperature from 20 °C and that it significantly decreased going from 16% to 21% moisture contents (Table 8). Although mean total mold count decreased after eight weeks of storage, it did not change significantly from eight to 12 weeks and from eight to 16 weeks of storage. The temperature by storage duration interaction effects were temperature-dependent (Table 8). A significant difference in mean mold count between four and eight weeks only occurred at the 30 °C temperature. However, at 20 °C, 12 or 16 weeks in storage significantly decreased the total mold count compared to counts at four weeks in storage. The mean difference in storage duration were not important for rice stored at 40 °C, the temperature having the lowest total mold count.

For total mold count, the magnitude of influence of the environmental effects presented above overwhelmed the effects of the antifungal treatments or differences in rice cultivars. Table 9 shows the means and standard deviations of total mold count for rice cultivars by antifungal treatment and for all antifungal treatments combined.

The similarity of the mean data for antifungal treatment and cultivar illustrates the negligible effects these factors had on total mold count in this study.

3.2.3 Mold and discoloration contrasts

Temperature affected microbial counts and discoloration very differently. At the highest temperature of 40 °C, mean percent discoloration was from six- to eleven-times greater than at 20 °C, for a given antifungal treatment or cultivar. In contrast, mold count decreased in an orderly manner as temperature increased, though the influence of increased temperature on total mold count was much less profound than the temperature effects on discoloration. While mean mold was significantly less at 21% than 16% MC, discoloration differences were too small to be detected. For the two variables, responses to storage duration were inverse, although responses were significantly affected by interactions between temperature and duration in both cases. Discoloration notably increased with storage duration at lower temperatures, but not at 40 °C. On the other hand, mold count generally decreased with storage duration and counts were lowest after 16 weeks. However, as with discoloration, there was not a significant storage duration effect on total mold counts at 40 °C. The sodium chloride treatment significantly reduced percent discoloration versus control at the two lower temperatures and its influence also appeared to be effective at 40 °C. Similarly, discoloration changed with cultivar where it was smaller for the XL753 cultivar than for Roy J and Titan. In contrast, antifungal treatments and cultivars did not exhibit detectable responses on mold counts. Relationships between the transformed discoloration data (cube root transformation) with total mold count were evaluated using linear regression for non-treated control data and the sodium chloride-treated data (Figure 4), as regressed over identical experimental conditions (i.e., same temperature, MC, duration, and cultivar). As expected, the regressions resulted in negative correlation between discoloration and total mold count, showing

decreased discoloration with increased mold count. The inverse relationship was stronger for the control treatment, having a better fit and a steeper negative slope than for the sodium chloride treatment. The regression relationship for natamycin data (not shown) was more like the sodium chloride than the control curve. The regression analyses provide general insight into the linkages between the two responses. However, there is not enough evidence to conclude that sodium chloride treated rice changed the relationship between discoloration and mold count from that of non-treated rice.

4. Discussion

This study demonstrated that all three rice cultivars, despite antifungal treatment, had highly detectable levels of both discoloration and mold during storage. While a wide array of causes for rice discoloration during storage, including microbial contamination, have been proposed by others (Schroeder, 1965; Troller and Christian, 1978; Bason et al., 1990; Trigo-Stockli and Pedersen, 1994), in this study it was shown that rice discoloration was highly affected by the storage conditions, temperature and duration. The impact of storage temperature was much more prominent on rice discoloration progress than MC. Untreated rice and rice treated with natamycin stored at 40°C approached total (100%) discoloration after 16 weeks of storage regardless of MC (Figure 1). Significant discoloration in stored rice kernels due to storage temperature and duration have been reported in other studies. High temperature-induced stress (50-70°C) was shown to cause significant kernel discoloration in rough, brown, and milled rice (Bason et al., 1990; Dillahunty et al., 2001; Belefant-Miller, 2009; Ambardekar and Siebenmorgen, 2012). Results here were consistent with rice storage studies by Dillahunty et al. (2001) who found that storage temperature and duration were the most important factors affecting rice color, whereas the effect of storage MC was not significant. They noted that although rice MC did not directly affect

yellowing, it does have an important interactive impact on the factors that influence discoloration. Discoloration level in the present study was found to be generally proportional to storage duration and duration effects were also dependent on temperature. De Dios et al. (2004) reported that the percentage of discolored kernels in stored rice of different degree of milling was slightly increased as the storage period progressed. However, Haydon and Siebenmorgen (2017) also showed discolored kernel area for several rice cultivars was significantly increased with increased temperature (20, 27, and 40°C) and increased storage duration (2 to 16 weeks, by 2-week intervals). It was reported slight differences in percent rice discoloration for storage temperatures that varied from 10 to 27 °C for hybrid long grain rice cultivar XL745. However, similar to our results, it was found that percent discoloration reached 80 to 100% when storage temperature was 40 °C after 16 weeks of storage, except at an MC of 12.5%. The study of Dillahunty et al. (2001) also reported high interaction effects between storage temperature and exposure duration on the rice discoloration, while the data presented by Haydon and Siebenmorgen (2017) show two- and three-way interactions between temperature, MC, and duration. Our results of the effect of MC on discoloration differed from literature findings in the respect that the slight rise found for discoloration going from 16% to 21% was not significant. However, increased rice discoloration was found by increasing rice storage MC from 18% to 22% (Trigo-Stockli and Pederson, 1994). Haydon and Siebenmorgen (2017) showed significant effects of MC on discoloration. However, in both papers the results were not consistent across temperatures showing no difference in discoloration by MC at low temperatures and stronger differences by MC at high temperatures.

In the present study, discoloration levels of stored rice varied by antifungal treatments and by cultivar (Table 5). Considering the three antifungals treatments, there was significantly less discoloration in rice samples treated with sodium chloride. We noted that the a_w of stored rice

treated with sodium chloride was also lower than control and rice treated with natamycin and considerably lower at the 21% MC (Table 6). Lower a_w in the cells of fungi may slow down the movement of substances in and outside fungal cells (Bason et al., 1990). Although sodium chloride was not found to be related to mold count in the present study, there was a connection between the lower a_w for sodium chloride-treated rice in the discoloration results. Findings suggest that the use of sodium chloride as a rice antifungal warrants further research evaluation. Discoloration also varied among cultivars, with mean levels greatest in Roy J, followed by Titan. Cultivar XL753 was significantly less susceptible to discoloration due to storage conditions. These results support the assumption that the long-grain hybrid rice cultivar XL753 may be more resistant to kernel discoloration as opposed to "purer" long-grain pureline and medium-grain varieties.

The total mold count observed in this study (overall mean ≈ 4.0 $\log_{10}$ CFU/g; Table 9) was comparable to those reported in recent studies in Arkansas (Atungulu et al., 2015; Atungulu et al., 2016). In the present study, the total mold counts of rice were signficantly reduced as storage temperatures increased from 20°C to 30°C to 40°C. Similarly, Atungulu et al. (2016) found storage temperature of 40°C reduced mean mold counts for several long-grain hybrid cultivars from those at temperatures from 10 to 27 °C, although mean mold differences between 27 and 40 °C were not signficant for all cases studied. In the present study, as MC increased from 16 to 21%, mean total mold count was noted to decrease signficantly (Table 8), albeit the mold decrease was less than 7.0%. In contrast, the mean mold count in the Atungulu et al. (2016) study, was relatively proportional and signficantly different going from MC levels of 12.5 to 21% MC. Trigo-Stockli and Pederson (1994) indicated a decreasing trend of storage fungi as MC increased from 18, 22, and 26%. They note, however, that the findings were almost exclusively related to elevated temperatures as MC increased. Our experiment indicated that total mold count was significantly

lower at 20°C and 30°C temperatures when storage duration was 8, 12, and 16 weeks than at four weeks of storage, while there was no difference in mold count with storage duration at 40°C (Table 8). Unlike our findings, Atungulu et al. (2016) indicated that the effects of storage duration on mold count generally interacted with MC, where at MC ≤17%, mold count remained relatively stable across all storage durations, whereas at MC >17% the mold count was initially low over six weeks, then increased linearly for the next four weeks, and then either declined or remained high.

We attribute the deviation between the MC effects on mold obtained in this study with other reports may have been due to the following processes. Initially, fungi were able to grow rapidly in the rice stored in sealed containers while oxygen and nutrients were abundant and available for microbes. But as storage duration increased, regardless of moisture content, oxygen depleted and was replaced with carbon dioxide, while microbes also now had limited nutrients to feed on and continue growing. It was shown by Navarro et al. (1992) that development of microorganisms in stored conditions are inhibited in limited oxygen conditions, which have also been associated with minimized damage of the stored product. Moreover, hermetic and sealed containers have been found to control microbes and pests in stored corn, e.g., maize weevils (Sitophilus zeamais) and larger grain borers (P. truncatus) (De Groote et al., 2013).

Although the mold count significantly decreased with temperature, kernel discoloration significantly increased. This inverse relationship and other findings suggest that discoloration may be due to chemical reactions, such as the Malliard reaction, rather than fungal contamination. As shown in the Bason et al. (1990) study, significant rice kernel discoloration occurred at high temperatures (60°C) and low water activities (aw=0.40, 0.60), i.e., conditions that would deactivate fungi growth. Moreover, Belefant-Miller et al. (2005) reported discoloration in rice field-treated with fungicide during development, which were fungus-free endosperm. They found

that discoloration penetrated into the endosperm without fungal hyphae detected into the kernels.

The mold growth in our study was also not suppressed significantly by the applied sodium chloride and natamycin treatments. According to previous studies, natamycin is a qualified antifungal for fungi inhibition because of its binding to cell membrane sterol, primarily ergosterol. The recommended concentration of 10 mg/L for natamycin use in rice (Pengfei et al., 2013) was also used in this study. However, Pengfei et al. (2013) emphasized that at higher concentration (>10 mg/L), natamycin entirely inhibits the growth of Aspergillus, Penicillium, Embellisia, Mucor, Fusarium, and Rhizoctonia spp on rice. Depending on environmental factors, Kogkaki et al. (2016) found that higher concentrations of natamycin may also be effective in delaying fungal growth. Minimal natamycin inhibitory impact on mold was observed in this study. Hence, we infer that increasing the concentration of applied natamycin may be more effective as an antifungal and submit that further research on the subject is needed.

The current study found rice cultivars did not respond differently to mold growth unlike the discoloration response, which was lower for the hybrid cv. XL753. While the overall mean mold counts for Roy J and Titan were slightly higher than for XL753 (Table 9), they were not significantly higher. The mold findings for our cultivars differed with the results of Atungulu et al. (2015) who found that the total mold counts on rice were significantly related to cultivars. In particular, long-grain hybrid rice cultivars were less susceptible to mold colonization in that study compared with medium-grain rice cultivars, and somewhat less susceptible compared to long-grain pureline (including Roy J) cultivars. Comparison of mold count differences for the two cultivars that were also used in the present study (i.e., XL753 and Roy J) shows a signficant mean mold difference between the cultivars occurred only in one of two years (Atungulu et al. (2015).

5. Conclusions

Rice quality factors, discoloration and total mold count, were evaluated under variable storage conditions representative of stored-rice practices in major US rice production areas, including the state of Arkansas. Storage conditions imposed were moisture content (16 and 21%), temperature (20, 30, and 40 °C), and duration (4, 8, 12, and 16 weeks). The experiment also included three rice cultivars, a long-grain hybrid (XL753), a long-grain pureline (Roy J), and a medium-grain (Titan). Additionally, samples from each rice cultivar were placed in jars and treated with two antifungal agents (natamycin and sodium chloride). Untreated sample for each cultivar were also retained for analysis. Discoloration and total mold count were measured for the treated and untreated cultivars at each of the 2 (MC) by 3 (temperature) by 4 (duration) storage combinations.

Results from this study imply the following conclusions: Discoloration levels in the storage environment were overwhelmingly dictated by temperature with rice stored at 40 °C having six- to eleven-times more discoloration than at 20 °C. Increased discoloration was also found to occur when storage duration was extended past four weeks. However, discoloration was not significantly different between storage MC levels of 21% and 16%. Discoloration was significantly lessened for rice treated with the antifungal sodium chloride, while natamycin appears to be ineffective, at least for the dose applied in the study. Hybrid long-grain rice was significantly less susceptible to kernel discoloration than long-grain pureline and medium-grain cultivars. Mold count, unlike discoloration, was found to decrease with greater MC, temperature, and duration. The opposite environmental effect trends for mold and discoloration imply that observed microbe levels were not the primary influential cause of the resultant discoloration, particularly at higher storage temperatures. Moreover, while sodium chloride and cultivar differences clearly impacted

discoloration, antifungal and cultivar had no effect on total mold count. Thus, findings suggest that the mechanism of discoloration under the storage and treatment conditions in these studies may have been more related to non-enzymatic browning, or Maillard reaction. The results provide rice producers and processors new and useful information on the effects of storage conditions on rice quality.

6. References

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7. Tables and Figures

Table 1. Summary of stepwise regression scores and probability level (p) for discoloration.

Effect	Chi-Square score	p value
Temperature	165	<0.001
Antifungal	47.6	<0.001
Duration	21.0	<0.001
Cultivar	14.7	<0.001
temperature by duration	11.0	<0.001
temperature by cultivar	7.0	0.008
temperature by duration by cultivar	4.2	0.039
duration by cultivar	4.0	0.046

Table 2. Summary of partial correlation coefficient (r) and probability level (p) between discoloration and temperature considering each antifungal treatment by cultivar.

	Antifungal					
	Control		Natamycin		Sodium chloride	
	r	p	r	p	r	p
Cultivar						
XL753	0.89	<0.001	0.78	<0.001	0.66	0.001
Roy J	0.86	<0.001	0.86	<0.001	0.63	0.002
Titan	0.89	<0.001	0.86	<0.001	0.77	<0.001

Table 3. Summary of partial correlation coefficient (r) and probability level (p) between discoloration and storage duration (weeks) considering each antifungal treatment by cultivar.

	Antifungal					
	Control		Natamycin		Sodium chloride	
	r	p	r	p	r	p
Cultivar						
XL753	0.11	0.619	0.24	0.292	0.22	0.320
Roy J	0.54	0.009	0.48	0.023	0.47	0.023
Titan	-0.00	0.997	0.16	0.483	-0.20	0.372

Table 4. Summary of partial correlation coefficient (r) and probability level (p) between discoloration and antifungal treatment by cultivar. In analysis, antifungal treatments were numerically-defined as 1, 0, and -1 for control, natamycin, and sodium chloride, respectively.

Cultivar	partial correlation coefficient (r)	probability level (p)
Roy J	0.50	<0.001
Titan	0.41	<0.001
XL753	0.39	0.001

Table 5. Means of discoloration as influenced by antifungal treatment and cultivar for three storage temperatures.

	Storage Temperature (°C)		
	20	30	40[£]
Antifungal			
Control	12.2a[*]	27.1a	88.2
Natamycin	9.4ab	23.6a	82.2
Sodium chloride	6.3b	9.3b	41.6
Cultivar			
Roy J	11.2a	26.0a	70.9
Titan	11.3a	19.6ab	75.4
XL753	5.5b	14.5b	65.7

[£]Analysis of variance, and therefore means separation tests, were not performed for the 40 °C temperature due to significant non-normality of discoloration data at that temperature.
*Means for antifungal treatment or for cultivar under a given temperature followed by different letters were significant at the 0.05 probability using Tukey's Honestly Significant Difference mean comparison test.

Table 6. Means and standard deviations (parenthesis) of water activity (a_w) by moisture content (MC) and antifungal treatment.

	Mean of water activity		
Treatment	16% MC	21% MC	Combined over MC
Control	0.764 (0.06)	0.899 (0.04)	0.832 (0.08)
Natamycin	0.783 (0.04)	0.899 (0.03)	0.841 (0.06)
Sodium chloride	0.726 (0.01)	0.743 (0.01)	0.734 (0.01)

Table 7. Summary of stepwise selection scores for total mold count and partial correlation coefficient (r) and probability (p) value for temperature, moisture content, duration, antifungal treatment, and cultivar with total mold count.

Effect	Chi-Square score	p value
temperature	64.9	<0.001
moisture content	5.43	0.020
duration	5.32	0.021
temperature by duration	5.29	0.022

Effect	r	p value
temperature	-0.600	<0.001
moisture content	-0.208	0.002
duration	-0.195	0.004
antifungal*	0.072	0.296
cultivar*	0.059	0.392

*Antifungal treatments were coded as -1, 0, and 1 for control, natamycin, and sodium chloride, respectively. Cultivars were coded as -1, 0, and 1 for Roy J, Titan, and XL753.

Table 8. Mean separation of total mold count (log 10 CFG/g) by storage temperature, moisture content, storage duration, and storage temperature by storage duration.

Main effects[t]								
Storage Temperature (°C)			Moisture content (%)		Storage duration (week)			
20	30	40	16	21	4	8	12	16
4.57a	3.85b	3.39c	4.07a	3.80b	4.20a	3.82b	3.94ab	3.78b

Interaction effects for temperature by week[*]												
@20 °C				@30 °C				@40 °C				
4	8	12	16	4	8	12	16	4	8	12	16	
5.00a	4.56ab	4.29b	4.41b	4.35a	3.73b	3.84ab	3.46b	3.25a	3.17a	3.69a	3.47a	

[t]Mold means in columns below either storage temperature, moisture, or storage duration with different letters were significantly different at $p<0.05$ using Tukey's Honestly Significant Difference mean comparison test.
[*]Mold means for temperature by week interaction in columns below a given temperature having different letters were significantly different at $p<0.05$ using Tukey's Honestly Significant Difference mean comparison test.

Table 9. Means and standard deviations (parenthesis) of total mold count for rice cultivars by specific antifungal treatment and when combined over all treatments.

	Mean of total mold count (log 10 CFU/g)[*]		
Treatment	XL753	Roy J	Titan
Control	3.94 (1.19)	4.15 (0.80)	3.98 (0.92)
Natamycin	3.87 (0.57)	3.87 (0.66)	3.88 (0.96)
Sodium chloride	3.86 (0.91)	3.92 (0.70)	3.95 (0.60)
All treatments	3.89 (0.91)	3.98 (0.72)	3.94 (0.83)

[*]Means were not significantly different between antifungal treatment or cultivar.

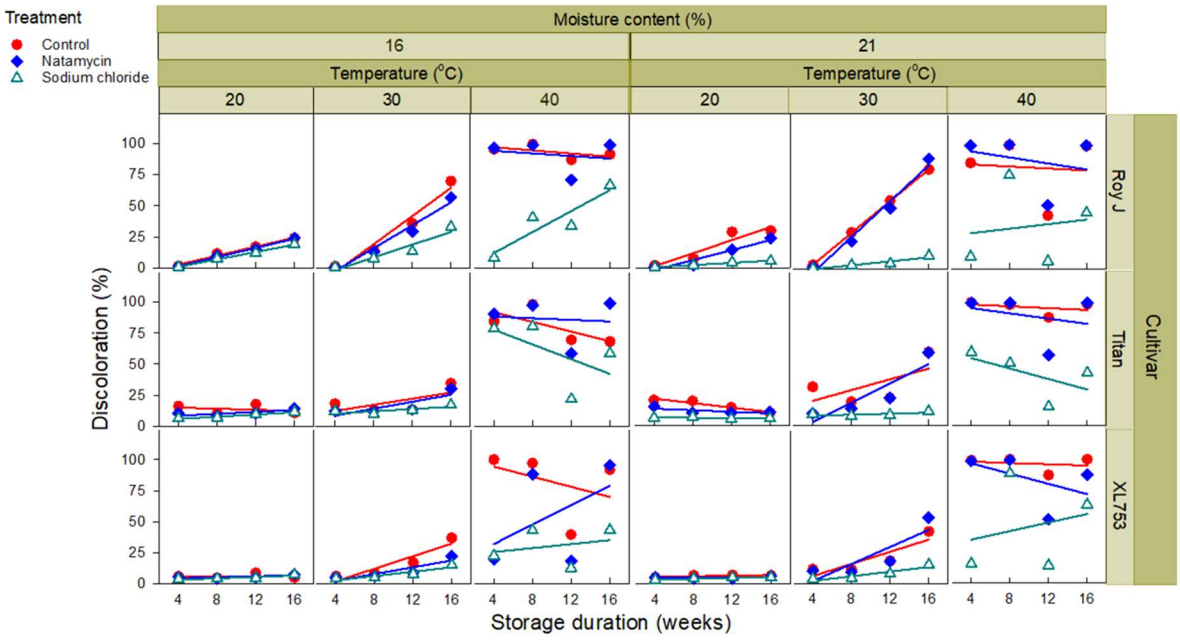

Figure 1. Discoloration of rice cultivars for treated and non-treated rice samples stored for 16 weeks at two moisture contents (%, wet basis) and three temperatures. Lines in each frame are linear regression curves of discoloration as a function of week.

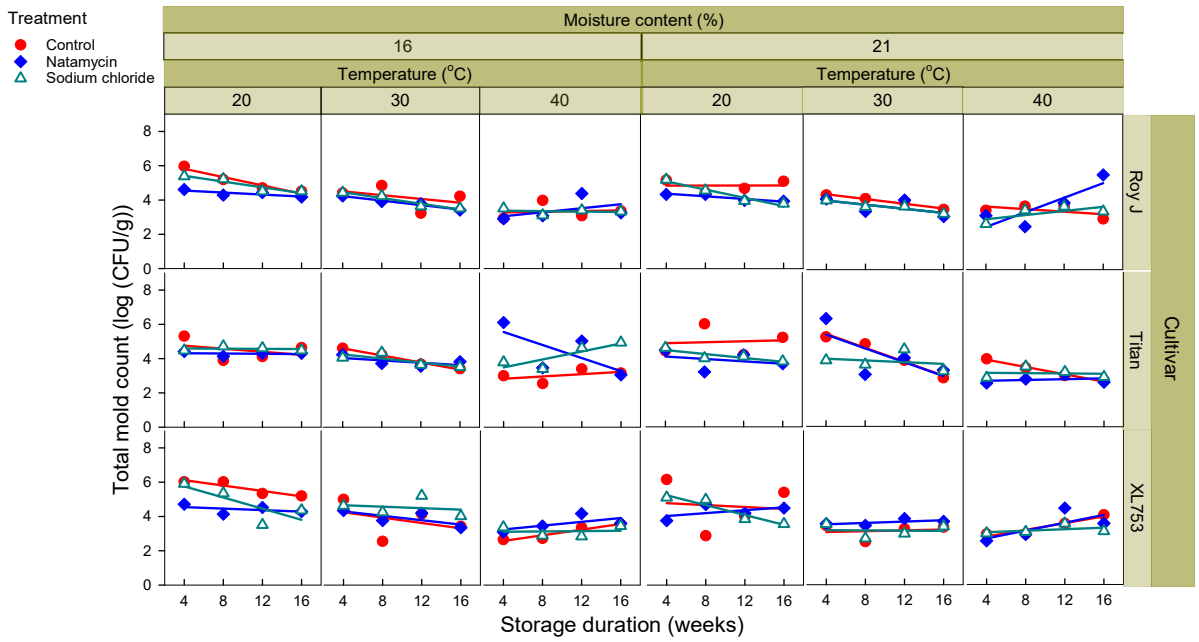

Figure 2. Total mold count of rice cultivars for treated and non-treated rice samples stored for 16 weeks at two moisture contents (%, wet basis) and three temperatures. Lines in each frame are linear regression curves of total mold count as a function of week.

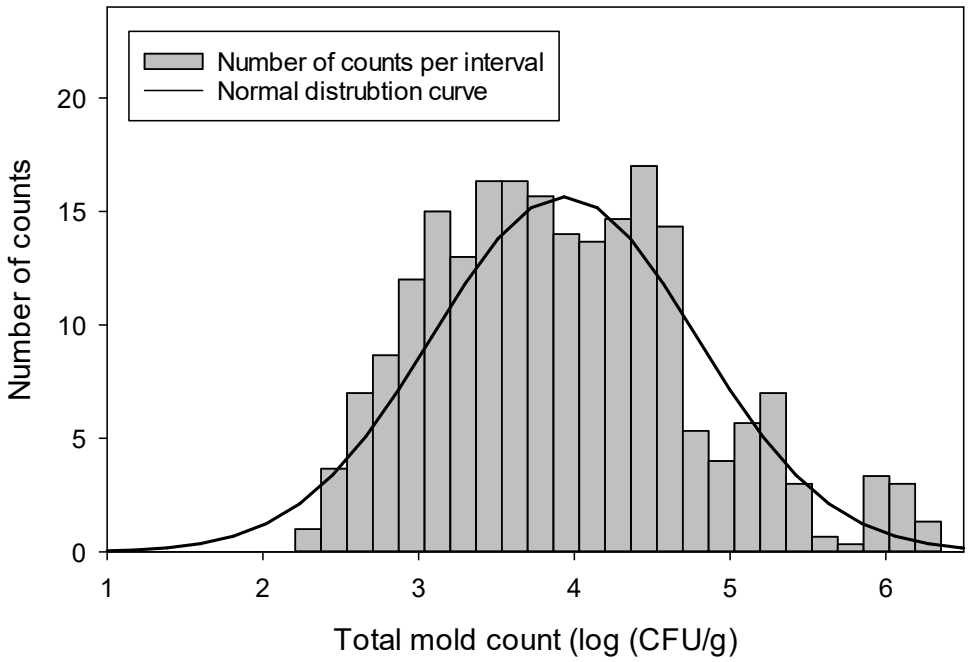

Figure 3. Frequency distribution and derived normal distribution curve of total mold count considering the entire experimental data set having a mean and standard deviation of 3.94 and 0.82 log 10 CFU/g, respectively.

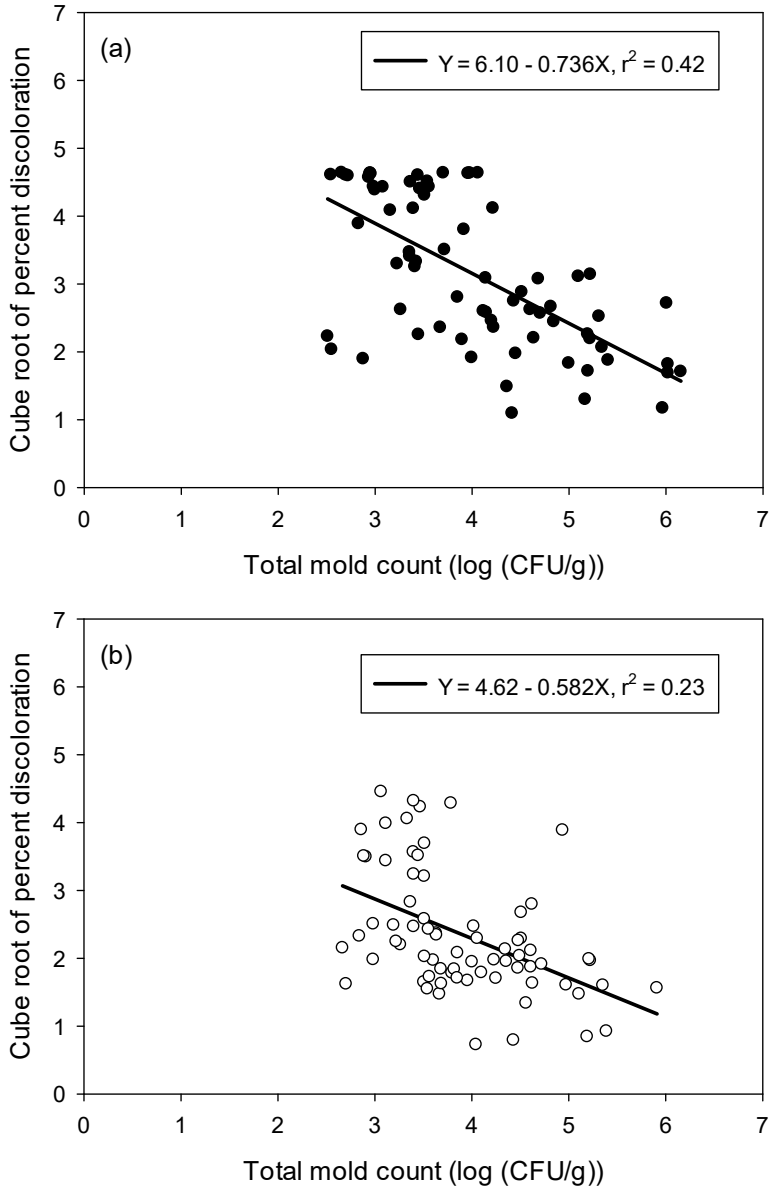

Figure 4. Cube root percent discoloration as a linear function of total mold count for data only including the (a) control treatment and (b) sodium chloride treatment. Discoloration and total mold count were regressed over experimental identical conditions i.e., same temperature, MC, duration, and cultivar. Linear regression results are in the legends for each treatment.

Chapter 3: Physical Integrity of Long-grain Hybrid, Pureline, and Medium-Grain Rice Kernels as Affected by Storage Conditions

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Abstract

Rice kernel physical integrity directly correlates with rice milling yield and quality. In this study, the impact of storage conditions on rice kernel physical integrity was examined by assessing changes in head rice yield (HRY) and kernel microstructure. Long-grain hybrid (XL753), long-grain pureline (Roy J), and medium-grain (Titan) rice were stored at different storage moisture contents (16 and 21% MCs, wet basis), storage temperatures (20, 30, and 40 °C), and storage durations (4, 8, 12, and 16 weeks); the samples were also treated with antifungals, sodium chloride and natamycin. Results showed that kernel physical integrity was highly dependent on the rice storage environment. At the same storage conditions, the physical integrity of medium-grain cultivar was significantly higher than that for the two long-grains. Generally, the long-grain hybrid rice exhibited more resilience to breakage than pureline under the same storage conditions. An average of 4% reduction in HRY was seen for the three cultivars when 16 % MC rice was stored at 40 °C compared to at 20 °C; however, the HRY reduction was 12% for rice stored at the highest MC (21%). Decreases in kernel physical integrity were also associated with unique microstructural changes in rice kernels. Sodium chloride treatment of rice kernels significantly and negatively impacted their physical integrity during storage compared to natamycin treatment and untreated control samples. Multiple regression models, developed for each cultivar, were applicable for predicting changes in rice kernel physical integrity as a function of studied storage conditions and fungal treatment.

1. Introduction

Proper post-harvest processing and storage of rice are essential to maintain rice kernel physical integrity. Unlike most grains that are ground, rice is mainly consumed as intact kernels. The degree of the rice kernel physical integrity correlates to the rice milling yields, quality, and hence the market value. During milling, rice kernels get exposed to abrasive and shear forces. Typically, less integral and weak kernels break easily during the milling process. Literature has reported that rice kernel physical integrity may be influenced by intrinsic factors related to the rice cultivar type, and extrinsic factors, which includes pre-harvest and post-harvest storage conditions (Daniels et al., 1998; Lanning and Siebenmorgen, 2011). For example, harvest moisture content (MC), nighttime harvest air temperature, and drying and storage treatments all have shown an impact on rice kernel physical integrity as evaluated through head rice yield (HRY) measurements.

In order to maintain rice kernel physical integrity (for optimum HRY), most rice producers prefer to use on farm in-bin systems. These systems dry the rice very gently by using low air temperatures (usually ambient to 10 °C above ambient). However, challenges to maintain the physical integrity of rice kernels during the in-bin drying process still exist (Atungulu et al., 2019). For example, during rainy or high humidity conditions, the top layers of rice in bins with underdesigned aeration systems (i.e. less than 1 cfm/bu airflow; grain depth > 22', high harvest MC >21%, and also without heater) may for prolonged periods remain at the high harvest MC and temperature. In the hot humid summer grain harvest season, the grain temperature may easily rise up to 40 °C; such conditions may lead to excessive fungal growth rate in the grain mass (Mohammadi Shad and Atungulu, 2019). Excessive fungal growth rate and contamination of the grain have been blamed for a decline

in rice kernel physical integrity and loss in desirable milled rice attributes (Haydon and Siebenmorgen, 2017; Atungulu et al., 2018; Shad et al., 2019).

Christensen and Kaufmann (1965) studied and reported on the impacts of storage conditions on rice physical integrity. Stored rice MC, temperature, storage duration, and interactions among these factors affect HRY (Pearce et al., 2001; Pereira et al., 2008; Haydon and Siebenmorgen, 2017). For example, HRY decreased with increased storage duration at high MC, but did not decrease with duration at low MC (Trigo-Stockli and Pederson, 1994). Pearce et al. (2001) showed 3-way interaction effects occurred for HRY at different MC, temperature, and storage duration. Moreover, the HRY varied among different rice varieties due to differences in milling properties.

Lanning and Siebenmorgen (2011) reported that differences in thickness of bran layer and chemical composition of bran or embryo are associated with variation in milling attributes. Some cultivars may require shorter or longer milling duration to achieve the same milling degree in terms of surface lipid content and color. Pureline rice cultivars generally were shown to have a smaller reduction in surface lipid content (SLC) than hybrid rice cultivars when milled for durations less than 20 seconds (Lanning and Siebenmorgen, 2011). Pereira et al. (2008) also reported differences in the milling attributes between pureline and hybrid long-grain cultivars.

Rice kernel structural modification during storage may provide crucial information to predict and preserve the rice kernel physical integrity and milling quality. Scanning electron microscopy (SEM) has been used to study changes in rice structure (Venkatesh Babu et al., 2018). The environmental scanning electron microscopy (ESEM) has also been used to provide three-dimensional visualization of grain microstructure at very high resolution (Venkatesh Babu et al., 2018).

Literature suggests that application of antifungals may preserve the physical integrity of stored rice kernels. Mohammadi Shad and Atungulu (2019) suggested the use of sodium chloride and natamycin as antifungals; the antifungals inhibited rice discoloration and helped to maintain good HRYs. While many of the cited studies have described the effects of storage environment on HRY, they typically focused on only two or three storage parameters; chiefly MC, temperature, and storage duration.

This study was conducted to expand understanding of how rice physical integrity varies with storage conditions, rice cultivar, and microbial activity. Three contemporary rice cultivars were examined and changes in HRYs documented. The ESEM was used to determine microstructure changes associated with studied variables.

2. Materials and Methods

2.1. Sample Procurement and Storage Conditions

The rice cultivars used in the study comprised two long-grains, a hybrid (XL753) and a pureline (Roy J), and one medium-grain (Titan). All lots were harvested at approximately 21-22% MC (MCs in this study are expressed on a wet basis (w.b.) unless otherwise stated). The Roy J and Titan rice were grown at the University of Arkansas System Division of Agriculture, Pine Tree Research Station, in Colt, Arkansas. The cultivar XL753 was grown at a commercial field owned by Florenden Farms in Burdette, Eastern Arkansas. Immediately after harvest, the rice samples were temporarily stored in a walk-in cooler set at 4 °C. Prior to experiments, samples of each cultivar lot were cleaned using a dockage tester (MCi Kicker Laboratory Grain Cleaner, Mid-Continent Industries, Inc., Newton, KS.). Rice samples were then spread on tarps and air dried at 25 ± 3 °C and 60 ± 5% relative humidity (RH) to achieve the target storage MCs, 16 and 21% MC. The rice

samples were mixed periodically to provide uniform drying and reached 16% MC w.b after 72 hours and 21% MC w.b. after 24 hours. These MCs represent the lower and upper bounds of harvest MCs typically witnessed in rice drying operations.

The rice MC was measured using a moisture meter (AM 5200, Perten Instruments, Hägersten, Sweden). The MC levels were verified by drying two, 15-g samples, from each cultivar, in a 130 °C oven (1370FM, Sheldon Mfg. Inc., Cornelius, OR) for 24 h (Jindal and Siebenmorgen, 1987). Control samples (at 0-week, having no difference in storage temperature or antifungal treatment) were taken for HRY analysis. One-third of the remaining samples at each MC level of each cultivar lot were mixed with sodium chloride at a ratio of 30% w/w, another one-third with natamycin at a ratio of 0.46% w/w, and the rest of the samples were untreated. 600-g samples of each rice cultivar at each MC and antifungal treatment (including control) were then transferred into labeled quart (0.95 L) glass Mason jars. The jars were then closed with an aluminum cap and sealed airtight with Parafilm M (Bemis Company, Inc., Neenah, WI, U.S.). The air-tight jars were stored in incubators set at 20, 30 or 40 °C, for 16 weeks. Every 4 weeks one jar of each cultivar/MC/temperature/antifungal treatment combination was removed for analyses.

2.2. Milling Quality

The rice sample was dried on a metal screen in a chamber with air conditions maintained at 25 ± 3 °C and 57 ± 5% RH by a temperature and relative humidity-control unit (AA5582, Parameter Generation and Control, Inc., Black Mountain, NC) to a MC of 12.5%. The HRY was determined in duplicate as follows: two 150 g sub-samples of each dried rough rice sample were measured for each storage treatment. Then, the samples were first dehulled using an impeller sheller (Model FC2K rice huller, Yamamoto Co., Ltd., Yamagata, Japan). Next, the resultant brown rice sample was milled using a laboratory mill (McGill #2, Rapsco, Brookshire, TX). The milling

machine had a 1.5-kg mass placed on the lever arm, 15 cm from the centerline of the milling compartment. The milling process removed about 90% of the surface lipid content (SLC) of brown rice to optimize HRY and maximize rice storability. Therefore, milling durations were selected for each cultivar to attain a head rice SLC of 0.4%, as measured by near-infrared reflectance (NIR) spectroscopy (DA 7200, Perten instruments, SE-141 05 Huddinge, Sweden); rice cultivars were milled at four durations of 15, 30, 45, and 60 s. After milling and sorting, the head rice samples at the mentioned durations were analyzed by NIR to measure SLC. Then, the required milling durations to achieve SLC of 0.4% for each were determined based on the relationships between milling durations and resulting SLC. To obtain 0.4% SLC, the milling durations were 15 s for Roy J, 27 s for XL753, and 35 s for Titan.

After milling, the sub-samples were aspirated to remove excess bran. Then, the broken rice kernels were sorted. The milled rice was sized on a laboratory-scale rice sizing shaker (Model 61, Grain Machinery Manufacturing Corp., Miami, FL) to collect the head rice. The head rice was considered as the milled kernel that has at least ¾ the length of whole milled kernel (USDA, 2009). The HRY was calculated as the mass percentage of the initial rough rice sample (150 g) remaining as head rice using the following formula:

$$\text{Head Rice Yield (HRY)} = \left(\frac{\text{Head rice mass}}{\text{Rough rice mass}}\right) \times 100$$

2.3. Environmental Scanning Electron Microscopy (ESEM)

Milled rice kernels were used for ESEM study. Five rice kernels were randomly examined from each storage combination. The rice kernels were analyzed intact or cross-section wise. To generate cross-sections, the kernels were manually fractured using a sharp metal blade. The intact and sectioned samples were processed for the ESEM (Model

Philips-XL30, Holland) microstructural evaluation. Before samples being placed on the Peltier stage of the ESEM, the rice samples were directly mounted on 9 mm diameter self-adhesive carbon discs attached to stainless steel stubs and sputter-coated with approximately 6 nm gold using Emitech-SC7620 sputter coater (SC7620 Mini Sputter Coater/Glow Discharge System).

2.4. Statistical Analysis

The duplicated HRY values that were determined for each jar were averaged, and the coefficient of variation (CV) was calculated for each pair. The HRY differences for paired samples were small with a mean for CVs of only 0.012 over all 216 paired samples. All statistical analyses were performed using the averaged data for each jar. Statistical analyses for HRY were performed using SAS 9.3 software. Stepwise regression (Neter et al., 1990) was used to determine the set of independent variables that represent the "best" set of predictors for HRY. Using the PHREG procedure in SAS with the 'stepwise' option, stepwise regression was run containing all single effects (i.e., storage temperature, MC, duration, antifungal treatment, and cultivar), and all 2nd to 5th order interaction terms. Selection of the single effects and interactions included in the best set were made in the procedure one-step-at-a-time. During the stepwise procedure, individual chi-square scores determined which one of the independent variables had the highest bivariate correlation with HRY. The procedure continued by selecting additional variables based on the next highest chi-square score and so on. For variables added to the best set, the chi-square statistic had to be significant at a p-value <0.05. Because cultivar had the highest effect on HRY, the data for each cultivar were evaluated in separate stepwise regression procedures to find the best set of explanatory variables for each cultivar. Once final best set of significant variables were selected in the stepwise regression procedures, further analyses of HRY treatment means were compared by computing exact p-values for pairwise mean differences (Agresti, 1992) using the SAS

NPAIR1WAY procedure. This nonparametric test procedure, valid for data for any distribution, computed the exact p-value based on Wilcoxon ranked scores. Analysis of multiple linear regression to develop HRY prediction models was performed using the REG procedure in SAS.

3. Results and Discussion

3.1. Primary Effects on Head Rice Yield

The percent head rice yield is shown graphically for each cultivar by storage temperature, duration, MC, and antifungal treatment (Figure 1). It is noted that the HRY for the medium-grain (Titan) appears to be somewhat higher than that for the other two cultivars at given treatment levels. The Figure also indicates lower HRY for rice treated with sodium chloride than that for control or natamycin and that the HRY for that treatment also was more affected by higher MC and longer duration of storage. However, differences due to temperature appear less notable than other effects. The stepwise regression revealed that all five single-order effects significantly influenced HRY but the most important was cultivar followed in importance by antifungal treatment, duration, MC, and temperature. Also, four, two-way interactions were selected with "cultivar by storage duration" the most important and then "antifungal treatment by MC", "MC by temperature", and "MC by duration". Thus, all other two-way and higher order interactions were deemed not to have significant effects on HRY. The following sections describe the main effects and interactions in detail.

3.2. Cultivar and "Cultivar by Storage Duration" Interaction

Figure 2 illustrates the HRY changed with storage duration for each of the three cultivars. The higher rice kernel physical integrity was observed in medium-grain cultivar,

Titan, as the mean HRY for Titan was significantly greater than that for both the long-grain hybrid (XL753) and long-grain pureline (Roy J) cultivars. Mean HRY for cultivars, averaged for all storage durations, were 57.9, 48.4, and 45.8% for Titan, XL753, and Roy J, respectively. The overall mean of HRY for XL753 was significantly greater than for Roy J; however, the HRY means between these two cultivars were not significantly different at either 8 or 16 weeks of storage. For Titan, head rice yield declined significantly after 8 weeks of storage (p-value = 0.0445) and continued to decline with duration to 54.5% at 16 weeks. In contrast, mean HRY for Roy J was highest after 8 weeks of storage (47.0%) but significant differences in HRY for this cultivar did not occur with storage duration until 16 weeks (44%). HRY differences with duration for XL753 were not significant for 4, 8, 12 weeks of storage and varied from 48.3% (8 weeks) to 50.1% (12 weeks). For XL753, storage for 16 weeks decreased HRY (mean of 44.9%), which was significantly different than HRY means for all other weeks.

The stepwise regression (Table 1) shows that the HRY response was dominated by differences in the storage MC for the long-grain pureline (Roy J). However, for the long-grain hybrid (XL753), HRY was most highly affected by storage temperature, while storage duration had the greatest effect on the medium-grain (Titan).

Sidhu et al. (1975) determined that rice physical integrity and thereby HRY were different based on rice variety and the degree of milling; where the thick variety in their study, IR 8, gave the highest milling yield. Also, Palman 579, a medium-fine variety was found to have higher integral kernels and resistance to breakage during milling (Sidhu et al., 1975). Haydon and Siebenmorgen (2017) also reported that HRY was significantly dependent on rice cultivars and harvesting time. Hybrid rice varieties like XL753, used in the current study, are usually more resistant to pests and diseases and have a greater yield than pureline varieties (IRRI, 1988).

During rice aging, physical characteristics of rice grains, including HRY, will be affected (Keawpeng and Venkatachalam, 2015). The increased HRY after 8 weeks for Roy J and after 12 weeks for XL753 may be related to the aging process for long-grain rice. Daniels et al. (1998) reported that HRY increased with storage duration (studied from 3 to 18 weeks) for both pre-dried (16.5-17.8 % MC) and dried (12.5% MC) rice samples stored at temperatures of 4, 21, and 38 °C. The most profound increases of rice HRY in the study by Daniels et al. (1998) occurred during the first 12 weeks of storage, which is supported by our results for the long-grains. A study by Pereira et al. (2008) also found increased HRY with increased storage duration, especially for a long-grain rice cultivar (Wells, 12.5–12.9% MC). In that paper, the HRY increased after 8 weeks storage at 30 °C; contrary to our results, they reported that HRY increased after 16 weeks of storage at all temperatures (4, 21 or 35 °C). In the current study, a significant decrease in HRY with storage duration at 16 weeks was found for the medium-grain, Titan.

3.3. Antifungal, Storage Moisture Content, Storage Duration, and Their Interactions

The differences in rice kernel physical integrity as related to HRY due to both antifungal treatment and rice MC are observed in Figure 3. At zero storage duration, the mean of HRYs were nearly identical at 16 and 21% MC, 53.6 and 53.4%, respectively. Afterwards, storage of rice particularly at higher MC resulted in less integral rice kernels; the overall mean HRY for samples stored at 16% MC was significantly reduced from 52.9% to 48.5% when rice was stored at 21%. The mean HRYs of control (mean = 52.1%) and natamycin treated samples (52.2%) were not different; but both were significantly greater than the mean HRY for rice samples treated with sodium chloride (47.8%). The interaction of antifungal and MC had a significant impact on HRY (Figure 3). At 16% MC, the

differences in mean HRY for the treatments were not significantly different. However, at 21% MC, there was no difference between the HRY of control and natamycin treated samples. The mean HRY for sodium chloride treated samples was significantly less than control (p-value < 0.0001). The reduction in HRY for the sodium chloride treatment compared to control was similar for all three cultivars; where average HRY reduction was 9% in Titan, 10% in Roy J, and 6% in XL753.

Diminished physical integrity and thereby weakness in rice kernels stored at higher MC was also reported in other studies. Pearce et al. (2001) reported that rice stored at 10% MC had higher HRY than rice stored at higher MCs (12 or 14%). Trigo-Stockli and Petersen (1994) found that HRY was significantly reduced for storage MC greater than 18%, including at 22 and 26% MCs. However, in contrast to current results, Haydon and Siebenmorgen (2017) reported no significant changes in HRY during storage of rice at MC of 12.5 to 21% MC for a long-grain rice stored over 16 weeks of storage. In the current study, however, mean HRY for control samples did not change significantly with MC, which would agree with the non-effect result of MC on HRY for untreated rice cultivars by Haydon and Siebenmorgen (2017).

In the current study, a significant interaction between MC and storage duration (Table 2) was found. Analyses of mean HRY for the "storage MC by storage duration" interaction indicated there was no change in mean HRY until the rice had been stored for 16 weeks for storage at either 16 or 21% MC (Table 2). Trigo-Stockli and Petersen (1994) also found that HRY decreased with storage duration, but they did not indicate it interacted with MC level.

The lowest HRY in the current study occurred in rice samples treated with sodium chloride stored at 21% MC and can be related to low water activity; where the mean of water activity was 0.74, as reported by Mohammadi Shad and Atungulu (2019). The low water activity was presumed to increase salinity stress, thereby making the sodium-chloride treated rice grains weaker during

the milling process. The sensitivity of rice milling quality to sodium chloride was reported in a study by Dionisio-Sese and Tobita (1998), although the salinity stress was studied in the field, prior to harvesting.

3.4. Storage Temperature Effects on Head Rice Yield

Compared to storage at 20 °C at the 16% MC, HRY for cultivars were reduced by an average of 17% for the 40 °C and 21% MC storage condition (Table 2). The storage temperature and MC had a significant interaction effect on the HRY. When stored at 16% MC, mean HRY did not change significantly regardless of storage temperature (Table 2). When stored at 21% MC, HRY decreased with increased temperature and was significantly reduced at the 40 °C temperature. Likewise, Borlagdan et al. (2017) reported that HRY was maintained better when paddy rice was stored at lower temperatures, such as -5 and 18 °C, than at 28 °C through 8 weeks of storage. That study also found a significant interaction effect between MC and temperature on HRY; the HYR was highest at the 18% MC at the lowest temperature of -5 °C, but it was highest at the 14% MC when temperature increased to 28 °C. When both temperature and MC are high, broken rice grains increased during the rice milling process (Borlagdan et al., 2017). Conversely, Pereira et al. (2008) found that the HRY was significantly higher for long-grain rice (Wells) stored at 35 °C after 16 weeks compared to a non-stored control treatment and to Wells stored at 4 and 21 °C for all weeks. However, in that study, HRY for Jupiter (a medium-grain rice) was not significantly changed by storage temperature until storage duration was increased to 16 weeks (Pereira et al., 2008).

3.5. Head rice yield prediction models

Table 3 shows the multiple regression results for HRY as a function of storage and

treatment effects for the three cultivars. The highest coefficient of determination, R^2, of the three regression models was for the medium-grain rice, Titan (R^2 =0.80). This result was not unexpected since the effects of storage conditions, particularly temperature and duration, strongly influenced HRY for Titan than the two long-grain cultivars. For Roy J and XL753, the effects in the regression models accounted for ≈67% of the variation of HRY. The model residuals (i.e., predicted HRY minus measured HRY) were found to be normally distributed for all three cultivars. The root mean square error (RMSE) as shown in Table 3 varied from 2.3% for Titan to 2.8% for Roy J, indicating very reasonable predictions of HRY for the range of measured HRY in the experiments.

The predicted and modeled data for the three cultivars (Figure 4) illustrate similar prediction performance for the Roy J and XL753 cultivars, though the independent variables were not the same for these two cultivar models. The correlation between predicted and measured HRYs for Titan had a steeper slope than for Roy J and XL753 (Figure 4).

The regression model coefficients for each cultivar could be used to assess HRY for other combinations of storage condition of MC, temperature, and/or duration within the ranges of those measured in the experiment. For example, an estimate of HRY for long-grain hybrid XL753 could be made for a MC of 19%, a temperature of 27 °C, and at a storage duration of 10 weeks. Computing HRY for those storage conditions for rice having no antifungal treatment would give an HRY of 51.2%. For comparison with modeled HRY in the current study, data of Haydon and Siebenmorgen (2017) showed that HRY for a non-antifungal treated long-grain hybrid rice (CL XL745) at those same storage conditions varied from ≈50% to 58%. However, if treated with sodium chloride under those same storage conditions, estimated HRY for XL753 would decrease to 46.8%. Comparably, other researchers have modeled HRY of rice as a function of storage conditions. The optimization process of rice storage and aging was investigated by Rayaguru et al.

(2011) for long-grain Basmati rice. They used a similar regression approach to predict the optimum temperature, RH, and aging period to improve cooking and organoleptic characteristics of rice grains. The authors found HRY for long-grain Basmati could be successfully modeled by the duration, temperature, MC and their interactions (Rayaguru et al., 2011). Abud-Archila et al. (2000), combined heat and mass balances in a simulation model to predict HRY given dryer storage temperature and MC for an Ariete variety rice grown in France. In that paper, experimental data were used to calibrate the model for a range of temperatures between 40 to 80 °C and a range in RH between 3 and 60%.

3.6. Impact of storage treatments on microstructure of stored rice kernels

The ESEM images as depicted in Figure 5 (A, B, C) show a smooth outer surface on rice kernels when stored at low temperature and MC. However, Figure 5 (C, D, E) shows cracks and fungal spots on kernels when stored at higher temperatures > 30 °C, i.e., 40 °C, and 21% MC. For the latter images, roughness of the surface with clearly visible fine cracks or fissures within the kernels was evident. The high storage temperature and MC conditions that showed increased fungal spots and cracked rice were also found to reduce rice kernel physical integrity and HRY which coincided with significantly increased rice discoloration (Mohammadi Shad and Atungulu, 2019; Mohammadi Shad et al., 2019). In contrast, in cases where antifungals (sodium chloride) significantly reduced fungal count and discoloration for all three rice cultivars, the HRY was also reduced.

In the ESEM micrographs shown in Figure 6, it was also possible to see the presence of fungal hyphaes penetrating the rice kernels stored at temperatures of 30 °C and 40 °C and at 21% MC which might diminish the rice physical integrity and HRY. These were not present at 20 °C (not shown). These structural differences are due to the

susceptibility of moist rice kernels to be contaminated by various fungal pathogens that may attack rice earlier in field or during storage. Interestingly, the rice kernels which showed fungal hyphaes penetration were also associated with highly discolored rice (Mohammadi Shad and Atungulu, 2019). The consequences of the high temperature and high MC storage environment were fracturing of rice during the milling process. The current results agree with Siripatrawan and Makino (2015), who found fungal hyphae (mycelium) over rice kernel surface when brown rice kernels were inoculated with Aspergillus oryzae and stored for 10 days at 30 °C and at high humidity of 85% RH. They also reported discoloration and fuzzy appearances on rice grains that had a network of fungal hyphae (Siripatrawan and Makino, 2015). The starch in rice kernels provides nutritional support to fungi. According to Bauriegel et al. (2011) and Jackowiak et al. (2005) organic foods are capable of providing substrates for fungal growth on the surface of food. The ESEM scan of brown rice showed that mycelial growth was more evident on the rice embryo than endosperm because of the higher nutrient contents such as proteins, minerals, and vitamins (Siripatrawan and Makino, 2015). Based on another study, the optimum temperatures for fungal growth were found to be between 25 and 30 °C, and at this temperature, values of fungal activities were around 10 times higher than at 0 °C (Pietikainen et al., 2005). Another study showed significantly more Aspergillus growth at a temperature of 42 °C than at 10 °C, and higher Penicillium at 32 °C than at 5 °C (Ayerst, 1969).

Therefore, both fungal attack and rice kernel discoloration in rice stored at higher MCs could deteriorate the physical integrity of rice kernels and thus lower the fracture strength which can adversely affect milling yields (Houston et al., 1957; Mohammadi Shad et al., 2019).

Rough rice stored at 21% MC and temperatures higher than 27 °C for 16 weeks were contaminated with visible fungi; the fungal contamination weakened the rice kernels causing more breakage during the milling process (Haydon and Siebenmorgen, 2017).

4. Conclusion

Three contemporary rice cultivars were examined to characterize the effects of variable storage moisture content (MC), temperature, and duration on kernel physical integrity as indicated by head rice yield (HRY) and kernel microstructure. The study also examined whether antifungal treatment could impact kernel physical integrity. Rice physical integrity as assessed by head rice yields during storage was significantly greater for 1) medium-grain rice (Titan) compared to long-grain hybrid (XL753) and pureline (Roy J), 2) rice at 16% MC than 21%, 3) rice at 20 and 30 °C than 40 °C temperature, and 4) rice stored between 4-12 weeks compared to that at 16 weeks. The worst storage condition in the study was for rice of MC of 21% stored at 40 °C for 16-weeks. The results suggest that HRY will not change significantly when rice is stored at 16% MC for temperatures less than 30 °C, and durations up-to and within 4 to 12 weeks. Antifungal treatments had a negative impact on HRY of all the three studied cultivars, particularly the sodium chloride treatment, which reduced HRY compared to control by 6 to 10 percentage points. Multiple regression models were developed for each of the three rice cultivars to predict HRYs based on storage conditions. The models provide a means to estimate HRY under other discrete storage conditions within the ranges of the experimental conditions. The study provided a connection between rice microstructural changes and milling characteristics following storage. It was revealed that rice microstructure was impacted by storage at 21% MC and temperatures higher than 30 °C. The deterioration of rice structure at the high temperature

corresponded with reduced HRY and increased kernel discoloration. These findings warrant further investigation to completely understand the impacts of storage condition on rice kernel structural changes during storage. The findings from this study provide useful information and support for rice farmers and industries regarding appropriate practices to be used to lessen deleterious effects on rice physical integrity that can occur in postharvest storage.

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7. Tables and Figures

Table 1. Summary of stepwise regression p-values for the effects on head rice yield for Roy J, Titan, and XL753 cultivars.

	Cultivar		
Effect	Roy J	Titan	XL753
Storage temperature	0.0487	< 0.0001	0.0005*
Storage moisture content	< 0.0001*	< 0.0001	0.0035
Storage duration	0.5070	< 0.0001*	0.0183
Antifungal treatment	0.0001	< 0.0001	0.0059
Antifungal by Moisture	0.0138	0.0004	0.0008
Temperature by Moisture	0.4494	0.0041	0.2641
Duration by Moisture	0.1044	0.1434	0.0193

[*] Indicates the highest-level p-value found for each cultivar, as based on Chi-Square Scores in the stepwise regression analysis. (Note that only interactions that were found significant for one or more cultivars are given in the table).

Table 2. Means of head rice yield (HRY, percent) by storage temperature, storage moisture content (MC, wet basis), storage duration, "storage moisture content by storage temperature", and "storage moisture content by storage duration".

Single effects [a]

Storage Temperature (°C)			Storage moisture content (%)			Storage duration (week)			
20	30	40	16	21	a	4	8	12	16
52.5a	51.3a	48.3b	52.9a	48.5b		52.3a	51.6a	51.2a	47.8b

Interaction effects for storage moisture content by storage temperature [b]

@16% MC			@21% MC		
20 °C	30 °C	40 °C	20 °C	30 °C	40 °C
53.9a	53.1a	51.7ab	51.2ab	49.5b	44.9c

Interaction effects for storage moisture content by storage duration [b]

@16% MC				@21% MC			
4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	12 weeks	16 weeks
54.0a	53.1ab	53.5a	51.1bc	50.7abc	50.0abc	49.0c	44.5d

[a] HRY means in rows below single effects of storage temperature, moisture, and storage duration were significantly different at $p<0.05$ using exact p-values computed for Wilcoxon two-sample rank sums tests.

[b] HRY means in rows below moisture content by temperature interaction and in rows below moisture content by duration interaction were significantly different at $p<0.05$ using exact p-values computed for Wilcoxon two-sample rank sums tests.

Table 3. Summary of head rice yield multiple regression model coefficients and statistics for Roy J, Titan, and XL753 cultivars (The multiple regression models are constrained within a storage temperature range between 20 and 40 °C, storage MC range between 16 and 21% (wet basis), and storage durations from 4 to 16 weeks).

	Cultivar		
Model coefficients	Roy J	Titan	XL753
Intercept	70.91	59.34	60.73
Storage temperature	-0.156	0.575	-0.196
Storage moisture content	-1.102	0.595	-0.202
Storage duration	n/a	-0.553	0.646
Antifungal treatment	9.50	7.51	10.05
Antifungal by Moisture	-0.648	-0.544	-0.644
Temperature by Moisture	n/a	-0.044	n/a
Duration by Moisture	n/a	n/a	-0.048
Summary of model statistics			
Coeff. of determination (R^2)	0.678	0.802	0.668
Root MSE (Percent)*	2.77	2.29	2.46
Coeff. of variation	6.05	3.96	5.04

n/a indicates not applicable since the particular effect was not included in multiple regression as based on the selection of effects from stepwise regression shown in Table 1.
*MSE is mean square error.

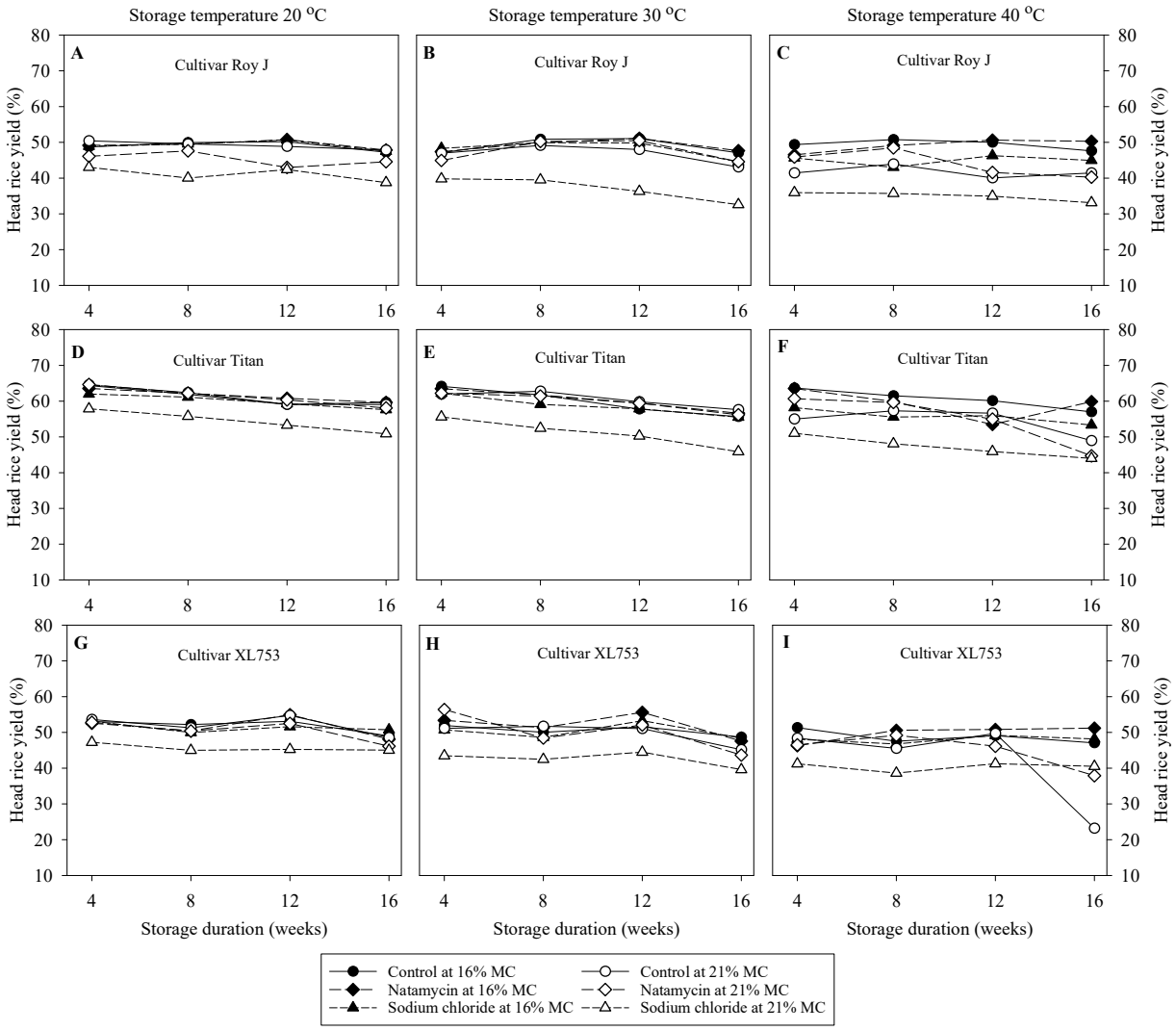

Figure 1. The percent head rice yield (HRY) for cultivars Roy J (A, B, and C), Titan (D, E, and F), and XL753 (G, H, and I) as affected by 16 and 21% storage moisture content (MC) and antifungal treatment (Control, Natamycin, and Sodium chloride) at indicated storage temperature (20, 30, and 40 °C) and storage duration.

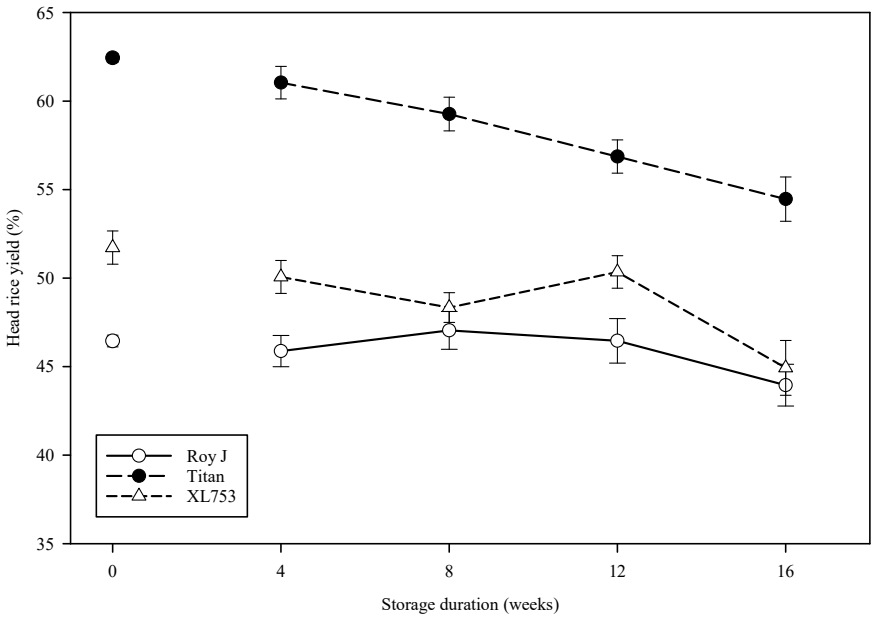

Figure 2. Head rice yield (HRY) for three rice cultivars as affected by storage duration. The HRY data are the means for cultivars at each week, where the error bars indicate plus and minus one standard deviation from the mean. Mean HRY data at zero time (pre-experiment) are also shown for each cultivar.

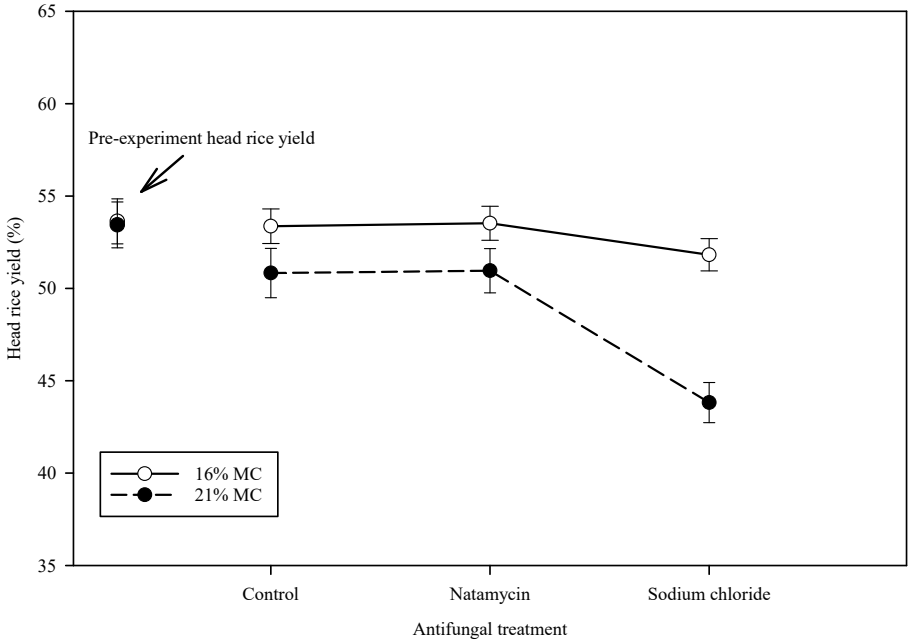

Figure 3. Head rice yield (HRY) for samples treated with antifungal as affected by storage moisture content (MC). The HRY data are the means for antifungal treatment at two MC levels, where the error bars indicate plus and minus one standard deviation from the mean. Mean HRY data prior to the experiment (zero time) are also shown for 16 and 21% moisture content (wet basis).

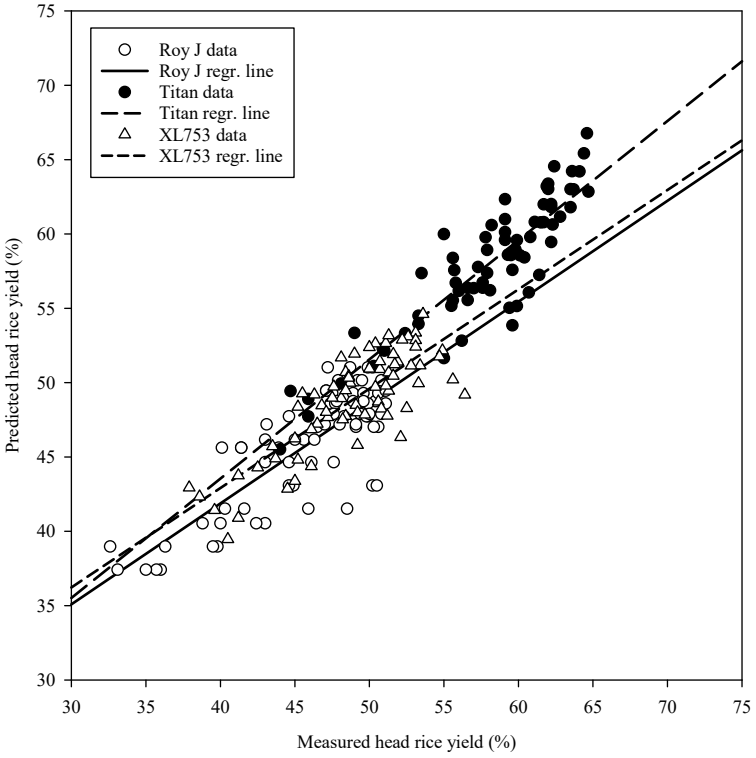

Figure 4. Predicted against measured head rice yield (HRY) for three rice cultivars. The predicted HRY for each cultivar are based on the multiple regression models of table 3 and, therefore, the prediction is constrained within a storage temperature range between 20 and 40 °C, storage MC range between 16 and 21%, and storage durations from 4 to 16 weeks.

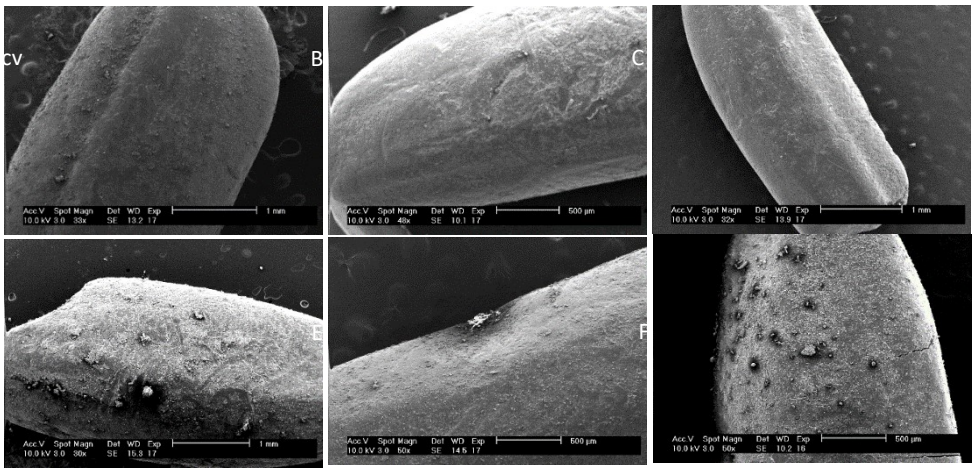

Figure 5. Environmental scanning electron microscopic (ESEM) images of outer surfaces of rice kernels Titan (A), Roy J (B), and XP753 (C); storage at low temperature, (≤30°C), and 16% moisture content (MC, wet basis); Titan (D), Roy J (E), and XP753 (F) stored at high temperature, (>30°C), and 21% MC (wet basis).

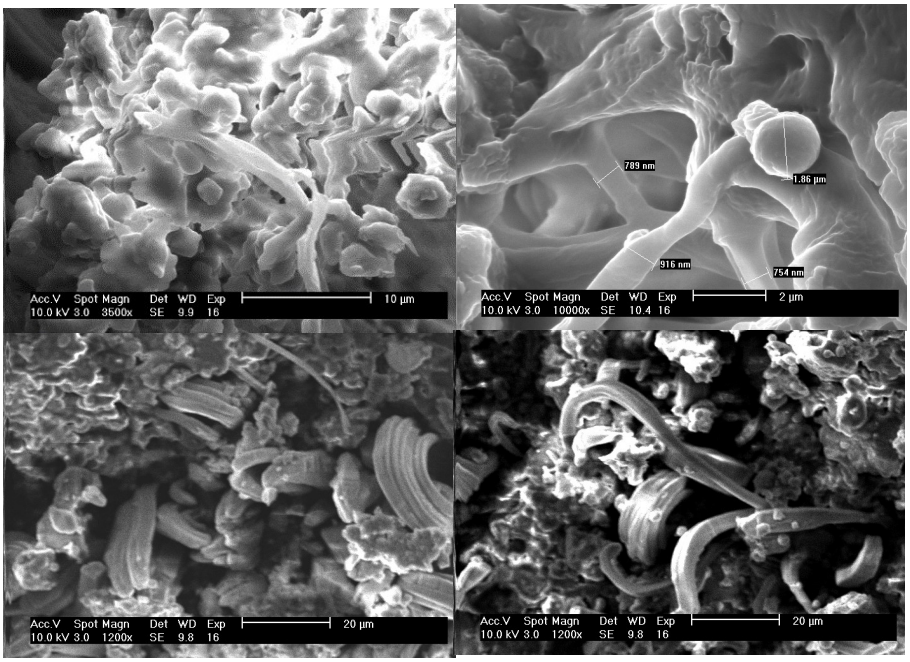

Figure 6. Environmental scanning electron microscopic (ESEM) images of rice kernels stored at 21% moisture content (wet basis) and temperatures >30°C.

Chapter 4: Identification of fungal population in stored rice using traditional and high throughput sequencing techniques

Mohammadi Shad, Z., & Atungulu, G. G.

Abstract

Rice preharvest and postharvest conditions can promote the growth of fungi in-field as well as in stored grains. Fungal growth in rice grains has been associated with low-quality rice grain, such as discolored rice grains. This study assessed the fungal composition on rice grains and its relationship with rice discoloration during storage. For these objectives, grain samples for three rice cultivars, including XL753, Roy J, and Titan at the high moisture content (21% w.b.), were used and stored at 20, 30, and 40°C. Before storage, the rice samples were divided into two treatments; non-treated control; and treated with the antifungal, sodium chloride, as a means to discourage fungi and maintain rice quality. Rice samples after four and 16 weeks of storage were analyzed for microbial diversity using conventional and metagenomics techniques. The study revealed that a diverse group of fungal genera were present on rice before storage, which changed in relative abundance during storage. Alternaria, Penicillium, Aspergillus, Nigrospora, and Fusarium were identified as the most persistent genera on rice samples. However, the relative abundance of these fungi changed considerably, influenced by storage conditions such as storage temperature and storage duration. For Titan, Aspergillus was found predominantly on rice samples stored at 20°C and 30°C compared to 40°C. However, the relative abundance of Aspergillus in non-treated Roy J rice samples was found in a greater percentage at 40°C than 20 and 30°C storage temperatures. The decreasing trend in relative abundances over storage temperatures was seen for Alternaria and Fusarium genera. Although sodium chloride was effective in reducing the relative abundances of Aspergillus and Penicillium, the presence of other fungi can produce pigments and

change rice color during storage, dependent on storage temperature, which also varies with specific rice cultivars. These results suggest that the development and implementation of particular fungal prevention strategies may be useful in reducing rice discoloration during storage to some degree.

However, consistent with previous findings presented in Chapter 2, microbe presence was not notably correlative to the observed discoloration under these experimental-study conditions.

1. Introduction

Rice is harvested at a high moisture content (MC) for optimum quality attributes such as maximum head rice yields. However, high MC rice undergoes quality degradations such as rice discoloration during prolonged drying and storage. Rice discoloration after harvest may also be affected by unknown factors that can happen in the field before harvesting, e.g., insect damage and pathogenic contamination (Haydon, 2016). Microbial contamination has been blamed for causing rice discoloration. Several studies listed in Table 1 have reported fungal contamination as the root cause of rice yellowing and other discoloration classes. As per these studies, different fungi have led to different color shades in rice (Table 1). The role of fungi on the discoloration of rough and milled rice was speculated by Quitco et al. 1982. The authors studied changes in sterilized and inoculated rough grain. Rice with different fungal species showed different patterns of discoloration (Quitco et al., 1982). The authors suggested that fungi could lead to discoloration of the grain directly by their metabolites, or indirectly, by increasing the grain temperature through respiration (Quitco et al., 1982).

In a study of milled rice, discoloration increased from 0-0.5% to 4.5-5.5% during storage (Philips et al., 1988). It was suggested by the authors that rice discoloration was related to the fungal contamination during the drying period, in which metabolite of fungi produced before drying led to yellowing of the grains during storage. In a later study by the same lead author

(Philips et al., 1989), it was found that rice of MCs more than 20% began heating-up after one day, and that visible fungal contaminations were detectable two days later; yellowing of the rice grains was reported after five days under these circumstances (Philips et al., 1989).

Rice discoloration was seen to be progressive in rice grains; starting from one point on the grain and then covering the whole surface of the grain (Borlagdan, 2008). Fungal contamination has been suggested as a cause of rice discoloration because discoloration in milled rice did not happen uniformly in all the grains; the argument is that if it was a biochemical reaction, it should have occurred in all the grains evenly and at the same pace (Borlagdan, 2008).

There is limited information to elucidate the primary fungal causes of discoloration of rice. It is reasoned that by knowing the specific fungal communities occurring in stored rice, how these change with storage conditions, and their effects on rice color, more effective prevention strategies could be developed. Such information would be beneficial to the rice industry's goals of minimizing fungal contamination and the associated color change during storage.

The objectives of this study are to identify fungi on rice during storage using the metagenomics technique and to elucidate relationships between the fungal genera associated with rice discoloration for different rice cultivars and storage conditions.

2. Material and Methods

2.1 Rice samples

Rice samples of three cultivars, XL753 as a long-grain hybrid rice, Roy J as a long-grain pureline rice, and Titan as a medium grain, were used for this study. All lots were harvested at approximately 21-22% MC (MCs in this study are expressed on a wet basis (w.b.) unless otherwise stated). The Roy J and Titan rice were grown at the University of Arkansas System Division of

Agriculture, Pine Tree Research Station, in Colt, Arkansas. The cultivar XL753 was grown at a commercial field owned by Florenden Farms in Burdette, Eastern Arkansas. Immediately after harvest, the rice samples were temporarily stored in a walk-in cooler set at 4 °C. Prior to experiments, samples of each cultivar lot were cleaned using a dockage tester (MCi Kicker Laboratory Grain Cleaner, Mid-Continent Industries, Inc., Newton, KS.). 600-g rice samples of both non-treated (defined as control) or treated with sodium chloride at a ratio of 30% w/w were transferred into labeled quart (0.95 L) glass Mason jars. The jars were then closed with an aluminum cap and sealed airtight with Parafilm M (Bemis Company, Inc., Neenah, WI, U.S.) stored at 20, 30, and 40°C for storage durations of 0, 4, and 16 weeks. The reason for using non-treated/control and sodium-chloride treated rice samples was due to the previous finding that non-treated rice samples had shown more discoloration during storage than those treated with sodium chloride (Mohammadi Shad and Atungulu, 2019).

2.2 Microbial analysis using conventional culture-based identification

A slide culture is the one method for observing sporulation characteristics of most fungi for identification purposes. This method is based on the morphology of fungi and its spore and mycelium characteristics. The potato dextrose agar (PDA) medium was used in culturing the fungi. To prepare the medium, 10 g of agar powder was dissolved in 1 L of distilled water and boiled. A 50 mg/liter chlortetracycline/chloramphenicol as antibiotics was added to media to inhibit bacterial growth. The media was sterilized at a temperature of 121°C for 15 min and then allowed to cool at room temperature to solidify. Suspicious rice kernels with fungal contamination observed by microscope were selected and placed on the agar media plates. Then, the plates were sealed well using parafilm and incubated at room temperature (25°C) for five to seven days allowing fungi to

grow with abundant spores. A maculating loop was flamed and allowed to cool and then it was used to separate individual colonies and Hyphal-tips from the fungi which were then transferred to PDA plates and sealed with parafilm. After five to seven days of incubation at room temperature (25°C), pure cultures of the isolated fungi were obtained for further investigation. The pathogens were initially identified morphologically on the basis of colony color, shape and size (Sharma et al., 2016). Later microscopic examination was performed to confirm the pathogens on the basis of mycelial growth (Kozel et al., 2014).

2.3 Metagenomic Analysis

DNA extraction, PCR amplification and sequencing

A 10 g sample of milled rice was mixed with 90 mL phosphate-buffered dilution water in a sterile stomacher bag and masticated. The solution was centrifuged at 10,000 × g for 10 mins at 4ºC. After centrifugation, the solution was discarded leaving a pellet at the bottom of the tube. Genomic DNA was extracted from the rice pellet using DNeasy Plant Pro kit (Geneaid, USA) following the manufacturer's instructions. The quality of the DNA was examined by 1 % (w/v) agarose gel electrophoresis and the concentration measured with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The extracted, diluted DNA was stored at -20°C until use in the sequencing analysis.

Next, the DNA samples were amplified with polymerase chain reaction (PCR). The PCR reactions (25 µL) contained 2.5 µL of Buffer II, 0.1 µL of AccuPrime™ Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, United States), 3 µL of DNA, and 1 µL of each dual-index primer combination. Each primer (ITS1 and ITS2) consisted of the appropriate Illumina adapter (AATGATACGGCGACCACCGAGATCTACAC for ITS1 and CAAGCAGAAGACGGCATACGAGAT for ITS2), an 8-nt index sequence (each index being

different from each other), a 10-nt pad sequence (TGTGGTGGCC for ITS1 and ACTGCGTCAT for ITS2), a 2-nt linker (GT for ITS1 and AT for ITS2) and the gene-specific primer (CTTGGTCATTTAGAGGAAGTAA and GCTGCGTTCTTCATCGATGC for ITS1 and for ITS2, respectively). The PCR was performed at 94°C for 2 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 1 min; and a final extension at 72°C for 5 min.

Then the pooled and purified PCR product was used to prepare the Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines. Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes, then sequences <150bp removed, i.e., sequences with ambiguous base cells were removed. Sequences were denoised, OTUs generated and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

3. Result and Discussion

3.1 Fungal isolates

The examination of the infected rice kernels under the microscope revealed the presence of different surface contaminating fungal genera isolated from stored rice samples. The fungi were identified according to the shape of the colony as shown in Figure 1. Fungi usually change in color from the center outward, as seen in Figure 1 (a-g).

The average incidence of fungal infection of the discolored rice samples at the three storage temperatures is presented in Figure 2. As it is shown in Figure 2, various genera of fungi, including Alternaria, Aspergillus, Cladosporium, Fusarium, Monilia, Penicillium, and Rhizopus were

identified, among other genera. Genera recovered from the rice samples in the order of highest frequency were Rhizopus, Penicillium, and Aspergillus.

Rice can be contaminated with field fungi such as Fusarium species preharvest, while during storage, rice can be infected with storage fungi like Aspergillus and Penicillium. Tonon et al. (1997) found rice surface mycoflora was dominated by storage fungi, notably Penicillium and Aspergillus. Field fungi usually get replaced by storage fungi during prolonged storage duration. Similar to our study, Penicillium, Aspergillus, and Fusarium were found in high frequency from polished rice in Korea (Park et al., 2005). In another study in Nigeria, rice samples in storage were frequently contaminated with Aspergillus, Penicillium, Fusarium, Alternaria, Mucor, Rhizopus, Trichoderma, Curvularia, Helminthosporium and Cladosporium (Makun et al., 2007). Notably, for rice cultivars in this study, incidence was highest for Rhizopus.

3.2 Fungal community composition

Taxonomically, the primary 13 fungal genera, namely Alternaria, Microascus, Thermomyces, Nigrospora, Fusarium, Wickerhamomyces, Penicillium, Aspergillus, Hypochnicium, Ustilaginoidea, Podospora, Rhizopus, and Cladosporium were observed in all rice samples. As shown in Figure 3a, the relative abundances of the dominant fungal genera for XL753 rice were varied for different storage conditions. In addition, the relative abundance of the fungal genus was unique among rice cultivars.

In XL753 rice, prior to storage (at zero storage duration), Alternaria (21.35%), Penicillium (15.40%), Fusarium (9.70%), Hypochnicium (6.58%), Cladosporium (4.75%), Rhizopus (4.70%), Microascus (4.23%), and Ustilaginoidea (3.16%) were the most abundant, followed by Nigrospora (1.39%), Aspergillus (0.06%), Wickerhamomyces (0.04%), Thermomyces (0.02%), and Podospora (0.02%). As shown in Figure 3a for XL753, the relative abundances of the major genera

varied for different sample conditions. In non-treated XL753 rice, the relative abundance of most fungal genera decreased from initial abundance. For example, the relative abundance of Alternaria genus was reduced from an initial 21.35% to a minimum relative abundance of 0.48% when rice was stored at 40°C after 16 weeks of storage. At the same storage condition, a similar trend was seen for genera of Fusarium, Penicillium, and Hypochnicium, in which their initial relative abundances of 9.79%, 15.40, and 6.58% were reduced to 0.18%, 0.32%, and 0.04%, respectively. The relative abundance of Penicillium in stored rice was more at 20°C than at 30°C and higher at 30°C than that at 40°C. However, the relative abundance of some other fungal genera on rice increased over storage at 40°C. For example, the initial relative abundance of Microascus (4.23%), Thermomyces (0.02%), and Aspergillus (0.06%) increased to the maximum levels of 52.54%, 41.46%, and 3.81% at 40°C, respectively.

Similar to non-treated samples, in treated XL753 rice samples with sodium chloride, the abundance of fungal genera varied during storage. Likewise, the initial relative abundance of Alternaria genus (21.35%) reduced after 16 weeks of storage at 30°C (7.94%) and 40°C (11.76%). In contrast, the initial relative abundances of Aspergillus and Penicillium increased from 0.06% and 15.40% to 5.30% and 39.31%, respectively, after 16 weeks at 40°C (Figure 3b).

Various fungi were found in this study, which can produce different pigments as their secondary metabolite. The type of pigment produced by fungi varies by strain, media composition, and growth condition and is greatly affected by the media composition (Jung et al., 2003). In the current study, more than 90% relative abundance of fungi in non-treated XL753 rice stored at 40°C for 16 weeks was related to Microascus and Thermomyces. Also, rice stored at 40°C for 16 weeks had the highest discoloration rate compared to rice stored at a lower temperature. Thermomyces have been found to produce yellow pigments, which have been applied in textile dyeing. The

pigment produced by Thermomyces is stable from acidic to moderately alkaline conditions, pH 5.1 and 8.0 (Rao et al., 2017). It is reported that yellow pigments produced by Thermomyces sp are stable at high temperatures up to 150°C (Poorniammal and Gunasekaran, 2015). In general, the higher relative abundances of Penicillium, Alternaria, and Fusarium were seen in non-treated XL753 rice stored at 20°C and 30°C than at the higest temperature. It has been reported that Ascomycetes such as Aspergillus, Cercospora, Penicillium, and Aschersonia produce carotenes. Carotenes absorb ultraviolet (UV), violet, and blue light and scatter orange or red light, and (in low concentrations) yellow light. Penicillium sp such as Penicillium oxalicum produce red pigments named Arpink redTM (Rao et al., 2017). Alternaria sp can produce β-carotene and accumulate a red pigment or a red-brown pigment by the mycelium depending on the medium and strain (Häggblom and Unestam, 1979). Also, Fusarium species such as F. oxysporum, F.fujikuroi, and F. verticilloides produce pigments among which bikaverin, norbikaverin, and other compounds like nectriafurone and O-demethylanhydrofusarubrin are found; bikaverin and its derivatives are red in color (Palacio-Barrera et al., 2019).

In sodium chloride-treated XL753 rice stored at 20°C and 30°C, the abundance of Penicillium was relatively lower compared to non-treated rice, which was reported to produce yellow, orange-red pigments by some strains. However, the relative abundance of Penicillium was high in sodium chloride-treated XL753 stored at 40°C for 16 weeks.

The same major fungal genera were identified contaminating Roy J rice cultivar at zero storage duration; in order of decreasing predominance were Alternaria (24.01%), Aspergillus (14.00%), Fusarium (8.00%), Nigrospora (4.00%), Cladosporium (3.21%), Microascus (3.00%), Podospora (2.81%), Hypochnicium (2.00%), and Penicillium (1.31%). Others include Rhizopus (0.52%), Ustilaginoidea (0.33%), Thermomyces (0.21%), and Wickerhamomyces (0.13%).

Depending on storage temperature and duration, the major fungi varied in relative abundance for non-treated Roy J (Figure 4a). The initial relative abundance of Alternaria, Fusarium, Hypochnicium, and Cladosporium reduced to a minimum of 4.7%, 1.01%, 0.21%, and 0.30%, respectively, in Roy J rice stored at 40°C for 16 weeks. However, the relative abundance of Aspergillus genus considerably increased from the initial 14.00% to 42.00% and 82.42% in rice stored at 40°C for four and 16 weeks of storage, respectively.

In Roy J rice treated with sodium chloride (Figure 4b), the relative abundance of Alternaria genus was also reduced to its minimum of 7.36%. The initial relative abundance of Fusarium first considerably increased to 17.28% in rice stored at 20°C for four weeks, followed by a reduction to 2.53% at 40°C storage temperature for 16 weeks. The initial relative abundance of Wickerhamomyces increased in treated Roy J rice stored at temperatures more than 30°C for 16 weeks, thereby reaching its maximum abundance of 27.24% at a storage temperature of 40°C and storage duration of 16 weeks. A similar increasing trend was seen for genera of Penicillium, Aspergillus, and Ustilaginoidea when Roy J rice treated with sodium chloride was stored at temperatures ≥ 30°C. For example, Aspergillus reached to the highest relative abundance of 47.66% in rice stored at 30°C for 16 weeks.

The relative abundance of Aspergillus in non-treated Roy J rice was higher than that for non-treated XL753. Also, the relative abundance of Aspergillus sp. was less in sodium chloride Roy J (except in rice stored at 30°C for 16 weeks) compared to that of non-treated Roy J. In non-treated Roy J stored at 40°C after four and 16 weeks of storage, the majority of genera consisted of Aspergillus. Some species of Aspergillus have been reported to produce pigments (He et al., 2012). For example, Aspergillus fumigatus can produce melanins, which are dark-brown or black pigments formed by the oxidative polymerization of phenolic compounds (Youngchim et al.,

2004). Some other species of Aspergillus such as A. niger produce colorful pigments, including brown aspergillin and a golden-yellow pigment (Reid, 1950).

The Titan rice at zero storage duration was infected with Alternaria (23.08%), Ustilaginoidea (9.12%), Fusarium (9.00%), Penicillium (6.23%), Microascus (5.00%), Cladosporium (4.86%), Aspergillus (4.26%), and Nigrospora (4.00%) followed by Hypochnicium (2.08%), Rhizopus (1.04%), Thermomyces (0.40%), Podospora (0.08%), and Wickerhamomyces (0.06%). As for other non-treated rice cultivars, the initial relative abundance of Alternaria was generally reduced with increased storage duration and temperature with an exception of at 40°C and 16 weeks, in which its abundance slightly increased to 30.78% (Figure 5a). Ustilaginoidea genus was also reduced in relative abundance from the initial value over storage of rice with a large exception occurring when rice was stored at 30°C for four weeks, where it was at a maximum level of 58.02%. The initial relative abundances of Fusarium, Rhizopus and Cladosporium reduced to the minimum level at storage temperature of 40°C and duration of 16 weeks. On the other side, the relative abundance of Aspergillus generally increased during rice storage particularly in rice stored at 20°C after four weeks and 30°C and 40°C after 16 weeks. The relative abundance of Thermomyces increased to a maximum of 89.07% in rice stored at 40°C after four weeks, but slightly decreased at the same storage temperature after 16 weeks (less than one percent). Nigospora was relatively more abundant in non-treated Titan stored at 30°C and 40°C after 16 weeks. The relative abundance of Penicillium considerably increased in rice stored at 20°C; however, decreased to a minimum at 40°C.

In Titan treated with sodium chloride (Figure 5b), the relative abundance of Alternaria increased at 20°C and 30°C but it decreased when storage temperature was at 40°C. The relative abundance of Ustilaginoidea genus (9.11%) reduced at 20°C and 30°C followed by a significant

increase in rice stored at 40°C after four weeks (58.02%) and also after 16 weeks (15.31%). There was a reduction in the prevalence of Microascus, Fusarium (at 40°C), Hypochnicium, Rhizopus (minimum abundance at 40°C after 16 weeks), and Cladosporium (particularly in rice stored at 20°C and 30°C even after four weeks).

In non-treated Titan stored at 30°C, the relative abundance of Ustilaginoidea was predominant after four weeks. Some species of Ustilaginoidea, such as U. virens have been reported to cause rice false smut; white hyphae are produced by the Ustilaginoidea after the initial infection of the floral organs of the rice crop. As the infection matures with time, darker brownish-green chlamydospores are produced on the rice spikelets (Ashizawa et al., 2012).

3.3 Storage trends for five dominant fungal genera

The relative abundance of Aspergillus was initially highest for the Roy J cultivar compared to the other two rice cultivars. Notable features of this fungi during storage for Roy J were that relative abundance increased substantially as storage temperature increased and that sodium chloride treatment generally reduced relative abundance compared to non-treated rice (Figure 6a). The highest reduction in initial Aspergillus for the treatment conditions in this experiment occurred with treated rice at the storage of 20°C after four or 16 weeks of storage. Previous results for Roy J had shown no incidence of discoloration at this condition with 21% MC (Mohammadi Shad and Antungulu, 2019). For non-treated Roy J, a considerable reduction from initial was seen after 16 weeks of storage at 30°C (Figure 6a), although the rice was highly discolored at that point for the control treatment. The initial Aspergillus abundance in Titan was not reduced for the control except at 30°C and 40°C temperatures after four weeks of storage (Figure 6b). However, less than initial Aspergillus abundance occurred for treated Titan rice when stored at 20°C for 16 weeks, where discoloration was slight, and also at 40°C for 4 weeks, where discoloration was large. Minimal

initial Aspergillus occurred in the XL753 rice (Figure 6c) and it remained about the same level for the control and sodium chloride treatments when stored at 20°C for 4 weeks (Figure 6b). There was no appearance of an effect on Aspergillus level due to sodium chloride treatment for XL753.

Penicillium relative abundance was much less for Roy J than Titan or XL753 at the initial condition, unlike the cultivar trend for initial Aspergillus. At 20°C, Penicillium abundance was similar for the Roy J control and treated rice (Figure 7a). However, the only conditions that reduced Penicillium below the initial level was for the treated Roy J rice after 16 weeks at 20°C and after 4 weeks at 20°C, which was similar to that for Aspergillus in Roy J. For Titan, substantial reduction in initial Penicillium occurred in treated rice after four weeks of storage at 20°C and 30°C temperatures (Figure 7b). However, treated Titan rice when stored for 16 weeks led to a higher Penicillium relative abundance, especially as temperature increased. The most reduction in initial Penicillium abundance for non-treated Titan was at 40°C after 4 weeks. Treated XL753 rice had very reduced Penicillium abundance at 20°C after 4 or 16 weeks of storage compared to control (Figure 7c). In this cultivar, the untreated rice was profuse with Penicillium at 20°C at both four and 16 weeks storage, while as temperature increased above 20°C, the Penicillium decreased considerably. The optimal storage condition for minimizing the initial Penicillium level in XL753 was with sodium chloride treatment, four weeks of storage at 20°C. This condition was also associated with the lowest percent discoloration for XL753 rice (Mohammadi Shad and Antungulu, 2019).

Nigrospora, having low initial relative abundance in all three cultivars, was increased for each cultivar at storage of 20°C and 30°C, regardless of duration and antifungal treatment (Figure 8). Only when temperature was 40°C, was there a lessening of the initial Nigrospora for Roy J

(Figure 8a), Titan (Figure 8b), and XL753 (Figure 8c). The minimum Nigrospora was about the same for the treated and untreated rice.

Trends for the Fusarium fungi were similar in some ways to those seen for Nigrospora. The most reduction in the initial Fusarium occurs at 40°C, when at 16 weeks of storage for Roy J (Figure 9a), four weeks of storage for Titan (Figure 9b), and 16 weeks of storage for XL753 (Figure 9c). However, Fusarium was reasonably reduced for treated Titan and XL753 rice at 20°C after four weeks, but not treated Roy J at 20°C after four weeks. Further reductions in Fusarium occurred for treated Roy J and XL753 when stored for 16 weeks rather than four weeks at 20°C. The longer storage duration when at 20°C did not increase discoloration for those treated cultivars.

Alternaria had reductions for all cultivars with increased storage temperatures. After 4 weeks of storage at 20°C, the relative abundance of Alternaria was decreased below initial abundance in non-treated samples for Roy J (fig. 10a), Titan (fig. 10b), and XL753 (fig. 10c). Rice samples treated with sodium chloride had typically higher Alternaria than non-treated rice over all storage conditions and cultivars. In nearly all cases, relative abundance decreased below initial levels when stored at 40°C, regardless of storage duration (Figure 10a, b, c). Conditions found to be appropriate for rice discoloration were also reported to be good for fungal contamination as well (Atungulu et al., 2018). When considering increasing discoloration as rice storage temperature increases above 20°C, it is noteworthy to point out that several of these five major fungi can be reduced or at least held near initial relative abundance with storage at 20°C. Sodium chloride can also be effective in controlling fungi buildup during storage, as well as reduce discoloration compared to untreated rice. For all cultivars stored at 20°C and 21% MC, there is a minimal consequence of discoloration for treated rice when storage increases from four to 16 weeks. On the other hand, there may be better control of Aspergillus in Titan and XL753; Penicillium in Roy

J; Nigrospora in Titan and XL753; and Fusarium in Foy J, when treated rice is stored at a longer duration than four weeks. For other fungi, especially Alternaria in this study, storage treatment appears to be ineffective unless at 40°C, when discoloration was significantly higher than at 20 °C (Mohammadi Shad and Antungulu, 2019).

4. Conclusion

Rice stored at high MC was found to be contaminated with several major fungal genera. The relative abundance of the fungal genera varied based on storage temperature, rice cultivars, and antifungal treatment. The three fungal genera including Penicillium, Alternaria, and Aspergillus were found in most of the samples in high abundance. These three fungi grow in high MC stored rice and can produce colorful pigments as their secondary metabolite. However, since these fungi were present in all samples, the fungal pigments were likely not the primary reason for observed rice discoloration. However, as previous results (Chapter 2) made clear, microbe load and discoloration were not mutually related and, therefore, rice discoloration was more likely due to chemical or enzymatic reactions occurring at some point during these storage conditions. To improve rice value and reduce post-harvest losses, the storage temperature should be less than ambient temperature (30°C). Storage condition may be critical in determining the cause of rice quality degradation including rice discoloration. This study provides information to rice farmers, processors, and industries about safe storage conditions for rice grains in order to prevent grain loss caused by rice discoloration. The results also suggest that the implementation of particular antifungal treatments in storage may be useful in reducing rice discoloration to some degree. However, the mechanisms causing the antifungal effects on discoloration, as related to fungi contamination, will require further research exploration.

5. References

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6. Tables and Figures

Table 1. Discoloration caused by different fungi species during storage.

Discoloration	Fungi	Reference
Yellow	Fusarium spp, Aspergillus (A) flavus	Quitco et al., 1982
Light yellowish brown	Rhizopus spp	
Black	Curvularia spp, A. terreus, A. ochraceus	
		Philip et al., 1988
Dark brown to black	Curvularia spp	
Creamy to slightly yellow	Aspergillus flavus	
Creamy, opaque and friable	A.candidus,A.fumigatus	
Cream, bright lemon yellow,		Philip et al., 1989
Dull yellow, orange red,	Penicillium spp	
opaque and friable		Borlagdan, 2008
Yellow	Thermophile; Humicola lanuginose	Schroeder, 1965
	Thermotolerant sp; A. fumigatus , A. flavus, A.candidus,	
	Mucorales, Corynascus Sepedonium, Mesophilic Penicillium	
		Borlagdan, 2008
	Aspergillus flavus, Rhizpus stolonifer,	
Yellow	Penicillium sp	
	Fusarium chlamydosporium	
Red	Rhizopus sp, Rhizopus	
	Stolonifera, Aspergillus flavus,	
Yellow	Aspergillus niger, Penicillium sp	

Table 2. The storage conditions of rice samples subjected to mycoflora analyses.

Cultivar	Moisture content (w.b.)	Treatment	Temperature (°C)	Storage duration (weeks)
XL753	21%	Non-treated	20	0
Roy J			30	4
Titan		Sodium chloride	40	16

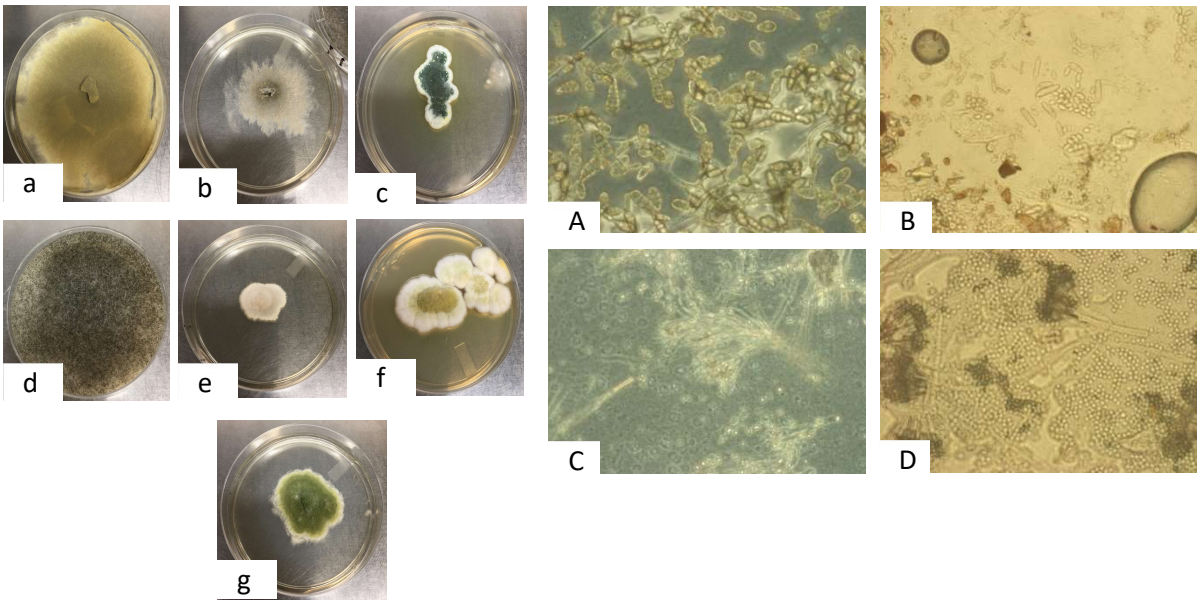

Figure 1. Right: Colonies of different fungal species on agar media after incubation at 25°C for 5 days. Aspergillus (a, b); Rhizopus (d); Penicillium (c,g); Fusarium (e,f); left: Photomicrograph of fungal slide cultures A) Alternaria sp. B) Cladosporium sp. C) Monilia so. D) Penicillium sp.

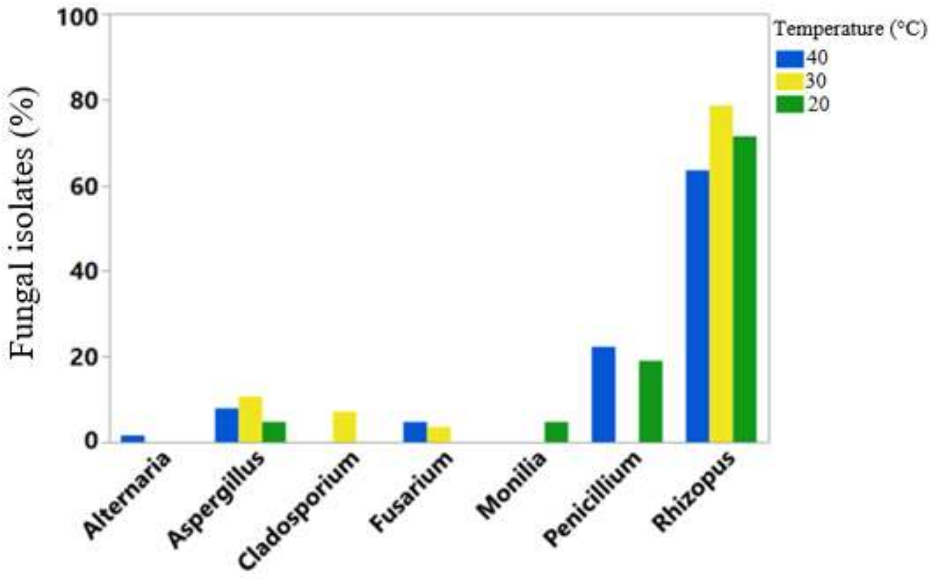

Figure 2. Frequency of fungal genera isolated from discolored rice obtained from rough rice stored at 20°C, 30°C, and 40°C.

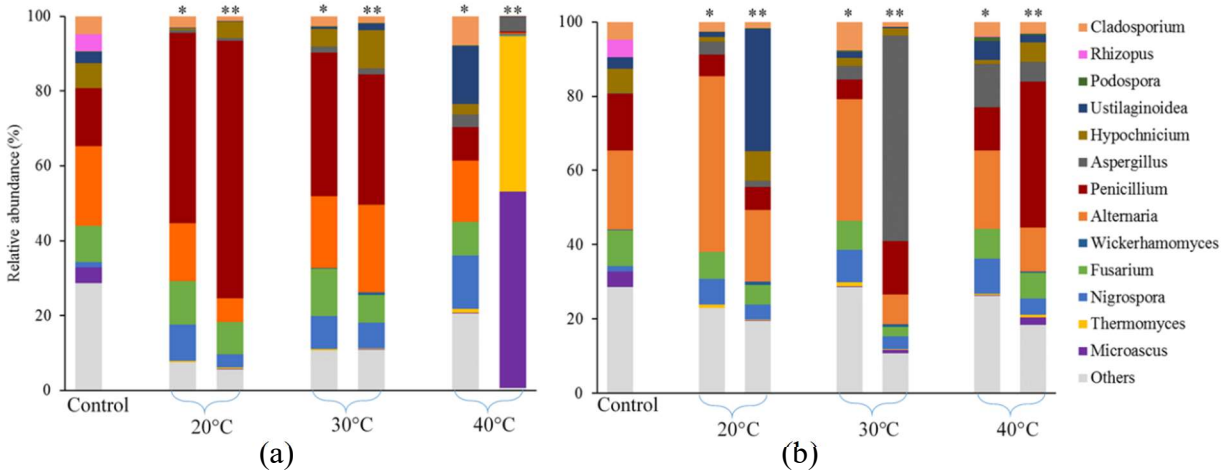

Figure 3. Relative abundances of fungal genera in stored XL753 rice (without antifungal treatment (a); and with sodium chloride treatment (b)) at 20, 30, and 40°C after four (indicated as *) and 16 (indicated as **) weeks of storage.

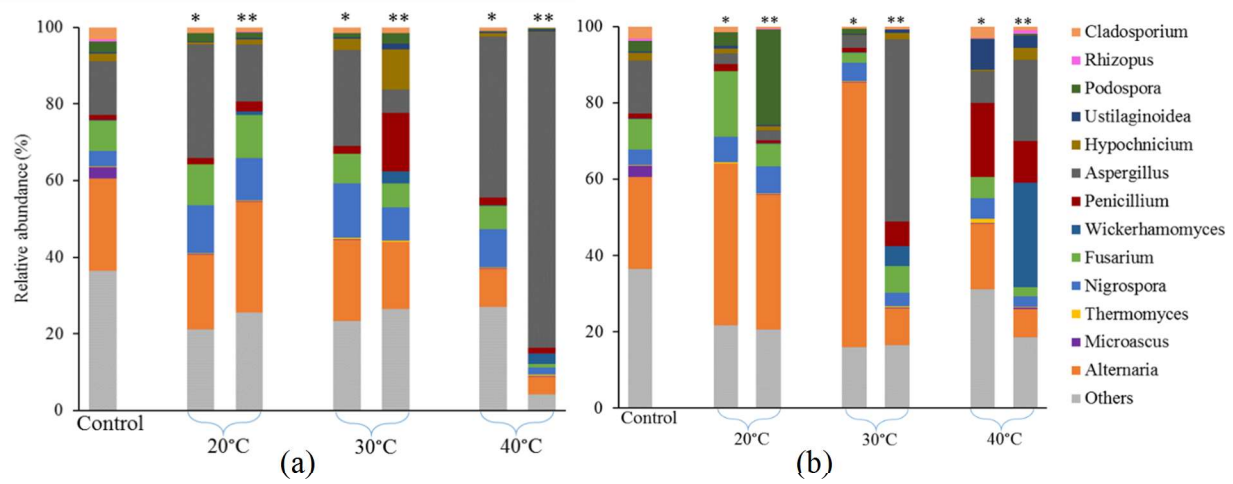

Figure 4. Relative abundances of fungal genus in stored Roy J rice (without antifungal treatment (a); and with sodium chloride treatment (b)) at 20, 30, and 40°C after four (indicated as *) and 16 (indicated as **) weeks of storage.

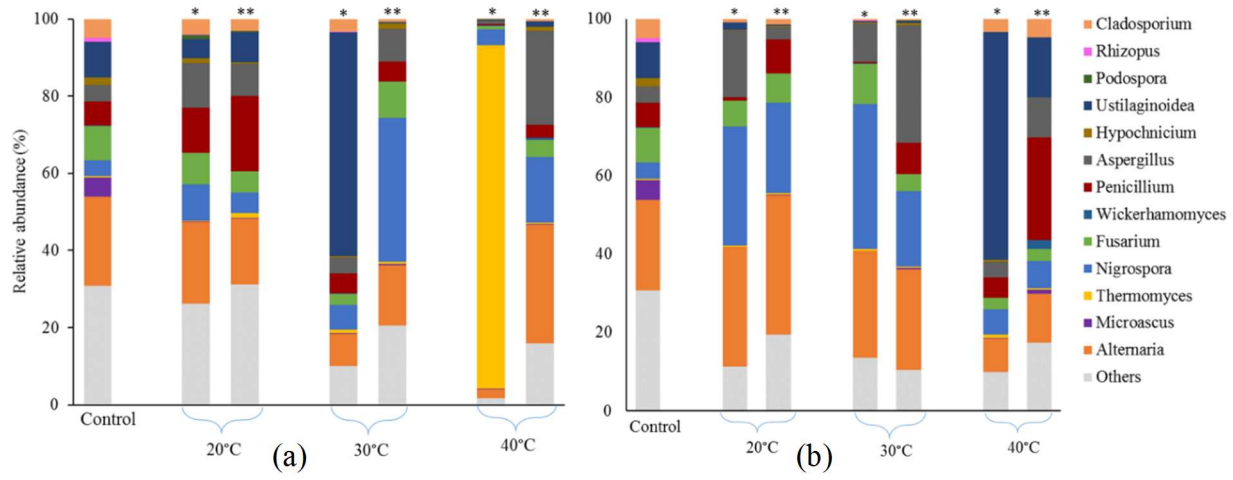

Figure 5. Relative abundances of fungal genus in stored Titan rice (without antifungal treatment (a); and with sodium chloride treatment (b)) at 20, 30, and 40°C after four (indicated as *) and 16 (indicated as **) weeks of storage.

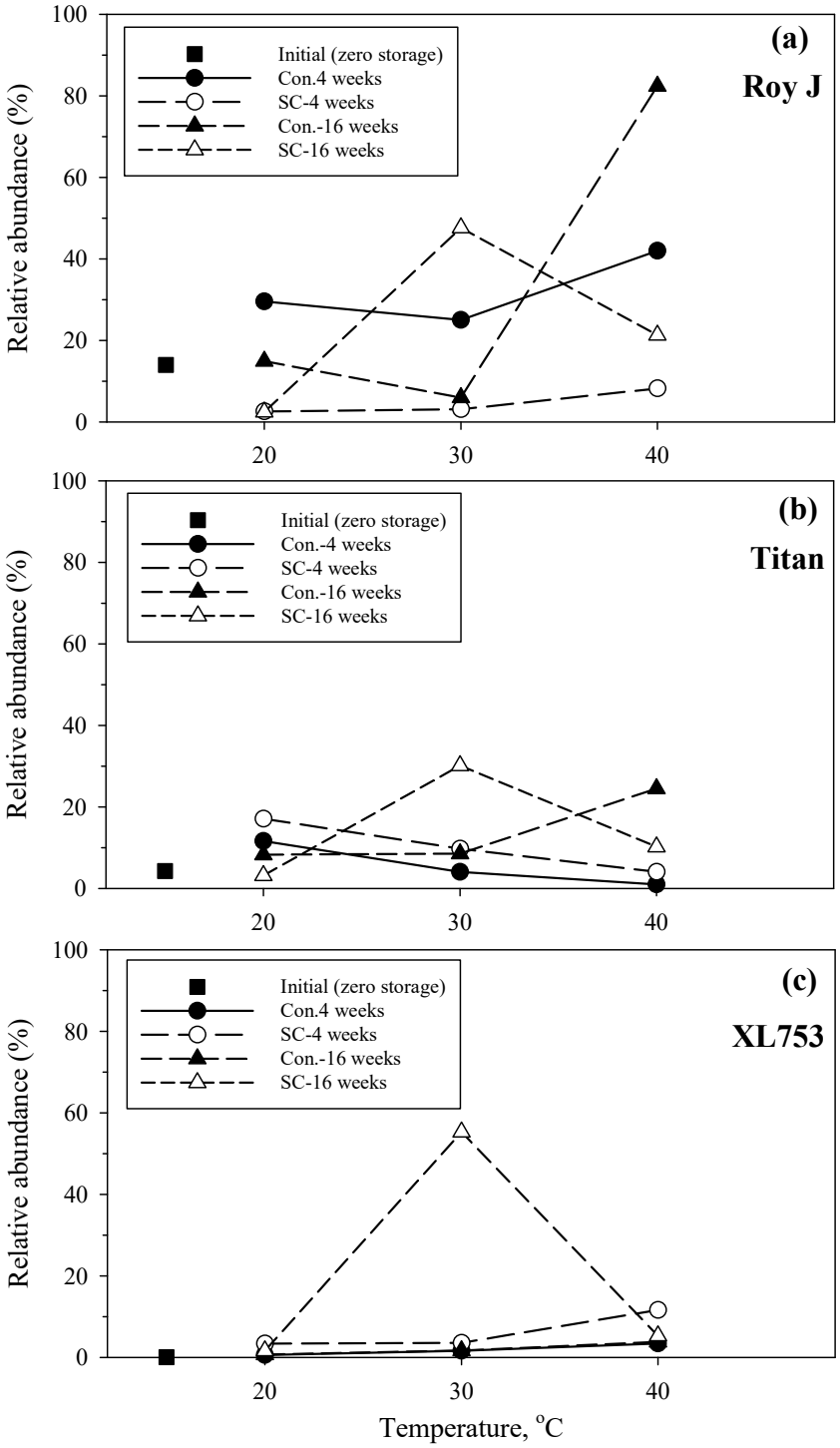

Figure 6. Initial and change in relative abundance of Aspergillus with storage temperature for treated and non-treated rice for Roy J (a), Titan (b), and XL753 rice, at 4- and 16-week storage durations (Con. and SC stand for non-treated/control and sodium chloride treated rice samples, respectively).

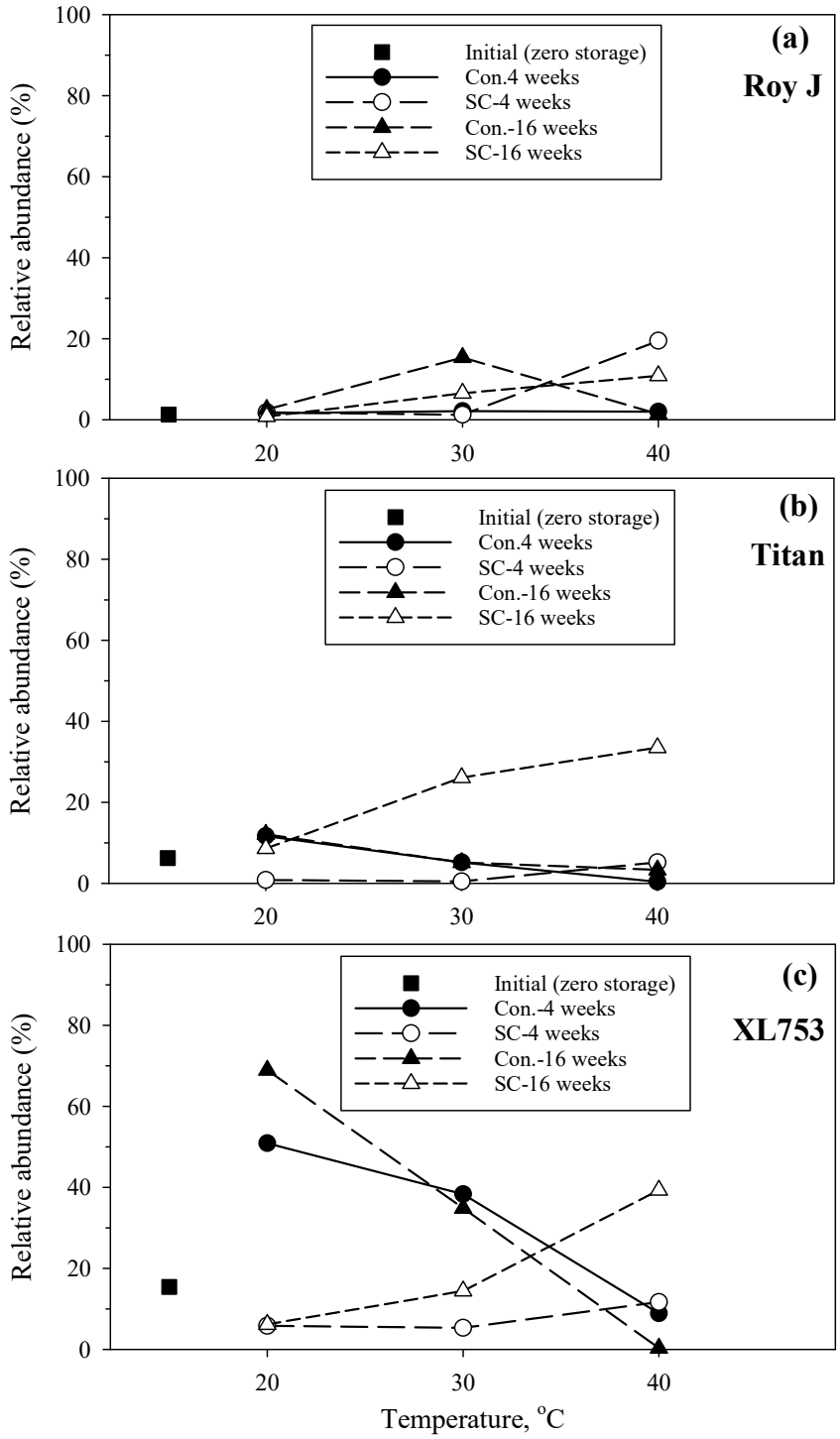

Figure 7. Initial and change in relative abundance of Penicillium with storage temperature for treated and non-treated rice for Roy J (a), Titan (b), and XL753 rice, at 4- and 16-week storage durations (Con. and SC stand for non-treated/control and sodium chloride treated rice samples, respectively).

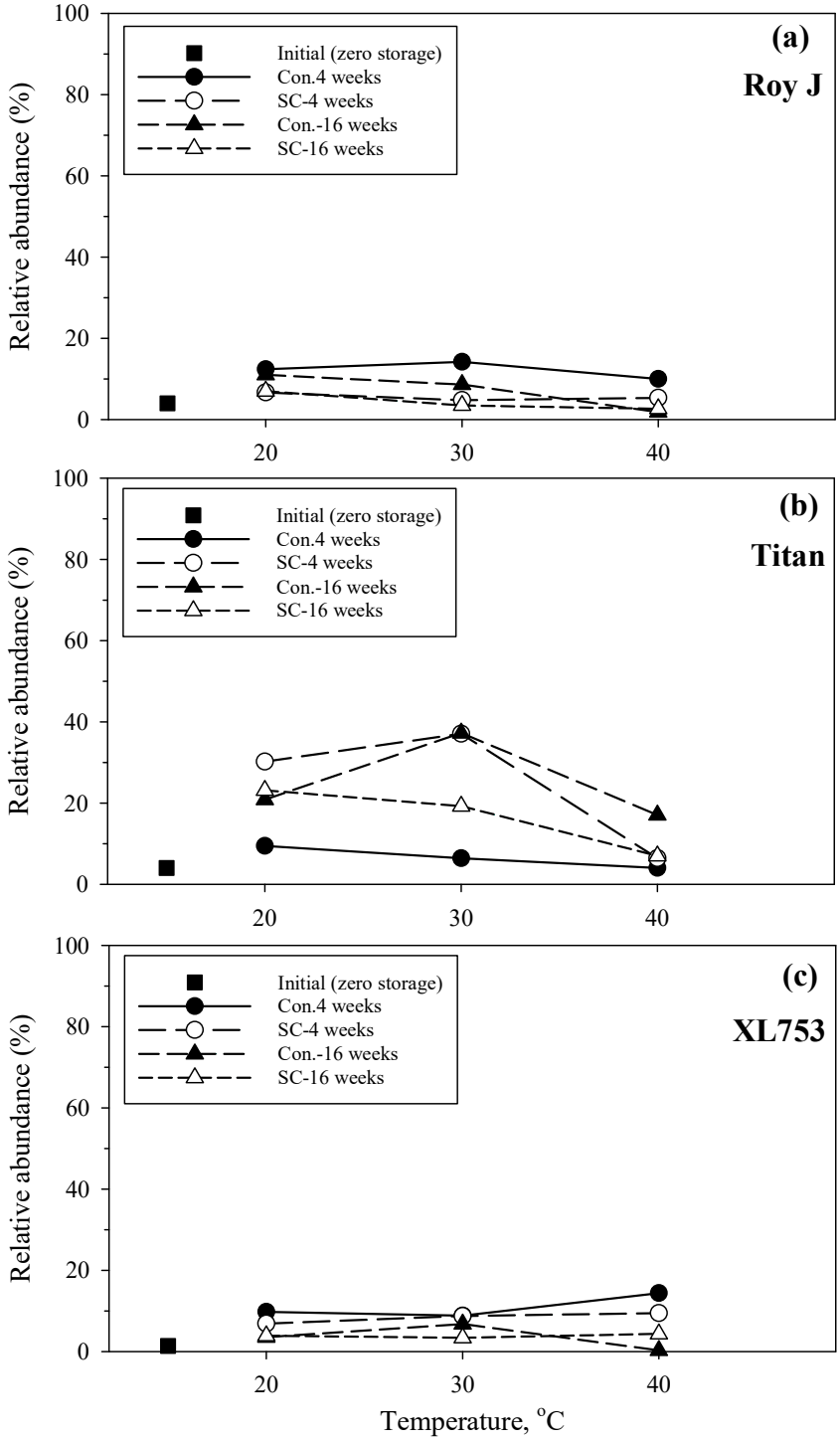

Figure 8. Initial and change in relative abundance of Nigrospora with storage temperature for treated and non-treated rice for Roy J (a), Titan (b), and XL753 rice, at 4- and 16-week storage durations (Con. and SC stand for non-treated/control and sodium chloride treated rice samples, respectively).

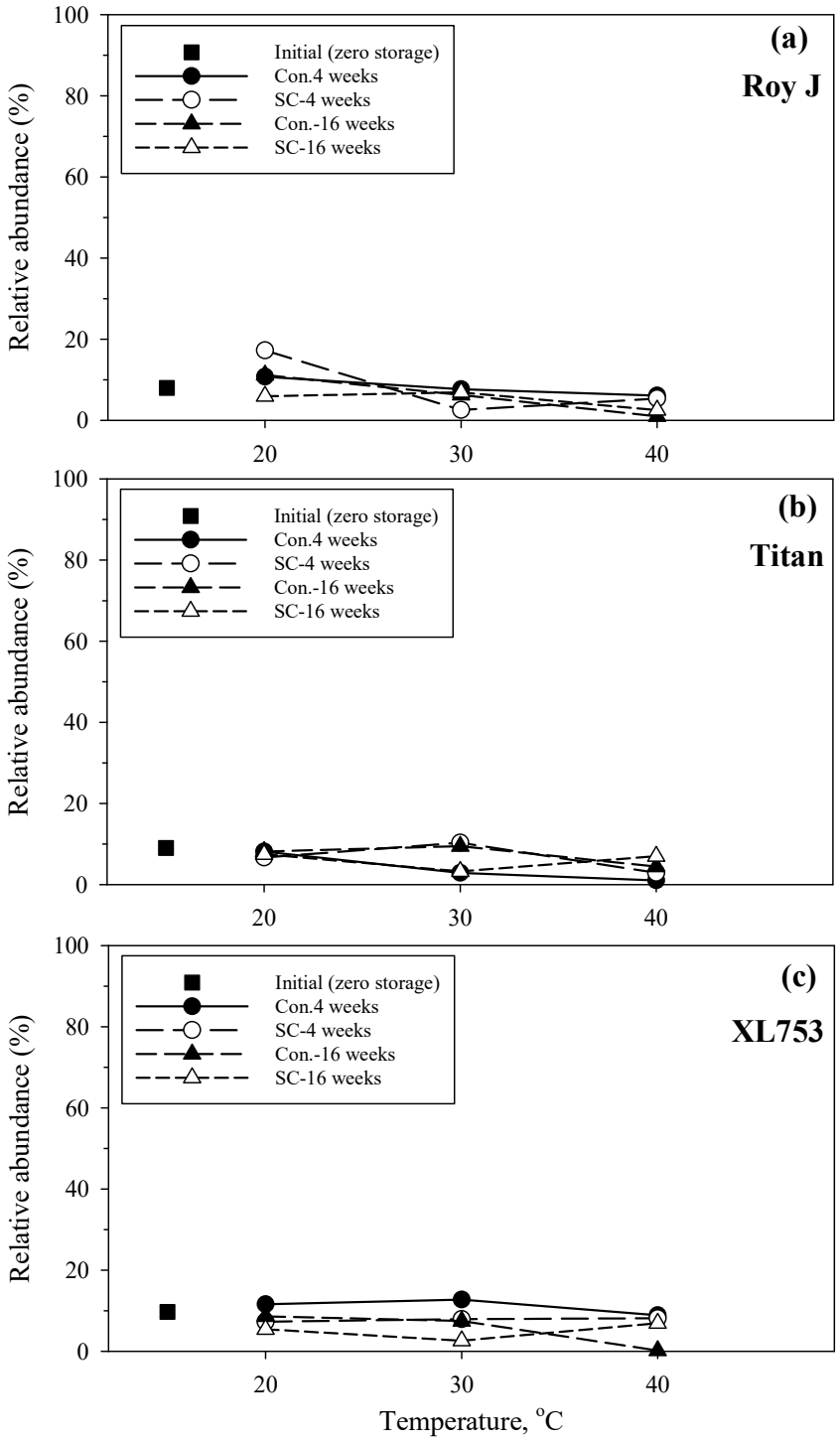

Figure 9. Initial and change in relative abundance of Fusarium with storage temperature for treated and non-treated rice for Roy J (a), Titan (b), and XL753 rice, at 4- and 16-week storage durations (Con. and SC stand for non-treated/control and sodium chloride treated rice samples, respectively).

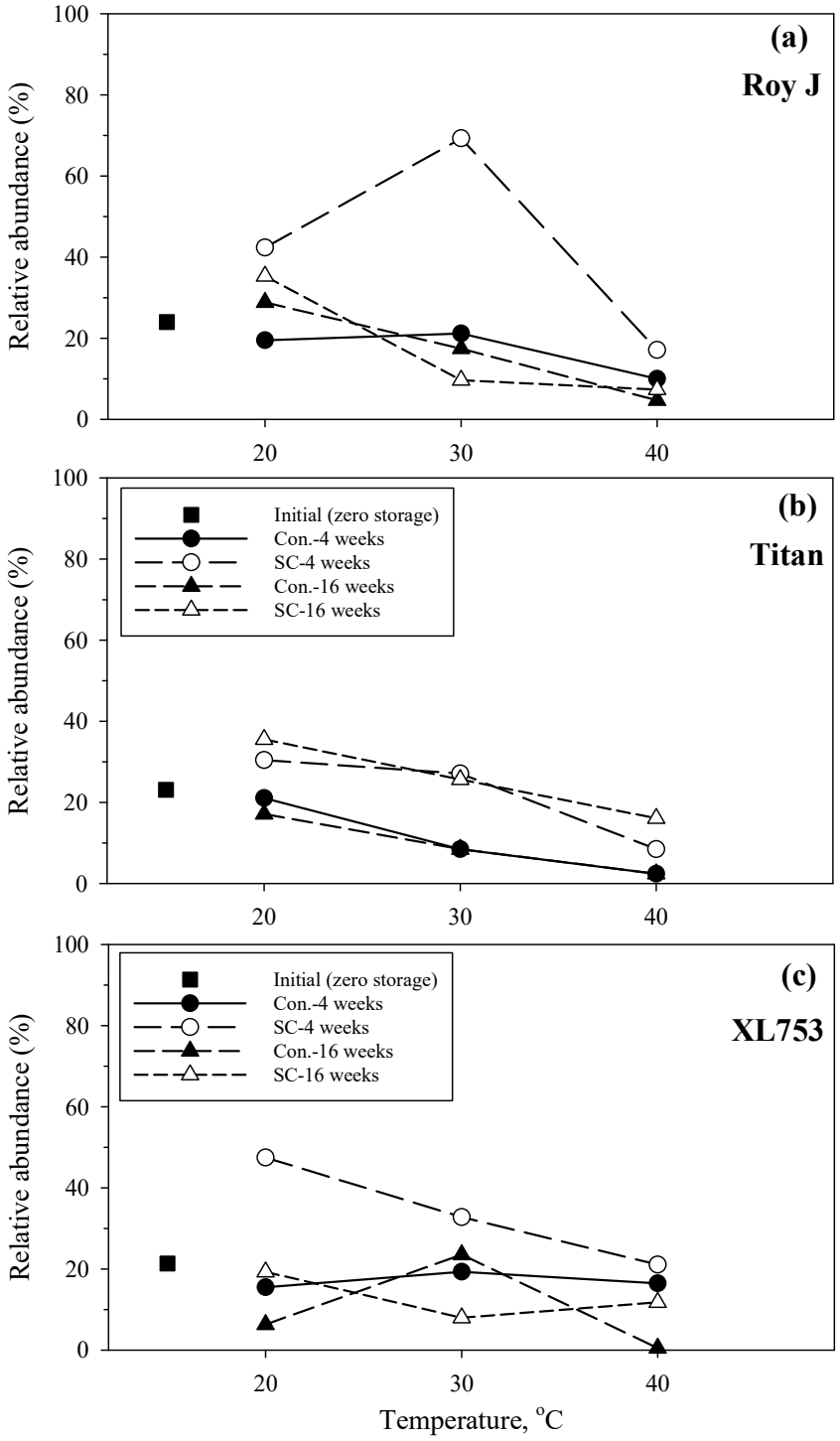

Figure 10. Initial and change in relative abundance of Alternaria with storage temperature for treated and non-treated rice for Roy J (a), Titan (b), and XL753 rice, at 4- and 16-week storage durations (Con. and SC stand for non-treated/control and sodium chloride treated rice samples, respectively).

Chapter 5: Biochemical changes associated with electron beam irradiation of rice and links to kernel discoloration during storage

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Abstract

Rice kernel discoloration during storage results in significant economic losses to rice growers and processors. This study aimed to elucidate the extent of chemical changes and microbial involvement on discoloration of rice kernels during storage. To segregate and/or diminish the effects of microbes, one lot of hybrid long-grain rice (XL753) samples was irradiated with non-thermal electron beam (EB) dose of 14 kGy. The irradiated and non-irradiated control samples of rice at a moisture content (MC) of 21% on a wet basis were stored at three temperatures (20°C, 30°C and 40°C) for 8 weeks. Samples were taken every 2 weeks for microbial and chemical analyses. Findings: A negative relationship was noted between discoloration and microbial load. The trend of increasing discoloration and chemical properties such as free sugars, free fatty acid and free 5-hydroxymethyl-2-furaldehyde (HMF), especially at higher storage temperatures and durations, suggested that biochemical changes were major drivers of the observed rice discoloration. The higher HMF in highly discolored rice (≥20%) explained non-enzymatic browning in the rice matrix during storage. From this study, it was drawn that the rice kernel discoloration was not directly related to the microbial load; the discoloration was seen in EBI rice even with 99% reduction in microbial load. However, it was clarified that the rice discoloration especially in EBI rice samples was related to the observed chemical changes, which were also storage temperature dependent. Milled rice discoloration during storage of rough rice is insufficiently understood. There is no information correlating changes in chemical attributes and microbial activity to discoloration of contemporary hybrid rices during storage. Therefore, the

results of the current study provide important fundamental information and also suggest storage conditions required to arrest discoloration and maintain quality of contemporary milled hybrid rice.

1. Introduction

Rice is usually harvested at high moisture content (MC), 21-22% MC on a wet basis (w.b.), and needs to be dried immediately to safe storage MC, 13-15% to arrest biodeterioration. Otherwise, the high MC favors the growth of insects, spoilage microbes (Mohammadi Shad & Atungulu, 2017; Atungulu, Kolb, Karcher, & Shad, 2019) and/or initiation of chemical reactions such as lipid oxidation/decomposition and discoloration, which can result in rice downgrading at the point of sale (Zhou, Wang, Si, Blanchard, & Strappe, 2015).

Rice discoloration, in particular, has drawn a lot of concern by rice processors and millers due to the associated economic losses; typically, discolored rice is severely discounted. According to the US standard, No. 1 grade milled rice should not have more than one heat-damaged kernel per 500-g sample (USDA, 2009). Discolored rice kernels, also generally referred to as "yellowed" or "stained" rice kernels, may comprise of kernels with colors such as yellow, salmon, black, pink, brown, and opaque.

Mostly, discoloration of rice kernels is associated with improper post-harvest management practices that promote excessive rice kernel respiration; for instance poor storage or delayed drying of the rice (Atungulu et al., 2019). Based on the literature, rice at 26% MC increased in temperature from 25°C to 63°C, over 30 days of storage, due to energy release by accelerated respiration (Trigo-Stockli & Pedersen, 1994). Moreover, storage of rice at high temperatures of 61.5 to 81.6°C (MC<14%), 60°C (aw=0.40, 0.60), and 60°C (25% MC) resulted in discolored rice (Bason, Gras,

Banks, & Eseteves, 1990; Dillahunty, Siebenmorgen, & Mauromoustakos, 2001; Belefant-Miller, Kay, & Lee, 2005). Also, rice drying itself can accelerate milled rice discoloration. The rice discoloration, yellowing, on milled rice can result from air drying of high MC rough rice at temperatures higher than 50 °C (Tong, Gao, Luo, Liu & Bao, 2019).

Rice kernel discoloration may also occur due to chemical reactions in the rice matrix. The chemical reactions may occur during storage or thermal processing of rice such as parboiling. For example, Maillard reaction in the rice matrix may be initiated by condensation between amino groups of proteins and reducing sugars. The extent of Maillard reaction can be monitored by products such as 5-hydroxymethyl-2-furaldehyde (HMF). The HMF is produced in the intermediate stage of the Maillard reaction when the Amadori products are internally broken. In previous studies, HMF production was highly correlated with an increase in redness of parboiled rice (Lamberts, Rombouts, Brijs, Gebruers & Delcour, 2008). Other studies have also correlated carotenogenesis in rice bran stored at 70°C with rice kernel discoloration (Belefant-Miller & Grunden, 2014). The findings related to the impact of biochemical reactions on rice discoloration were inconclusive (Belefant-Miller & Grunden, 2014); this warrants further investigation.

Schroeder (1964) suggested that contamination of rice with mold contributes to the head rice kernel discoloration. In the study, *Fusarium*-inoculated rice was stored at 22% MC and 30°C for ten days. However, subsequent investigations have refuted the role of mold in rice discoloration. For instance, Mohammadi Shad & Atungulu (2019); Belefant-Miller et al. (2005); Phillips, Widjaja, Wallbridge, & Cooke (1988); Schroeder (1965) revealed no correlation between the mold presence and rice discoloration. Hence the research community has no concession on the exact role of microbes on rice discoloration.

Different irradiation approaches have been evaluated to reduce the microbial load on grains (Atungulu, Mohammadi-Shad, & Wilson, 2018). For example, Wilson, Okeyo, Olatunde, & Atungulu (2017) used infrared heating to dry high MC corn (24%) and achieved 2.6-2.9 log reductions of microbes; Smith and Atungulu, (2018) used microwaves set at the 915 MHz to inactivate microbes on rice and achieved 4.56 and 2.93 log reduction of aerobic bacterial and *Aspergillus flavus*, respectively. Non-thermal EB irradiation has also been used to inactivate spoilage microorganisms on food (Molins, 2001). In EB irradiation, a concentrated bundle or beam of highly energetic electrons (beta-particles) is shot on a food product. In order to delineate the role of microbes on rice kernel discoloration, such irradiation approaches may be used to surface sterilize products before storage.

The current study focused on investigating the correlation of biochemical reactions occurring during rice storage and rice discoloration. Impacts of microbes were minimized by non-thermal EB irradiation of freshly-harvested rice. Changes in discoloration of milled rice and physicochemical properties such as total mold count (TMC), total protein, crude oil, rice carbohydrates (total starch, free sugars), free fatty acid (FFA), and free 5-hydroxymethyl-2-furaldehyde (HMF) were tracked over different storage durations and temperatures.

2. Materials and Methods

1.1. Rice samples

Long-grain hybrid rice (Oryza sativa L.) cultivar XL753 harvested in Burdette (Arkansas, U.S.) in 2018 was used. The rice was harvested at 22% MC on a wet basis (w.b.). The samples were cleaned using the dockage tester (Model XT4, Carter-Day, Minneapolis, MN, U.S.). After cleaning, the rough rice was conditioned to the desired MC of 21% by spreading the rice on a tarp

in a conditioned environment (26°C, 56% relative humidity). The MC was measured using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden). It was calibrated according to Jindal & Siebenmorgen (1987). Briefly, 15 g of sample was put in a 130°C oven for 24 h, cooled down in a desiccator for half an hour, after which the difference in weight was measured.

1.2. Electron beam (EB) irradiation

The conditioned rough rice was divided into two lots. One lot was irradiated with non-thermal EB and the other used as non-irradiated. The EB irradiation was used to decontaminate the rough rice samples and minimize the role of storage mold on rice discoloration. To accomplish the EB irradiation, rice sample portions of 600 g were evenly distributed in the vacuum polyethylene bags with a thickness of 3 cm and vacuum heat-sealed. The samples were placed on a conveyer belt with adjustable speed, which was set at 0.028 m/s. Alanine probes were placed on the top and the bottom of the samples. The irradiation was carried out under ambient temperatures using a 10 MeV, 18 kW Electron Beam Linear Accelerators (LINAC) at the National Center for Electron Beam Research (NCEBR) (College Station, TX, U.S.). Determination of the absorbed irradiation dose was performed using an alanine dosimeter. Average irradiation dose was 14 kGy.

1.3. Storage

Samples of 600 g from non-irradiated and electron beam irradiated (EBI) rough rice were filled in sterile glass jars (1 L), closed with an aluminum cap, and sealed airtight with Parafilm M (Bemis Company, Inc., Neenah, WI, U.S.). Storage took place at three storage temperatures (20°C, 30°C and 40°C) for four storage periods (2, 4, 6 and 8 weeks). The samples of zero-duration storage for the irradiated and non-irradiated rice samples were taken for analyses before storage.

The samples of zero storage duration were called controls in this study. There were three jars for each temperature condition by storage duration combination for both non-irradiated and EBI rice. Therefore, the experiments were performed in triplicates.

1.4. Chemicals

All chemicals used were obtained from VWR™ (Avantor, Radnor, PA, U.S.) unless stated otherwise.

1.5. Total mold count (TMC) and water activity of stored rice

Enumeration of the molds on the rice was done with the 3M Petrifilm Mold Count Plates (3M Microbiology Products, Minneapolis, MN, U.S.), following the AOAC Method 997.02 (AOAC, 1995). A 10 g sample of rough rice was mixed with 90 mL sterilized phosphate-buffered dilution water (0.5 M, pH = 7.2) in sterile stomacher bags and homogenized with a lab masticator (Silver Panoramic, iUL, S.A., Barcelona, Spain) for 240 s at 0.7 strokes s^{-1}. Sequential dilutions were made, and the 10^{-1} to 10^{-5} dilutions were plated and stored in an incubator for five days at 25°C. Total mold counts (TMC) were expressed as the logarithm (base 10) of colony forming units per gram (log CFU g^{-1}). The water activity (a_w) was measured using the AQUALAB water activity meter 4TE (METER Group, Inc., Pullman, WA, U.S.).

1.6. Rice Milling

After TMC and water activity measurements of the rough rice, the remainder of the samples were placed on a metal screen and dried in a controlled environment at 25 ± 3°C and 60 ± 5% relative humidity to attain 12.5 ± 0.5% MC. Dehulling was done using an impeller husker (Model FC2K, Yamamoto, Yamagata, Japan). Milling was done using a laboratory mill (McGill No. 2,

RAPSCO, Brookshire, TX, U.S.), having a 1.5 kg mass placed 15 cm from the centerline of the milling compartment on a 0.650 kg lever arm. Milling duration was standardized to 41 seconds to attain a head rice surface lipid content (SLC) of 0.4% as measured by near-infrared reflectance (NIR) spectroscopy (DA 7200, PERTEN Instruments, Hägersten, Sweden). After milling, the bran was collected for further analysis. The milled rice was aspirated to remove excess bran and then head rice kernels were separated from the broken kernels. A kernel was considered head rice if the kernel length is at least ¾ of the length of an intact and unbroken milled kernel (USDA, 2009). Head (whole) rice yield (HRY) was calculated as the mass of whole kernels to the mass of rough rice.

The head rice which resulted from milled rough rice was used for starch, free sugar, HMF, and discoloration analyses. The bran obtained from rough rice milling was used for crude oil, FFA, and protein analyses.

1.7. Gross chemical composition

Total starch was measured using the Total Starch Assay Kit (AA/AMG) from Megazyme (Megazyme u.c., Co. Wicklow, Ireland), according to the recommended procedure which is based on the American Association of Cereal Chemists (AACC) International Method 76-13.01. Rice bran oil (RBO) was extracted from the rice bran (30 g) mixed with hexane (250 mL) for 10 min according to Proctor & Bowen (1996). Only rice bran that could pass through a 0.50 mm sieve was used. The solvent/oil mixture was filtered over a Whatman® grade 1 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA, U.S.), and the solvent was evaporated using a rotavapor. The amount of RBO was measured gravimetrically. Protein content was measured with a micro-Kjeldahl method according to AACC International Method 46-13.01 on the defatted rice bran (cfr.

supra). For the digestion, 0.500 g of defatted rice bran was mixed with 5 mL concentrated sulfuric acid and a Kjeldahl digestion tablet (CT-37, Kelmate, Darmstadt, Germany) for 2 hr on a heater unit (Labconco 6000, Labconco Corporation, Kansas City, MO, U.S.). The nitrogen from the digested bran was captured with a distillation unit (Labconco Rapid Still Distillation System, Labconco Corporation, Kansas City, MO, U.S.) as ammonia in a 40 g L^{-1} boric acid receiver solution containing methyl red-bromocresol green as titration indicator. Titration was performed with 0.025 mol L^{-1} HCl. The nitrogen to protein conversion factor used was 5.95 (Juliano, 1972).

1.8. Free fatty acids

The FFA was measured on the extracted RBO (cfr. *supra*) from the recovered rice bran, immediately after milling. Determination of the FFA was done according to the AACC International Method 58-15.01. The extracted oil was dissolved in 75 mL isopropanol (≥99%). The mixture then was titrated with sodium hydroxide (0.25 M); phenolphthalein was used as an indicator. The FFA number is expressed as percentage oleic acid equivalents.

1.9. Free sugar analysis

Head rice was ground to a fine flour using a cyclone mill (Cyclone Sample Mill Model 3010-030, UDY Corporation, Fort Collins, CO, U.S.) to pass a 0.50 mm sieve. Free sugars were extracted from rice flour (0.100 g) with aqueous ethanol (80% v/v). Briefly, 5.0 mL of 80% v/v aqueous ethanol was added to the rice flour, vortex-stirred and incubated at 85°C for 5 minutes. Another 5.0 mL of 80% v/v aqueous ethanol was added, vortex-stirred again, and the suspension was centrifuged for 10 min at 2000 g. The supernatant, containing the free sugars, was removed and kept aside. A second extraction involved adding 10 mL of 80% v/v aqueous ethanol, vortex-stirring and centrifuging as above. The supernatant was carefully poured off and added to the first

extraction. The sugar determination and quantification of the rice extract was analyzed using a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). From the sugar extract, 1 mL was transferred into a 2.5 mL microcentrifuge tube, dried overnight at 40°C to remove the ethanol and dissolved with 1 mL of Millipore water. The sample was centrifuged at 5000 g for 5 min and 0.6 mL of the sample was injected into a Dionex ICS-3000 ion chromatography system (Dionex Corporation, Sunnyvale, CA, U.S.), equipped with an AS40 autosampler, a 50 mm AminoTrap guard column, and a 250 mm CarboPac PA 10 analytical column. The sample was eluted with 90 mM sodium hydroxide as the mobile phase at a flow rate of 1 mL min^{-1}.

1.10. Free 5-hydroxymethyl-2-furaldehyde (HMF)

The HMF content was quantified with a spectrophotometric assay using 2-thiobarbituric acid (TBA), based on Keeney & Bassette (1959). Rice flour (1.000 g) was defatted in two steps by adding 10 mL of hexane, stirring for 10 min and centrifuging for 10 min at 2000 g. The supernatant was discarded, and a second defatting was performed as above. The defatted rice flour was dried overnight under a fume hood after which it was mixed with 10.0 mL oxalic acid solution (0.15 M) to enhance the TBA dye-binding properties and placed on a shaker for 30 minutes. For deproteinization, 5.0 mL trichloroacetic acid (TCA) solution (40% (w/v)) was added, centrifuged for 10 minutes at 3000 g and 4.0 mL of the supernatant was mixed with 1.0 mL of TBA solution (0.05 M). After incubation at 40°C for 40 minutes, the absorbance was measured at 443 nm using a UV-Visible Spectrophotometer (DU 730, Beckman Coulter Inc., Fullerton, Calif., USA). An external HMF standard was made as above, replacing 1.000 g of defatted rice flour with 1.000 g of deionized water. The limit of detection (LOD) was 0.09, corresponding to 6 ppm according to the standard curve and was calculated as follows:

$$LOD = \frac{k \times SD_{blanks}}{Slope_{calibration\ curve}}$$

A factor of 3 was used for *k,* corresponding to a chance of 1.7% of measuring background noise as a correct value (Type I error) (Shrivastava & Gupta, 2011). SD is the standard deviation.

1.11. Discoloration analysis

The color of the head rice was measured using image analysis (WinSEEDLE Pro 2005a, Regent Instruments, Sainte-Foy, Quebec, Canada). The software was calibrated using nine specific milled rice colors: translucent white, opaque white, red/pink, black/brown, red/brown, three shades of yellow (yellow 1, 2 and 3) and salmon (Haydon & Siebenmorgen, 2017). Approximately 100 head rice kernels were spread (no kernel-kernel touching) on an acrylic tray holder (152 mm x 100 mm x 20 mm), covered with a blue plastic as background and scanned to create the analysis profile. The software determined each kernel and its area with a pixel-by-pixel assessment. The software measured the projected area of each of the nine defined colors per kernel. The sum of individual projected areas was regarded as the total projected area. The software reports the percentage of each color category per kernel and gives the total percentage of each color category for the total projected area. Percentage of discoloration is calculated as the sum of the discolored areas divided by the total projected area.

1.12. Statistical analyses

The results were expressed as the mean ± SD. Statistical analysis was performed using JMP Pro 14.0.0 (2018, SAS Institute Inc., Cary, NC, U.S.). All variables were tested for normal distribution and homoscedasticity applying the Shapiro-Wilk test and the Brown-Forsythe test, respectively. For parametric variables, analysis of variance (ANOVA) was used to determine

significant differences and Tukey's HSD (honestly significant difference) test was used as a post-hoc analysis. For non-parametric variables, the Kruskal-Wallis test was used and the Steel-Dwass test was used as a post-hoc analysis. Results were considered significant when $p < 0.05$. All the variables were considered parametric unless otherwise stated.

For each rice sample, a storage environment was defined by a specific MC, storage temperature and irradiation treatment such as non-irradiated rice stored at 21% MC and 20°C.

2. Results and Discussion

3.1 Gross chemical composition

The protein, starch, and crude oil content of rice samples exposed to different treatments in this study are presented in table 1. Generally, the storage duration, irradiation, and storage temperature had no significant effect on the total starch, crude oil and total protein content. But few different cases are explained as follows: as shown in table 2, the average of total protein content for the irradiated rice samples stored at 40°C was significantly lower ($p = 0.04$) than the other rice samples. The mean amounts of total starch and RBO in the same sample were 74.6 ± 2.45% and 14.1 ± 1.02%, respectively (data not shown). The mean total protein content in irradiated rice stored at 40°C for 2, 4, 6 and 8 weeks was 12.2 ± 0.16%, while 13.6 ± 0.30% for the non-irradiated rice stored at the same condition (table 2). The minimal changes of total starch and protein during rice storage were also reported by Swamy, Sowbhagya & Bhattacharya (1978). Important to mention here is the nature of the starch quantification method; the enzyme kit measures the amount of hydroperoxide, which is the stochiometric equivalent of the amount of D-glucose after a full hydrolysis of the starch. Ananthaswamy, Vakil & Sreenivasan (1970) talked about starch hydrolysis caused by storage ageing and radiolysis, respectively. This results in the

formation of maltodextrins, which are eventually transformed into D-glucose in the total starch assay.

The significant lower total protein content in the EBI rice stored at 40°C can be explained by the aggregation and cross-linking induced by the irradiation, enhanced by the high temperature; that could lead to an underestimation of the nitrogen content during the Kjeldahl analysis (Stewart, 2001).

3.2 Water activity and total mold count on stored rice

The water activity for all the samples ranged from 0.91 to 0.95. The water activity was not significantly affected by EB irradiation and storage conditions. The consistency of water activity during storage at different temperatures and storage durations is in agreement with a study done by Mohammadi Shad and Atungulu (2019) on the same hybrid rice used in the current study.

The non-irradiated controls had a TMC of 5.6 ± 0.07 log CFU g^{-1}. However, the EBI control rice samples showed a TMC of 2.2 ± 0.08 log CFU g^{-1}. The TMC of non-irradiated and EBI rice stored at higher temperatures decreased significantly. As seen in Figure.1, the trend for non-irradiated rice is a decrease in TMC after 2 weeks of storage compared to the control. This decrease is larger with increasing temperatures. During the first 2 weeks of storage, TMC decreased with 1.5 log-units, 2.1 log-units and 2.6 log-units at 20°C, 30°C and 40°C storage temperature, respectively. However, the opposite trend was seen for EBI rice. After 2 weeks of storage, the TMC increased with 0.9 log-units, 1.1 log-units and 0.2 log-units at 20°C, 30°C and 40°C storage temperature, respectively. After 8 weeks of storage, TMC in EBI and non-irradiated rice are the highest with the lowest storage temperature and vice versa.

The efficiency of EB irradiation on microbial decontamination is variable as reported by different studies. For example, Cuero, Smith & Lacey (1987) were able to sterilize rice grains with only 12 kGy of irradiation fully. However, the TMC of rice reduced with 3.4 log units using only 7.5 kGy of EB irradiation in a study done by Sarrías, Valero & Salmerón (2003). This variation can be explained by the load of initial TMC on rice grains which was quite high in the current study (5.6 log CFU g^{-1}). Similarly, Atungulu, Zhong, Thote, Okeyo, & Couch (2015) found TMC of 6.26 and 5.97 log CFU g^{-1} for the XL753 rice, harvested in 2013 and 2014, respectively. The same level of initial TMC in rice was also reported by Mohammadi Shad & Atungulu (2019) who found TMC in a range of 5.3 to 6.3 log CFU g^{-1}.

The pattern of increasing TMC after irradiation of the rice samples, followed by a stationary phase agrees with the microbial growth curve. The opposite is seen for the non-irradiated samples; a decrease in TMC after 2 weeks of storage, followed by a stationary phase. Since molds are aerobic microorganisms, the depletion of oxygen in the jars could be the reason for the seen reduction pattern. The jars were sealed air-tight and there was as good as no volume of head space. This could have resulted in the reduction of the initial TMC towards a level where the molds immediately transitioned into the stationary phase.

3.3 Head rice yield (HRY)

Neither storage temperature, EB irradiation nor any interactions had a significant effect on the HRY ($p = 0.97$). The overall mean HRY was $52.3 \pm 2.18\%$. Pereira, Cooper & Siebenmorgen (2008) reported that HRY was dependent on rice cultivar and milling duration, but not on storage duration for up to 4 months. Similarly, HRY, during the first sixteen weeks of storage, did not

show any significant changes, independent of storage temperature and MC (Haydon & Siebenmorgen, 2017).

3.4 Free fatty acids (FFA)

Table 3 shows the levels of FFA in rice bran obtained from stored rough rice. The control samples did not show a significant difference in the FFA between non-irradiated and EBI rice. In a study by Rhodes & Meegungwan (1962), enzymes were not deactivated by radiation up to 50 kGy. In non-irradiated rice stored at 20°C and 30°C, the percentage of FFA in the bran did not increase with more than 0.3% compared with the control samples. However, non-irradiated rice stored at 40°C had a significantly higher amount of FFA for every increasing storage duration, reaching 5.6 ± 0.19% of FFA after 8 weeks of storage. The trend of increasing FFA for non-irradiated and EBI rice stored at 40°C was similar. The highest amount of FFA was found in the EBI rice stored at 30°C, having 7.7 ± 0.08% of FFA in the bran after 8 weeks of rough rice storage. This is possibly caused by different types of lipases, each with its optimal temperature of activity. Researchers found 2 types of rice bran lipases (lipase I and lipase II) with an optimal temperature of activity of 37°C and 27°C, respectively (Aizono, Funatsu, Fujiki, & Watanabe, 1976; Aizono, Funatsu, Sugano, Hayashi, & Fujiki, 1973). The relative activity of both enzymes at 30°C is 80% or higher, but at 40°C, the activity of lipase II drops drastically to below 60%. The release of these lipases could be facilitated by radiolytic breakage of cell membranes (Patrick, 1977). It was noted that increasing the storage temperature to 40°C increases the fluidity of the membranes, as the phospholipids become less rigid. This increases the permeability by which the mobility of enzymes, lipases, in this case, increases. This is also true for the spherosomes that contain the lipids (Chandler, 2018).

3.5 Free sugar analysis

Figure 2 shows the amounts of sucrose, glucose and fructose. The mean amount of sucrose, glucose and fructose for the non-irradiated control samples were 586 ppm, 81 ppm and 85 ppm, respectively. Considering EBI control samples, these mean amounts were 637 ppm, 202 ppm and 96 ppm, respectively. The high level of glucose found in EBI rice confirms the theory proposed by Ananthaswamy et al. (1970) that ionizing irradiation induces cleaving in glycosidic bonds from starch. The amounts of glucose and fructose in the non-irradiated rice, stored at 20°C and 30°C did not change significantly during the storage and stayed in the range of 80 ppm to 120 ppm. For EBI rice samples stored at 20°C, a slight increase in both of these sugars was noted after 8 weeks of storage, but still in amounts less than sucrose. No sucrose was detected after 8 weeks of storage in non-irradiated rice stored at 40°C and EBI rice stored at 30°C and 40°C. Sucrose levels after 8 weeks in non-irradiated rice stored at 30°C decreased to 130 ppm, while still 348 ppm of sucrose was measured for 20°C non-irradiated stored rice. The highest amounts of free sugars detected were glucose and fructose in EBI rice stored at 40°C, with maximum amounts of 3755 ppm of glucose after 6 weeks and 3634 ppm of fructose after 2 weeks. In general, the amounts of glucose followed the same pattern as the amounts of fructose for every storage environment; if the amount of glucose increased during storage, so did the amount of fructose and vice versa. The general trend of decreasing sucrose and increasing glucose and fructose found in the current study was similar to what was seen by Tran et al. (2005), who stored brown and milled rice for up to 10 months. Enzymes (e.g. invertase) and high temperature induced reactions (e.g., isomerization) can explain the changes in the sugar content during rice storage. Patrick (1977) mentioned the damage of cell membranes caused by irradiation, therefore facilitating the movement of enzymes towards substrate molecules. The invertase enzyme found in rice could be responsible for the decrease in

sucrose and a simultaneous increase in glucose and fructose since this enzyme catalyzes the irreversible hydrolysis of sucrose to glucose and fructose (Ji, Van den Ende, Van Laere, Cheng, & Bennett, 2005).

Apart from sucrose, glucose and fructose, raffinose was also found in some samples. Raffinose was only found in non-irradiated samples stored at 20°C and 30°C after 2, 4, 6 and 8 weeks of storage in various amount, ranging from 63 ppm to 323 ppm. Raffinose was reported in rice bran in amounts of 7-13% by Pascual, Singh, Juliano (1978) and 5% by Saunders (1985). Most of the free sugars were found in the aleurone layer which is partially removed during the milling process, depending on the degree and duration of milling. Therefore, the following scenarios can explain raffinose presence in non-irradiated rice versus EBI rice: Hayashi, Okadome, Toyoshima & Todoriki, (1998) suggested that electron-degraded starch near the surface of the rice kernel could be removed more easily during milling compared to non-irradiated rice. Thus, the same milling duration could result in the removal of the raffinose present in the aleurone layer of the kernel. Also, the higher temperatures induced starch breakdown due to an increased amylase activity, as shown by Li, Zhang, Fu, Li & Li (2017), leading to a higher removal of aleurone layer. Furthermore, α-galactosidases found in rice have a high affinity for raffinose, hydrolyzing the α-1,6-bond (Fujimoto, Kaneko, Momma, Kobayashi & Mizuno, 2003). The high temperature could increase their enzymatic activity, as Zhang et al. (2015) found fungal α-galactosidases with the highest relative activity at 60°C.

3.6 Free 5-hydroxymethyl-2-furaldehyde (HMF)

Figure 3 shows the levels of HMF found in the head rice. The levels of HMF in controls of non-irradiated rice and EBI rice were 0.5 ppm and 3.9 ppm, respectively. All the measured

concentrations of HMF in the stored non-irradiated rice were below the LOD. For the EBI rice, only rice stored at 30°C for 4, 6 and 8 weeks and at 40°C for 2, 4, 6 and 8 weeks had HMF levels above the LOD. Levels of HMF in non-irradiated rough rice stored at 20°C and 30°C did not exceed 0.8 ppm. Non-irradiated rough rice stored at 40°C, however, showed a slight increase in HMF, increasing from 0.5 ppm in the control up to 5.7 ppm after 8 weeks of storage. Notable increases in HMF appeared in the EBI rice stored at 30°C and 40°C where amounts of 9.5 ± 0.26 ppm and 29.1 ± 0.43 ppm, respectively, were measured after 8 weeks of storage.

The HMF is an indicator of non-enzymatic browning occurring in irradiated and stored rough rice. In past studies, the HMF has been used as a good indicator of the extent of the Maillard-reaction (Lamberts et al., 2008). It could, therefore, be concluded that high ionizing radiation induced non-enzymatic browning in rice. The HMF amounts found in the current study also corresponded to the values measured by Lamberts et al. (2008) in brown rice parboiled for 30 min at 50°C followed by steaming.

3.7 Discoloration and its link to biochemical changes

Figure 4 shows the percentage of discoloration and the corresponding color categories. The initial kernel discoloration in controls was 0.8 ± 0.14% and 28.6 ± 2.31% in non-irradiated and EBI rice, respectively. As illustrated in Figure 4, the non-irradiated rough rice stored at 20°C and 30°C did not exceed more than 5% of discoloration after 8 weeks of storage. But when non-irradiated rice was stored at 40°C, discoloration of 23.1 ± 2.50% occurred just after 2 weeks and approached 100% discoloration after 8 weeks of storage. The discoloration trend was consistent with the rice storage study by Mohammadi Shad & Atungulu (2019) who found complete discoloration in milled rice (XL753) after 16 weeks of the rough rice storage, regardless of MC.

Discoloration of the EBI rice stored at 20°C and 30°C for 2 weeks increased by 47.9% and 66.2%, respectively, compared to the control samples. The EBI rice stored at 40°C reached almost 100% of discoloration after 2 weeks of storage.

Yellowing was the predominant discoloration in rice stored at 20°C and 30°C for both non-irradiated and EBI rice, although in higher absolute amounts for the latter one. Non-irradiated rice stored at 40°C was mainly discolored with yellow kernels during the first 4 weeks, but this amount shrunk during the subsequent 2 weeks, where the darker colors salmon and red/brown increased and became more prevalent. EBI rough rice stored at 40°C had a complex color pattern with approximately equal amounts of yellow, pink/red and red/brown. The results of discoloration are in agreement with others found during rough rice storage at different temperatures and MCs (Phillips et al., 1988; Haydon & Siebenmorgen, 2017; Shad & Atungulu, 2018).

In the current study, rice discoloration was not directly related to the mold growth. The correlation coefficient showed negative and significant correlation of discoloration with TMC, r-value was -0.50 ($p<0.05$). For instance, at a higher temperature where the discoloration was more predominant, there was less amount of mold count. Similarly, Mohammmadi Shad & Atungulu (2019) found an inverse relationship between rice discoloration and TMC; they observed a higher discoloration at higher storage temperature (>30°C) where the TMC was less.

The multivariate analysis of correlation illustrated that rice discoloration was highly and positively correlated with free sugar contents (glucose and fructose), FFA, and HMF concentartion. The r-values range from 0.63-0.72 with all correlations significant at the 0.05 level. Therefore, changes in physicochemical properties in rice can explain the discoloration found in the current study. High levels of HMF formation was observed in rice samples treated and stored at environments that promoted high rice discoloration. For example, the high discoloration in non-

irradiated rice stored at 40°C corresponded with high levels of HMF. Also, there were high levels of glucose and fructose, both reducing sugars, in samples with discoloration of more than 20%. Reducing sugars like glucose and fructose are the main ingredients that participate in non-enzymatic browning. The discoloration of rice kernels as a result of parboiling process was correlated with Maillard reaction where off-white color of rice was correlated with higher levels of HMF content. (Xu et al., 2019).

Furthermore, the high percentages of discoloration in non-irradiated rice stored at 40°C, happened with significantly higher amounts of FFA. These FFA are more susceptible to oxidation reactions, which are linked to browning reactions (Hidalgo & Zamora, 2000). Non-starch lipids in rice have been suggested to contribute to the discoloration of stored rice (Juliano, 1985).

In general, chemical changes were affected greatly by storage temperature which played a significant role in rice discoloration. At storage temperature more than 30°C, the HMF was clearly higher in highly discolored rice samples (≥20%) compared to low discolored rice (<20%). In addition, the higher amount of HMF was linked with observed trends for free sugars; at rice samples with less than 20% discoloration, the glucose and fructose were significantly less than rice samples with more than 20% discoloration. However, the opposite trend was seen for sucrose. Sucrose is a non-reducing sugar which is unable to undergo non-enzymatic browning. However, a high abundance in glucose and fructose, both reducing sugars, can create a pool of reagents for the non-enzymatic browning. The non-enzymatic browning reactions involve several condensation reactions that can result into discoloration (Lamberts et al., 2008).

3. Conclusions

The biochemical properties of non-irradiated and EBI long-grain rice during storage and their links to rice discoloration were studied. The EB irradiation of 14 kGy caused the microbial reduction of 99.9% in EBI rice samples. However, there were no noticeable changes in HRY, total protein, crude oil, and starch content between non-irradiated and EBI rice even after 8 weeks storage. Interesingly, the initial higher rice discoloration in EBI rice than non-irradiated rice was found to be as a result of the soaring FFA and HMF in the samples. Also, the FFA increased in the bran of non-irradiated rice stored at 30°C and EBI rice stored at 30°C and 40°C. The amounts of free sugars and HMF in control samples of EBI rice was higher than that in non-irradiated rice; and amounts continued to increase in both, non-irradiated and EBI rice, stored at higher temperatures where a higher rate of discoloration was also found. The effect of storage temperature on discoloration was significant in both non-irradiated and EBI rice. In general, there was a significantly higher level of browning markers such as FFA and HMF in highly discolored rice samples. The current study confirms that there was a significant influence of chemical reactions such as non-enzymatic browning on discoloration observed on rice following storage specially when rice was irradiated with EB. The results indicated that reactions such as lipid oxidation and non-enzymatic browning were more linked to observed rice discoloration as opposed to microbial activity.

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5. Tables and Figures

Table 1. Total protein in bran, crude oil content in bran and starch in head rice resulted from rough rice stored at different conditions and with different treatments (N=3)

(A)	Storage Duration (weeks)				
	0	**2**	**4**	**6**	**8**
Protein (%)	13.6 ± 0.09	13.6 ± 0.75	13.6 ± 0.68 ± 0.73	13.43 ± 0.60	13.63
Total starch (%)	72.9 ± 2.00	73.5 ± 1.17	74.8 ± 2.49	76.0 ± 2.64	75.7 ± 2.29
Crude oil content (%)	13.9 ± 0.66	14.6 ± 1.27	14.5 ± 1.16	13.8 ± 0.79	13.6 ± 0.77

(B)	Irradiation		Temperature (°C)		
	***EBI vs. Non-irradiated**		**20**	**30**	**40**
Protein (%)	13.5 ± 0.77	13.7 ± 0.23	13.8 ± 0.30	13.9 ± 0.29	13.1 ± 0.71
Total starch (%)	74.4 ± 2.16	74.4 ± 2.72	74.9 ± 2.59	74.1 ± 2.47	74.7 ± 2.26
Crude oil content (%)	14.1 ± 1.01	14.1 ± 1.04	14.5 ± 1.08	14.2 ± 0.94	13.5 ± 0.71

* EBI indicates electron beam irradiated rice samples.

Table 2. Mean and standard deviation of rice bran protein content (% ± SD) of stored rice. Different lowercase letters mean a significant difference in a row, different uppercase letters indicate significant difference in the same column with the given p-value (N=3)

Storage Temperature (°C)	Bran Protein content (%)		P-value
	Non-Irradiated	Electron Beam Irradiated	
20°C	13.7 ± 0.26 ^{aA}	14.0 ± 0.36 ^{aA}	0.0810
30°C	13.9 ± 0.18 ^{aA}	14.1 ± 0.34 ^{aA}	0.1558
40°C	13.6 ± 0.30 ^{aA}	12.2 ± 0.16 ^{bB}	< 0.0001
P-value	0.1818	< 0.0001	

Table 3. Mean free fatty acids (% ± SD) found in rice bran during storage of rough rice. Different letters in a row indicate a significant difference with the given p-value (N=3)

Storage (weeks)	Non-Irradiated			P-value
	20°C	30°C	40°C	
0	1.2 ± 0.08 ^{a}	1.2 ± 0.08 ^{a}	1.2 ± 0.08 ^{a}	1.0000
2	1.2 ± 0.08 ^{a}	1.4 ± 0.10 ^{a}	3.1 ± 0.15 ^{b}	< 0.0001
4	1.3 ± 0.06 ^{a}	1.3 ± 0.06 ^{a}	3.4 ± 0.08 ^{b}	< 0.0001
6	1.2 ± 0.04 ^{a}	1.2 ± 0.02 ^{a}	4.1 ± 0.01 ^{b}	< 0.0001
8	1.4 ± 0.04 ^{a}	1.4 ± 0.01 ^{a}	5.6 ± 0.19 ^{b}	< 0.0001
	Electron Beam Irradiated			
	20°C	**30°C**	**40°C**	
0	1.2 ± 0.02 ^{a}	1.2 ± 0.02 ^{a}	1.2 ± 0.02 ^{a}	1.0000
2	1.2 ± 0.02 ^{a}	2.0 ± 0.10 ^{b}	2.8 ± 0.05 ^{c}	< 0.0001
4	1.4 ± 0.03 ^{a}	3.6 ± 0.00 ^{b}	3.1 ± 0.12 ^{c}	< 0.0001
6	1.6 ± 0.09 ^{a}	5.7 ± 0.20 ^{b}	4.1 ± 0.05 ^{c}	< 0.0001
8	1.9 ± 0.00 ^{a}	7.7 ± 0.08 ^{b}	5.6 ± 0.18 ^{c}	< 0.0001

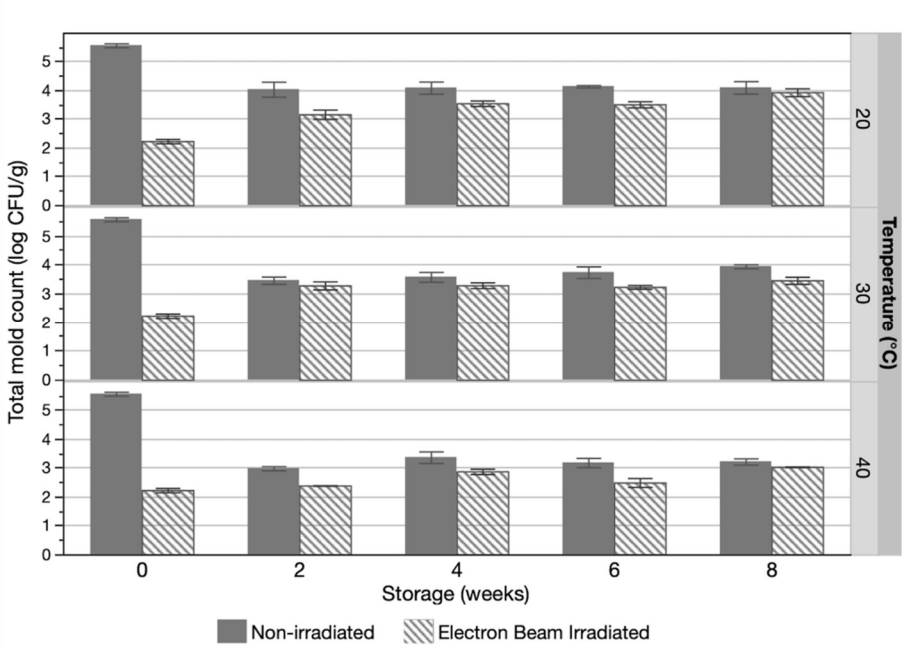

Figure 1. Total mold count means of the non-irradiated and electron beam irradiated rice during storage (error bars representing standard deviations)

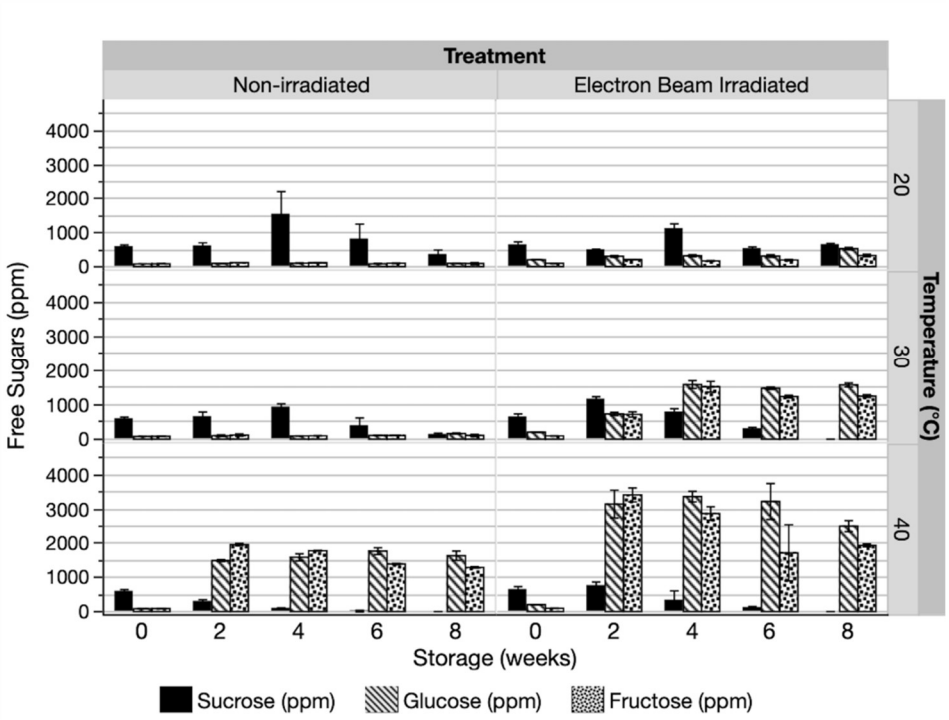

Figure 2. Amount of glucose, fructose and sucrose (ppm) found in head rice after storage of rough rice. (Error bars represent the standard deviations)

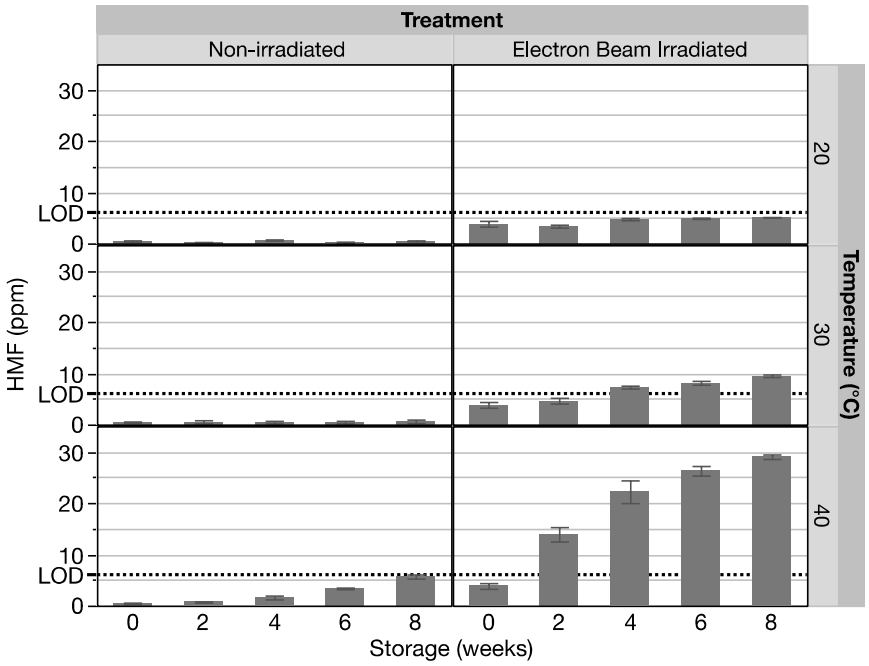

Figure 3. Levels (ppm) of 5-hydroxymethyl-2-furaldehyde (HMF) in head rice after storage of rough rice. Error bars are the standard deviations (LOD indicates the limit of detection).

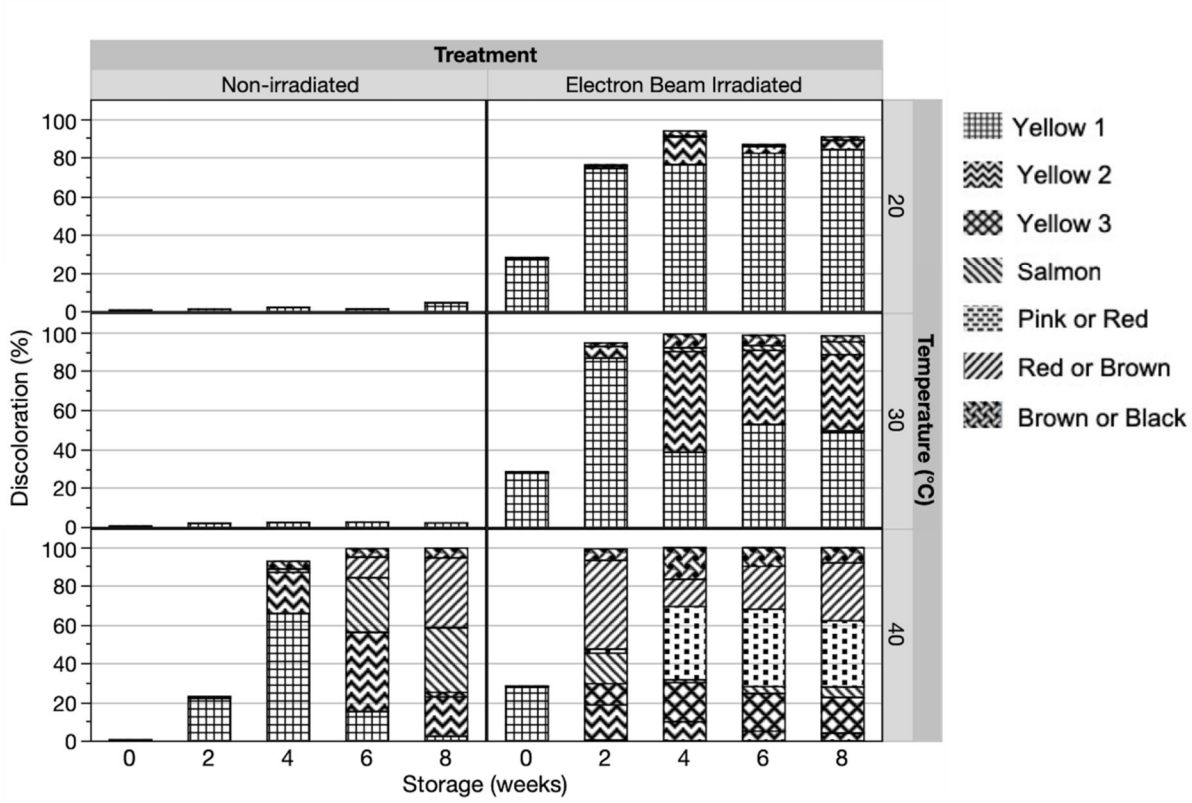

Figure 4. Distribution of discoloration on head rice stored at indicated temperature and storage duration for non-irradiated and electron beam irradiated samples.

Chapter 6. Conclusions

The study was a comprehensive investigation of biochemical factors associated with rice discoloration. A broad range of factors are considered. These included temperature, rice MC, rice cultivars, storage duration, and application of antifungals. The study aimed to provide an overall understanding of the physiology of rice discoloration under the considered factors. Notably, the role of fungi and chemical reactions were explored in detail. This subject has not been previously studied exhaustively; literature to date is very inclusive.

The study showed that rice discoloration highly depends on storage conditions and most particularly on temperature. Considering the significant discoloration observed at higher temperatures, cooling of rice as a technology may provide a viable option for the short-term preservation of high-MC rice. Low-temperature storage was generally successful at preventing discoloration, depending on the cultivar. Rice discoloration could be slowed down by antifungal treatments during storage. Implementing such antifungal treatment as a practice could preserve and even enhance the quality of a farmer's rice. However, at higher temperature and storage duration, chemical browning reactions like Millard reaction cause rice discoloration.

Overall, rice discoloration is influenced by an interaction of different storage parameters and results from both microbial and chemical reactions. Cooling of rice could be used in on-farm in bin storage as an approach to maintain the grain quality. Moreover, mild antifungal treatments could further help the grain color and preserve other quality attributes including HRY.