MICROBIAL ASSESSMENT OF RICE SAMPLES COMMONLY SOLD IN MUBI MARKET, ADAMAWA STATE, NIGERIA (MANUSCRIPT NUMBER: (MANUSCRIPT NUMBER: 2021-198)

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Abstract

Rice can be a source of food poisoning because it can be contaminated with dangerous pathogens. The aim of this study is to assess the microbial quality of some rice sold in Mubi market. A total of Eight (8) different rice samples purchased from Mubi market were analyzed to study the microbial loads by standard microbiological techniques. The Moisture content was also investigated. Highest moisture content (11.5%) was obtained from local rice whiles the lowest moisture content (7.5%) was observed in foreign rice. The mean ACC for the cooked and uncooked rice samples ranged between 2.67 ± 0.39 to 3.19 ± 0.06log_{10} CFU/g and 2.69 ± 0.3 to 3.34 ± 0.03log_{10} CFU/g respectively. The ACC was found to be higher in foreign rice as compared to the local rice for both cooked [redacted] *and uncooked rice samples. For the fungal counts, the mean FCC of the cooked and uncooked rice samples ranged from 1.0 ± 0.0 to 2.45 ±0.64log_{10} CFU/g and 1.0 ± 0.0 to 3.15 ± 0.21log_{10} CFU/g in that order. The mean fungal count for the local rice was found to be higher as compared to the*

KEYWORDS: rice, pathogens, microbial assessment, food poisoning, *Bacillus cereus*

foreign for both cooked and uncooked rice samples. Salmonella species, Staphylococcus aureus, and E. coli were not detected in all the samples.

INTRODUCTION

Rice is the seed of the monocot plant *Oryza sativa* of the grass family *Gramineae* (Kuldeep, 2006). It is regarded as one of the three major crops in the world. It also forms a staple diet of one third of the world population. The global production of rice has been estimated to be at a level of 65 million tonnes. Asia is a leading rice producing nation accounting for 90% of the total rice production, 75% of which is consumed within the continent. This makes rice to be the most important food grain with respect to food security in Asia (Chem, 2005). Moreover, it is considered as one of the most nutritious food source with carbohydrate, protein, zinc and niacin being the most important components (Lairon, 2010; Jamil, 2016)

Rice cultivation is the principal activity and source of income for millions of households around the globe. Several countries of Asia and Africa are highly dependent on rice as source of foreign exchange earnings and government revenue. (Rice Trade [RT], 2011). Rice is the second largest produced cereal in the world after wheat; it is a crop that cuts across regional, religious, cultural, national and international boundaries with very high demand. Today, rice is grown and harvested on every continent except Antarctica (RT, 2011), where conditions make its growth impossible. Asian farmers (India, China, Indonesia, Vietnam, and Bangladesh) account for 92% of the world's total rice production. More than 661 million tonnes of rice is produced annually around the globe (United States Development Agency [USDA], 2009)

Rice is an increasingly important crop in Nigeria. It is relatively easy to produce and it is grown for sale and for home consumption. In some areas there is a long tradition of rice growing, but for many, it is

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considered a luxury food for special occasions only. With the increased availability of rice, it has become part of the everyday diet of many people in Nigeria. There are many varieties of rice grown in Nigeria; some of these are traditional varieties while others have been introduced into the country. Rice is grown virtually in all the agro-ecological zones in Nigeria (Akande, 2003). This is because, Nigeria have ideal climatic conditions which is akin to that of South East Asia where the crop is produced for export.

Cereals and cereal products contain microorganisms from insects, soil and other sources. *Bacillus, micrococcus* and molds like *Aspergillus, penicillum* are very common, ((Ray, 2001). Microbiological analysis of cereal grains is not routinely performed, nevertheless, the determination and count of total mesophilic aerobes, *coliforms* (*Escherichia coli*), moulds and yeast, *Bacillus cereus* and *Salmonella* have been used in order to ascertain hygienic-sanitary quality (Anderson, 1992). Rice, the staple diet in most Asian countries, may become contaminated during growth, harvesting and other agricultural operations such as processing and handling (Haque & Russell, 2005). In general, members of the *Pseudomonadaceae, coliforms*, endospore-forming bacteria, yeast and moulds are the most common members of the rice microflora. Worldwide there is increasing demand for high-quality and safe food, free of chemical and physical contaminants and pathogens.

Rice is arguably the most important foodstuff associated with *Bacillus cereus* food poisoning

(Ray, 2001), *B. cereus* comprises 10% of the soil micro flora in rice paddies (Varnam & Evans, 1991; Sarrias *et al*., 2002). *B. cereus* grows and produces emetic toxins in a relatively short time on cooked rice and other starchy foods stored at room temperature (Agata *et al*., 2002).

Rice can be a source of food poisoning because it contaminated with dangerous pathogens. In general, food poisoning can cause by contaminant bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* group (except *Salmonella typhi*), *Shigella, Vibrio, Escherichia coli, Campylobacter, Yersinia enterocolitis, Clostridium* (Bardale, 2017). Rice-based food poisoning more often caused by *Bacillus cereus* and *Staphylococcus aureus* based on several cases in the world (Park *et al*., 2009; Ankolekar *et al*., 2009; Delbrassinne *et al*., 2012; Tahir *et al.,* 2012; Rahimi *et al*., 2013; Bilung *et al*., 2018; Leka *et al*., 2019; Yu & Wang, 2020; Hartini, 2001; do Carmo et al., 2003; Su Kyung et al., 2007; Erza, 2010; Basavegowda et al., 2014; and Radji et al., 2019) Besides, *Bacillus cereus* and *Staphylococcus aureus* are significant sources of microbiological harm from cereal grains and related products Food and Drug Administration ([FDA], 2003).

Bacillus cereus can found in soil, plants, and the intestinal tract of insects and mammals. In poor environmental conditions, bacteria can turn into spore forms. *Bacillus cereus* in spore form can found hiding in raw rice. The bacteria move from the soil to the paddy fields, their spores persist for years, even surviving during cooking due to their resistance to extreme temperatures. However, if rice left at room temperature, in warm and humid conditions, the spores can turn into vegetative bacteria and produce toxins that can cause vomiting and diarrhoea (Montville & Matthews, 2005)

Staphylococcus aureus is naturally present in the human body, so these bacteria are one of the essential agents causing food poisoning that often occurs in society. The most significant cause of *Staphylococcus aureus* entry into the food chain (which then causes staphylococcal poisoning) is the low sanitation of workers handling food (Ray, 2001). According to the Food Standards Agency (FSA), there are nearly 900,000 food poisoning cases each year. The lifestyle that has changed in recent years has resulted in an increasing dependence on ready-to-eat food, eating out more than cooking, and busyness results in having less time to prepare and cook food. This habit is the reason that increases the number of cases of food poisoning (Parashnath & Indranil, 2016). Apart from that, environmental factors also influence the level of contamination. Food prepared under unfavourable conditions situation is due to differences in the level of sanitation between developed and developing countries (Ray, 2001)..

Rice, once cooked, is perishable. The spoilage of boiled rice is found to be associated with the presence of Gram-positive *Cocci* and *Bacilli* (Veda et al., 1980). Out of them, *Bacillus cereus* is the most prominent bacterium responsible for the cooked rice spoilage (Veda et. al., 1980), and the bacterium *B. subtilis* also has been isolated as a spoilage agent from cooked rice (Roh et al., 1996). Also Varadaraj et. al. (1992) reported the ability of *B. stearothermophilus, B. brevis, B. laterosporus, B. licheniformis* to grow in cooked rice.

Much research work on the spoilage of cooked rice and its prevention has been recorded in other part of the world however, the report on spoilage after cooking of rice varieties commonly consumed in Mubi is lacking. Therefore, the aim of this work is to assessed the microbial quality of cooked and uncooked of some common rice varieties sold in Mubi Market Local (Kurda brown, Excellent Parboiled Rice brown, & White Lily white) and Foreign (Dansarki white, Cap brown, Champion brown, , Riz Blanc white, & Marori brown)

Materials and Methods

Samples Collection

A total of eight different types of rice samples were purchased from Mubi main market (Kurda brown rice, Dan sarki white rice, Excellent Parboiled Rice brown rice, Cap brown rice, Champion brown rice, White Lily white rice, Riz Blanc white rice, and Marori brown rice)

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Preparation of the Rice Samples:

Fifty (50) grams of rice sample was added to 200 mL peptone water, and then blended in a stomacher for one minute at medium speed. Cooked rice samples were prepared by boiling 50g of raw rice in 225 ml pre-boiled water for 10 minutes.

Bacteriological Examination

Preparation of Samples According to the International Organization for Standardization (ISO) 4833 British Standard (BS) 5763 Part 1 (1991) Protocol

Sample homogenate was prepared based on ISO 4833 BS 5763 Part 1 (1991) Protocol. Twenty five grams of the samples were weighed under aseptic condition and transferred to a sterile Stomacher bag, 225 mL of sterile 0.1% peptone water was added and the contents were homogenized for 2 minutes. The mixture was allowed to stand for 5 minutes at ambient temperature and subsequently transferred into a sterile flask and thoroughly mixed. To prepare a tenth fold serial dilution of the sample, 1 mL was transferred into separate tube each, containing 9 mL sterile 0.1% peptone water. The prepared samples were subjected to the following bacteriological examination:

Determination of Total Aerobic Colony Count

This was carried out according to the method described by ISO 4833 BS 5763 Part 1(1991) on plate count agar.

***E. coli* Pour Plate Colony Count Method on Violet Red Bile Glucose Agar (VRBGA)**

The following pour plate method helps to suppress the growth of non-fermentative organisms. It is based on BS 5763 Part 10 and ISO 21528-3 (1986) Pink to red-purple colonies of diameter 0.5 mm or more with or without haloes of precipitation was counted as positive.

Staphylococcus aureus and other Coagulase Positive _Staphylococci_ Colony Count on Mannitol Salt Agar

This method is based on BS EN ISO 6888-1 which describes the enumeration of coagulase positive staphylococci using Baird–Parker medium with confirmation of colonies by a positive coagulase test result.

Isolation of _Salmonella_ and _Shigella Species_

Homogenized 25 g food sample or 25 mL of drink samples were transferred into 225 mL of lauryl sulfate tryptose (LST) broth (DIFCO, USA) after 24 hrs of incubation at 35 ˚C, 1 loopful of the broth was then streaked onto salmonella-shigella Agar (SSA) (DIFCO, USA). Distinguished grown colonies were then validated using triple sugar iron (TSI) Agar slant as per established procedures (Bacteriological Analytical Manual ([BAM], 2016).

Total Mould and Yeast Count on Potato Dextrose Agar

Based on International Organization for Standardization (ISO) 21527-1 (2008) Method

Fungal Identification and Enumeration

Lacto Phenol Cotton Blue Teased Mount Procedure for Identification

A drop of lactophenol cotton blue dye was placed on the slide, and a sterile iron needle was used to transfer a tiny piece of a colony into Lacto Phenol Cotton Blue Dye on the slide. The colony was then teased into very tiny pieces using an iron needle. The slide was covered with a coverslip with a magnification ×40 used. The identification of the fungi was done macroscopically (texture and colour in the plate) and microscopically by observation of their cultural and morphological features under the microscope. Molds and yeast that appeared were identified by their cultural and morphological characteristics using standard identification manuals (Moss, 1989; Samson et al., 2000).

Moisture Content:

The mass of dish was weighted and the mass recorded. The rice sample of 5g was weighted into the moisture dish and the mass was recorded. The lid was removed and the dish was placed in an oven chamber. The sample was dried at a temperature of 130°C for 1 hour. After the drying is complete the sample was removed from the oven and placed in a desiccator to cool. The mass of the sample and dish was weighed and recorded (Bultosa, 2013). After that time, sample was reweighed and percentage moisture was calculated. It was found that unbranded samples had high moisture content as compared to branded rice samples.

Statistical analysis

Data analysis

Procedures for microbial counts and moisture contents were carried out in triplicates, and data collected were subjected to a single-factor analysis of variance (ANOVA). Differences among means were separated using Tukey's test and significances were accepted at a 5% level ($p<0.05$) using Minitab version 19.0 for windows (Minitab Inc.). The analysis was done using the mean counts expressed in the standard forms which were transformed into logarithmic values and results reported as means ± standard deviation.

Results and Discussion

The moisture content of the rice samples was investigated. The local rice samples showed higher moisture content as compared to the foreign rice samples (Figure 1). The highest moisture content of (11.5%) was observed in local rice samples while the lowest moisture content

(7.5%) was recorded in foreign rice samples. Tahir et. al. (2012) reported a moisture contents of (14.0%) and (8.4%) from unbranded and branded rice samples respectively from local market in Lahore, Pakistan. The difference in moisture contents in this study and the one reported in Pakistan might be due to their climatic conditions.

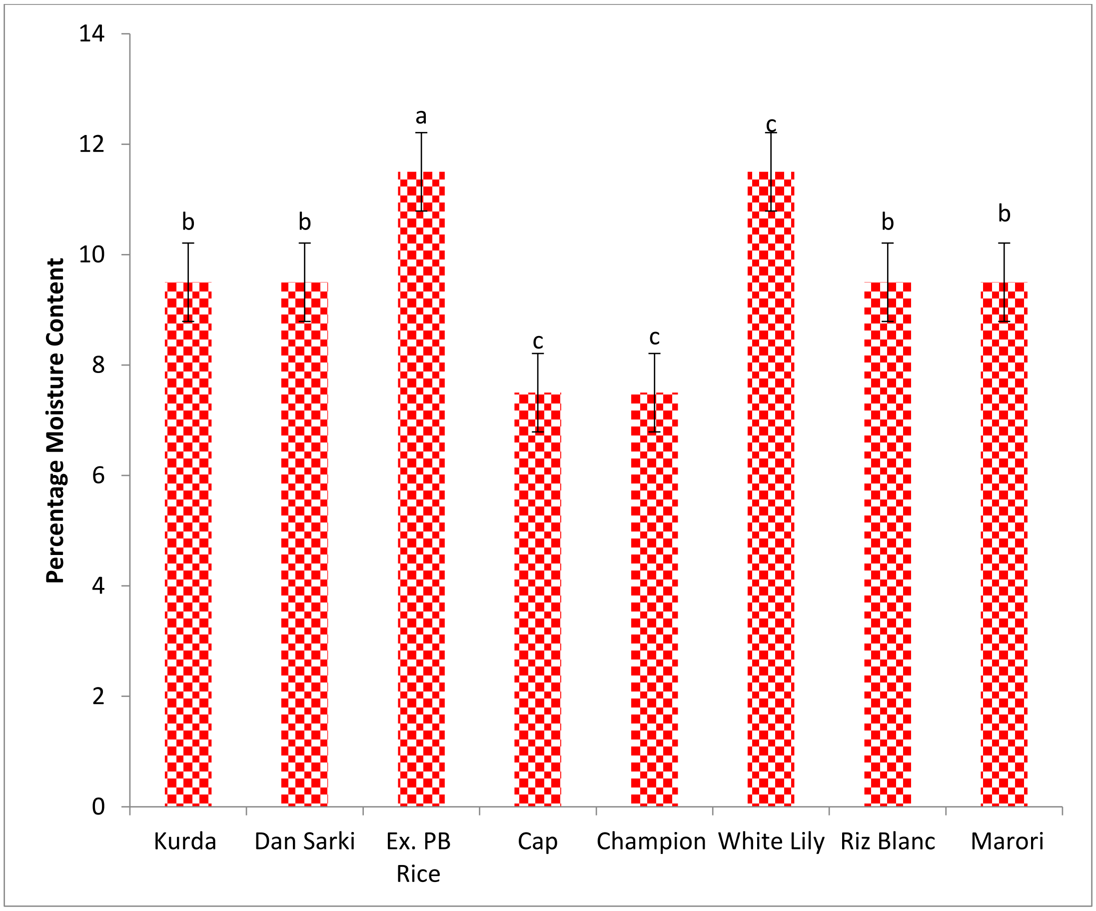

Figure 1: Percentage moisture content of different uncooked rice varieties. Error bars shows ± standard deviation. Mean with the same type of letter are not significantly different from each other ($p>0.05$).

Moisture content plays an important role in determining the shelf life of foods (Ebuchi & Oyewole, 2007). The difference in genetic make-up and the climatic condition in which they are cultivated determine the moisture content in rice varieties. As can be seen from Figure 1, the moisture content of brown rice varieties Champion and Cap is variable from 7.5% to 11.5%, the moisture content of white rice is ranged between 9.5% to 11.5%. Under humid storage conditions, the grains may deteriorate rapidly, resulting in qualitative and quantitative losses and this deterioration is accelerated at higher temperatures. Qualitative losses include appearance changes, nutritional degradation, loss of germination capacity, presence of insect fragments and mold contamination (Sinha & Muir, 1973).

The aerobic colony count (ACC) is an important factor for the evaluation of microbial quality assessment in food product and is an indicator of the overall degree of microbial contamination of foods International Commission for Microbiological Specification for Foods (International Commission for Microbiological Specification for Foods [ICMSF], 1996). It is recommended for all products for its usefulness as an indicator of utility and the condition and length of storage of products. ACC does not measure the entire bacterial population but rather the number of bacteria growth in the presence of oxygen and at mesophilic temperature (Shaltout et al., 2017). Figure 2: reveals the aerobic colony count of cooked and uncooked rice samples. For the cooked rice, Champion had the lowest mean aerobic plate count of 2.57$\log_{10}$ CFU/g and Dan sarki had the highest mean aerobic plate count of 3.19$\log_{10}$ CFU/g while for the uncooked rice samples, marori had the lowest mean aerobic count of 2.69$\log_{10}$ CFU/g and Dansarki had the highest aerobic count of 3.34$\log_{10}$ CFU/g. The aerobic plate count for the cooked and uncooked rice samples are within the minimum recommended level of 5.7$\log_{10}$ CFU/g (PHLS, 2000). All the aerobics organisms isolated are represented by *Bacillus species*; *B. cereus* 70%, *B. subtilis*, 10%, *B. megaterium*, 10% and *B. fluorescens* 10%.

Rice can be a source of food poisoning if it is contaminated with dangerous pathogens. In general, food poisoning can cause by contaminant bacteria such as *Bacillus cereus*, *Staphylococcus aureus*,

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Salmonella group, *Shigella, Vibrio, Escherichia coli, Campylobacter, Yersinia enterocolitis, Clostridium* (Bardale, 2017). Several cases of rice-based food poisoning more often caused by *Bacillus cereus* and *Staphylococcus aureus* were reported globally. Park et. al. (2009). Found out that 15(37%) of 83 samples of brown rice, 23(37%) of 63 samples glutinous rice collected in Korea contain *B. cereus*. Also Ankolekar et. al. (2009) reported that 94(52.8%) of raw rice samples collected in America contain *Bacillus species* spores with an average concentration of 32.6CFU/g. Tahir e.t al. (2012) in Pakistan, noted that cooked and raw rice samples collected showed the presence of *B. cereus*, with the highest number obtained as 1.52$\log_{10}$CFU/g. Rahimi et. al. (2013) in Iran observed the presence of *B. cereus* and its enterotoxigenic gene in infants diets made from rice. Bilung et. al. (2018) reported the number of *B. cereus* in local unhusked rice in Sarawak, Malaysia in all samples to be more than 1100MPN/g. Leka et. al. (2019) also noted that about 21% of yellow cooked rice in Indonesia was contaminated with B. cereus. Similarly, Yu et. al. (2020) reported 59 out of 119 rice noodles (50%) were positive for *B. cereus* in China. All the samples analyzed in this study showed the presence of *B. cereus.* There was no *staphylococcus* aureus and *Escherichia coli* isolated from both the cooked and uncooked rice samples.

Cereal grains accumulate a large and varied microbiota during growth in the field. Molds are among the common contaminants of grains during this time, and some of these molds are toxigenic. Modern grain harvesting and storage practices typically prevent further molds growth. Crops are cut, threshed, and winnowed by mechanical harvesters in order to separate the grain from the chaff, and then the crop is mechanically dried before storage. Grain crops can be stored for 1 year or longer before being processed (Legan, 2000; Sauer, 1992)

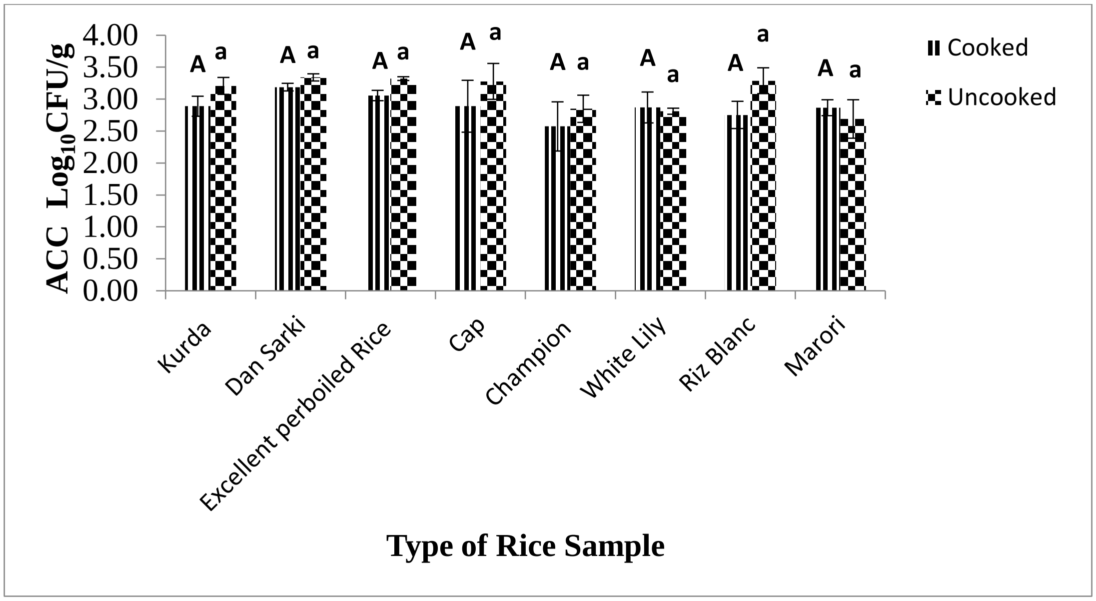

Figure 2: Mean Aerobic Colony Count (ACC) (log_{10} CFU/g) of Cooked and Uncooked Rice Samples. Error bars shows ± standard deviation. Mean with same type of letters uppercase letters, compare cooked rice means; lowercase letters compare uncooked rice means. Each set were analyzed separately. Means with the same letters are not significantly different at ($p>0.05$).

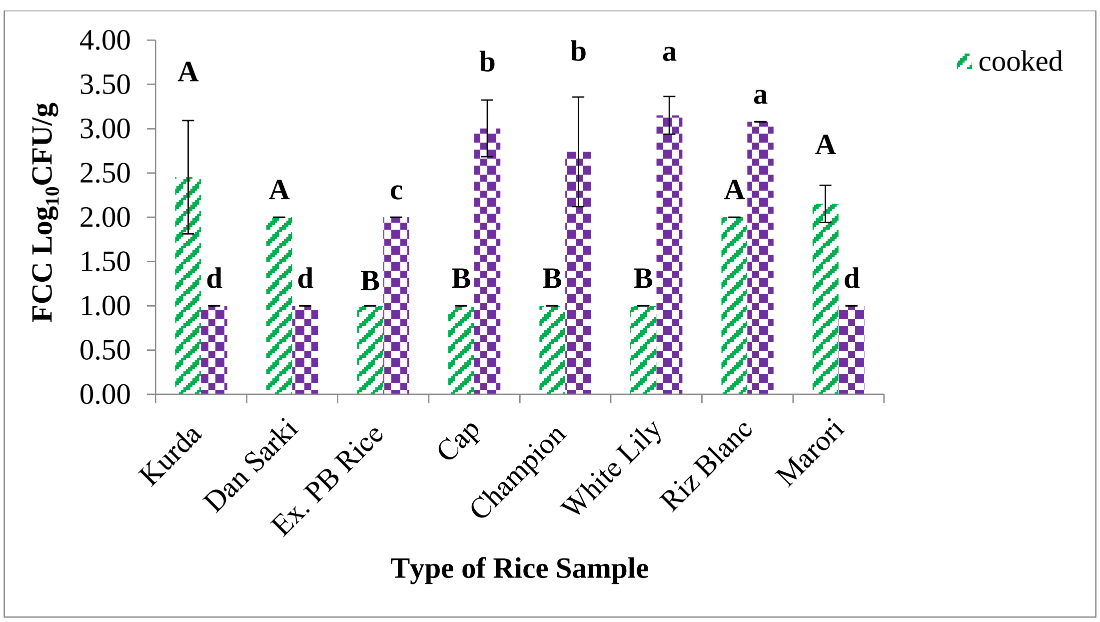

Figure 3: Mean Fungal Colony Count (FCC) (log_{10}CFU/g) of cooked and uncooked rice samples. . Error bars shows ± standard deviation. Mean

with same type of letters uppercase letters, compare cooked rice means; lowercase letters compare uncooked rice means. Each set were analyzed separately. Means with the same letters are not significantly different at ($p>0.05$).

During this storage period, the grain should be stored in a manner to ensure the exclusion of water, birds, insects, and rodents (Legan, 2000; Sauer, 1992). Grains that are properly dried to moisture levels of 12 to 14% will not molds during storage provided these moisture levels are maintained. Figure 3, shows the molds count of cooked and uncooked rice samples with excellent parboiled rice, Cap, champion, and white lily for cooked rice had the lowest molds count of 1$\log_{10}$cfu/g and Kurda had the highest count of 2.45$\log_{10}$cfu/g. For uncooked rice samples Kurda, Dansarki and marori had the lowest molds count of 1$\log_{10}$cfu/g while white lily had the highest molds count of 3.15$\log_{10}$cfu/g. The Fungal genus isolated from cooked and uncooked rice samples were found to be in the order of *Rhizopus* spp. (66%), *Aspergillus flavus* (57%), *Mucor* spp. (54%), *Penicillium* spp. (21%).

Conclusion

The study showed that highest moisture content (11.5%) was obtained from local rice while the lowest moisture content (7.5%) was observed in foreign rice. The mean ACC for the cooked and uncooked rice samples ranged between 2.67 ± 0.39 to 3.19 ± 0.06$\log_{10}$ CFU/g and 2.69 ± 0.3 to 3.34 ± 0.03$\log_{10}$ CFU/g respectively. The ACC was found to be higher in foreign rice as compared to the local rice for both cooked and uncooked rice samples. All the samples analyzed in this study showed the presence of *B. cereus. Salmonella species*, *Staphylococcus aureus*, and *E. coli* were not detected in all the samples. For the fungal counts, the mean FCC of the cooked and uncooked rice samples ranged from 1.0 ± 0.0 to 2.45 ±0.64$\log_{10}$ CFU/g and 1.0 ± 0.0 to 3.15 ± 0.21$\log_{10}$ CFU/g

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in that order. The mean fungal count for the local rice was found to be higher as compared to the foreign for both cooked and uncooked rice samples. The Fungal genus isolated from cooked and uncooked rice samples were found to be in the order of *Rhizopus* spp. (66%), *Aspergillus flavus* (57%), *Mucor* spp. (54%), *Penicillium* spp. (21%).

In general, prevention by proper handling of raw materials, controlling the temperature of cooking and storing rice, and personal hygiene of food handlers with prevent the contamination of both cooked and uncooked rice samples.

Recommendation

During the storage period, the grain should be stored in a manner to ensure the exclusion of water, birds, insects, and rodents. Grains that are properly dried to moisture levels of 12 to 14% will not molds during storage provided these moisture levels are maintained.

In general, prevention by proper handling of raw materials, controlling the temperature of cooking and storing rice, and personal hygiene of food handlers with prevent the contamination of both cooked and uncooked rice samples.

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