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MICROBIAL ASSESSMENT OF UNCOOKED AND COOKED RICE SAMPLES AVAILABLE IN LOCAL MARKETS OF LAHORE

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Abstract

The aim of this study is to assess microbial quality of rice in Lahore.A total of 20 rice samples purchased from different markets were analyzed to study the density of microorganisms by standard plate count (SPC), fungal count (FC), *coliform* counts (CC) and prevalence of *Bacillus cereus*. The Moisture Content was also investigated. Highest moisture content (14.9%) was found in the samples purchased from Samnabad while the lowest moisture content (8.46%) was observed in "Flora Basmati Rice". The microbial load was found to be higher in unbranded rice samples. The Fungal genus isolated from cooked and uncooked rice samples were found to be in the order of *Rhizopus* spp. (76%), *Aspergillus flavus* (67%), *Mucor* spp. (64%), *Aspergillus* spp. (42%), *Penicillium* spp. (31%). All the samples showed the presence of *B. cereus,* the highest count ($3.34x10^1$ CFU/ml) was observed in unbranded samples taken from Sadar while the lowest count ($0.933x10^1$ CFU/ml) was recorded in Flora Basmati Rice. *E. coli* was absent in all samples analyzed.

Introduction

Cereals and cereal products contain microorganisms from insects, soil and other sources. *Bacillus, micrococcus* and molds like *Aspergillus,penicillum* are very common, (Lammerding & Poali, 1999).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 and 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 grown in kharif or wet season in Pakistan. Frequent and heavy rainfall and floods particularly near harvest in coastal areas in eastern, southern and western regions of the country wet the crop and make panicles more prone to invasion by *Aspergillus* spp. (Reddy *et al*., 2004). Rice is arguably the most important foodstuff associated with *Bacillus cereus* food-poisoning (Adams and Moss, 2000). *B. cereus* comprises 10% of the soil microflora in rice paddies (Varnam and 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). Mycotoxins, produced by molds growing on cereals and oilseeds present a second including (Epstein *et al*., 1970). Mycotoxins pose a serious health risk to both humans and animals (Van Rosenburg, 1977).

Materials and Methods

Preparation of the rice samples: Fifty g of rice sample was added to 200 1 peptone water, and then blended in a stomacher for one minute at medium speed. Cooked rice samples were prepared by boiling 25 g of raw rice in 225 ml pre-boiled water for 10 minutes.

Microbiological analysis: Microbiological evaluation included enumeration of total bacteria, yeasts and molds. Serial dilutions up to 10^5 were prepared for the determination of Total Viable Count (TVC) and mold count. Pour plate method was employed for the isolation of microorganisms. 0.5 ml of serially diluted sample was poured in each petriplate with 25ml of nutrient agar and potato dextrose agar for the isolation of bacterial and fungal load respectively. For *coliform* bacteria, Petri dishes with mackonkey agar medium were inoculated aseptically with 1ml of stock solution.

The petriplates for Total Viable Count (TVC) and *coliform* were incubated at 37^{o}C for 24 hrs while plates for fungus were incubated for 48 hrs. *Bacillus cereus* Selective Agar was used for *B. cereus* enumeration (Holbrook & Anderson, 1980). Colonies with diameter greater than 0.5 mm were counted and the microbial load of the rice samples was calculated per gram of sample. The analyses were accomplished in triplicate trials.

Moisture content: Moisture content of the rice samples was determined by placing 1.00 g of the samples in an oven at 100^{o}C overnight. 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 was analyzed statistically for Mean and Standard deviation by using the mini-tab software. Mean values of different microbial parameters were calculated and the data was presented in the form of tables and figures.

Results and Discussion

Unbranded rice samples showed higher moisture content as compared to branded rice samples. (Figs. 1 and 2)The highest moisture content (14.9%) was observed in unbranded rice samples purchased from Samnabad while the lowest moisture content (8.4%) was recorded in Flora

Basmati Rice (branded). The moisture content recorded in other unbranded rice samples was also significantly high. 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).

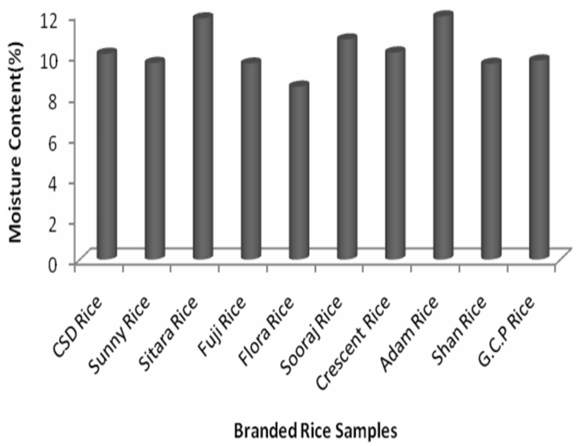

Fig. 1. Moisture content of branded rice samples.

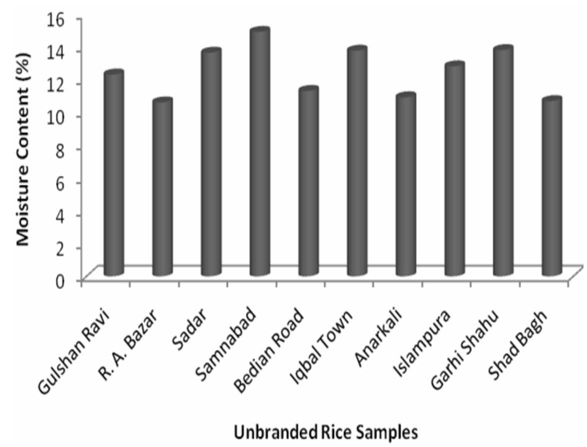

Fig. 2. Moisture content of unbranded rice samples.

Highest TVC ($16.09x10^5$ CFU/ml) was observed in unbranded (uncooked) rice samples purchased from Samnabad while the lowest TVC ($4.13x10^5$ CFU/ml) was observed in "Flora Basmati Rice" (Figs. 3 and 4). The Total Viable Count (TVC) of the unbranded (uncooked) rice samples was found to be significantly higher than branded (uncooked) rice samples. It may be due to the higher moisture content of the unbranded rice samples. High amount of moisture allows the growth of microorganisms at a higher rate. The high Total Viable Count (TVC) also reflects the inappropriate handling and storage of the product (Ramaday & Elnaby, 1962). Low occurrence of contaminants on branded rice samples could be because the branded rice samples are treated properly. The Total Viable Count (TVC) of the unbranded (cooked) rice samples was found to be highest ($6.40x10^5$ CFU/ml) in rice samples purchased from Anarkali while lowest count ($2.4x10^5$ CFU/ml) was recorded in Flora Basmati Rice. The TVC in cooked rice samples after boiling was found lower than in uncooked rice samples and indicated the presence of spore formers.

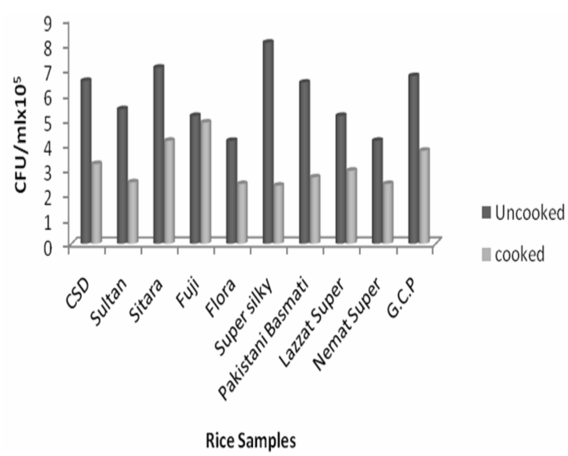

Fig. 3. Total viable count (TVC) in branded rice samples.

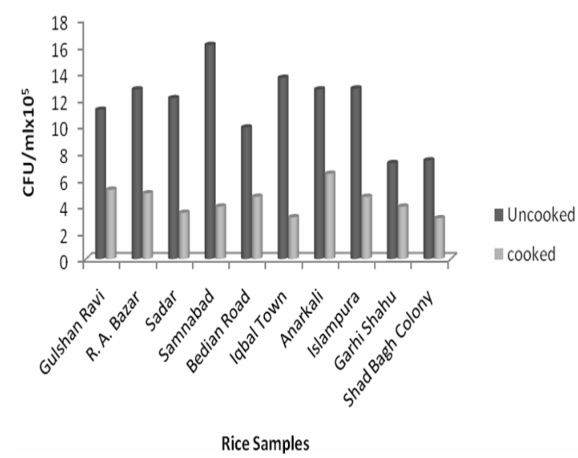

Fig. 4. Total viable count (TVC) in unbranded rice samples.

All the rice samples, both branded and unbranded, showed a high level of fungal contamination in uncooked (raw) form though their number decreased noticeably after cooking/boiling. Highest Fungal count ($16.73x10^5$ CFU/ml) was observed in unbranded (uncooked) rice samples purchased from Samnabad while lowest fungal count ($5.33x10^5$ CFU/ml) was observed in "Flora Basmati Rice". The highest fungal count ($11.73x10^5$CFU/ml) in unbranded (cooked) rice samples was observed in the samples purchased from Samnabad. While the lowest fungal count ($2.867x10^5$ CFU/ml) was recorded in Sitara Basmati Rice (Figs. 5 and 6). The high level of fungi obtained in this study can be associated with the water activity of rice samples and the physiology of the contaminating fungal genera. The growth of fungi is dependent on temperature and water activity (Christian, 1963). As the water activity decreases, the growth of microbes is slowed (Scott, 1957; Troller, 1973).

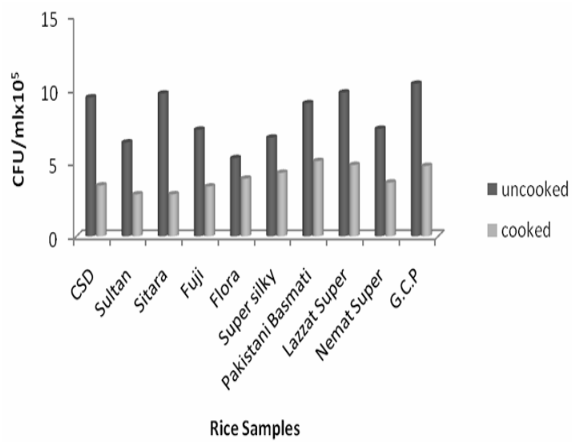

Fig. 5. Total fungal count in branded rice samples.

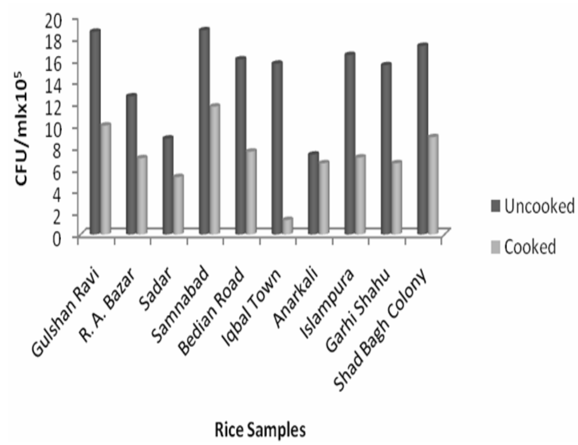

Fig. 6. Total fungal count in unbranded rice samples.

Fungal spp., isolated from branded and unbranded rice samples (uncooked and cooked) were, found in the order of, *Rhizopus* spp. (76%), *Aspergillus* spp. (67%), *Aspergillus flavus* (42%), *Mucor* spp. (64%) and *penicillium* spp. (31 %). *Rhizopus* spp. and *Aspergillus* spp., were found to be the predominant fungal species. This work is in agreement of other workers (Sundaram *et al*., 1988; Reddy *et al*., 2004). The occurrence of fungi could be a source of high pathogenicity as reported by other workers (Pitt *et al*., 1994). These organisms not only can cause several infections on their own; but also produce mycotoxins that are of public health importance to humans. Aflatoxin B_1 (AFB_1), the most potent aflatoxin produced mainly by *Aspergillus flavus*, is extremely toxic, mutagenic, carcinogenic and teratogenic to both humans and livestock and chronic exposure to low levels of AFB_1 pose a serious health and economic hazard (Karlovsky, 1999; Mishra & Das, 2003). These metabolites can contaminate agricultural commodities due to the results of mold invasion before and during harvest, or during storage. The formation of aflatoxins is closely linked to fungal growth (Tsai & Yu, 1999). The results of the present study indicated that Total Viable Count (TVC) and fungal growth increases with the increase in moisture content. It may be due to the poor storage conditions in Rice hordes and Chawal Stores. Due to dampness in the storage areas and poor ventilation, the moisture content of the rice grains increases which allows the microbial growth. Branded and unbranded (uncooked and cooked) rice samples were also examined for the presence of *E. coli*. The data shows the absence of *E. coli* in all of the examined samples. *Coliforms*, can influence food safety and preservation because these organisms are an indicator of fecal contamination and can carry water borne pathogens (Lee *et al.*, 2007). So it is the matter of great satisfaction that the varieties of rice (branded and unbranded) commercially sold in local markets of Lahore were free from *coliform* contamination.

The highest *B. cereus* count (3.34×10^1 CFU/ml) was recorded in unbranded samples taken from Sadar. While the lowest *B. cereus* count (0.933×10^1 CFU/ml) was observed in Flora Basmati rice (Figs. 7 and 8).The count was found to be low due to heat shock. Estimates of the infective dose of *B.* cereus in food poisoning vary from 10^3 to 10^{10} CFU/g^{-1} of food (Notermans and Batt, 1998). Up to 10^4 CFUg^{-1}in a typical serving of food is considered the upper limit to “acceptable” levels of contamination (Montville and Mathews, 2004). Processing steps such as drying, husking and polishing reduced the number of *B. cereus* in the final product (Sarrias *et al*., 2002). The high microbial load of unbranded samples of Local Markets could be because of unhygienic conditions of local market & exposure during transport facility, improper storage condition etc. that may add organisms.

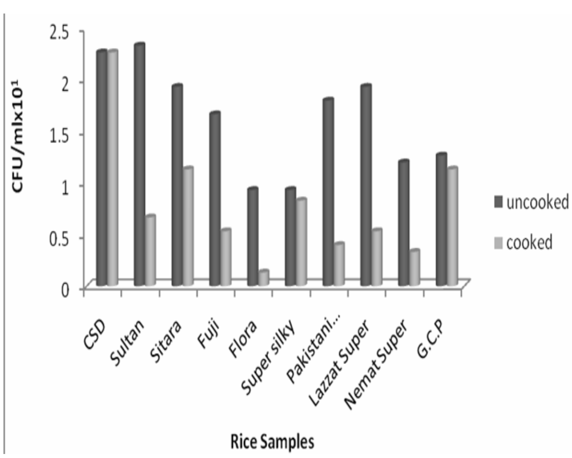

Fig. 7. *Bacillus cereus* count in branded rice samples.

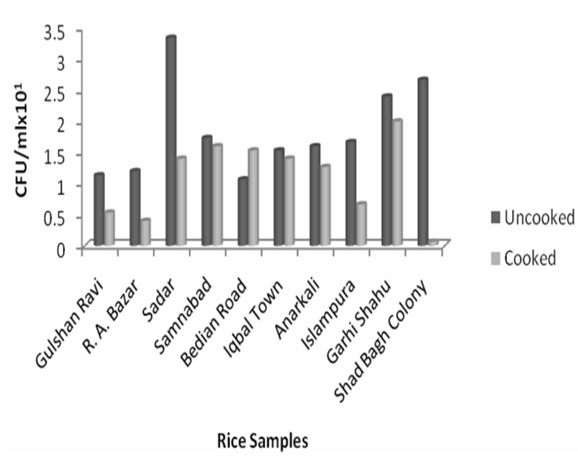

Fig. 8. *Bacillus cereus* count in unbranded rice samples.

Conclusion

The unbranded rice available in Lahore were found unsatisfactory in terms of microbial content. Unbranded rice samples have high moisture content may be due to the inferior quality of packaging material or poor storage conditions of rice. Unbranded rice samples of Local Market are harboring more microbial contaminants as compared to branded rice samples, indicating that these are protected from contamination while subsequent handling, packaging, storage and transporting. The presence of high fungal amounts especially *Aspergillus* spp. in rice samples draws attention of the agricultural authorities towards the health problems not reported yet but can be a future catastrophe, if the existing situation prolongs.

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