Vidyodaya J. of Sci. (2009) Vol. 14, pp 131-141

Spoilage after cooking of some rice varieties commonly consumed in Sri Lanka

S. Waduwawara & Pathmalal M. Manage

Department of Zoology,
University of Sri Jayewardenepura
Nugegoda, Sri Lanka.

Received on : 15-10-2008
Accepted on : 28-11-2008

Abstract

Rice once cooked is perishable. In the present study we investigated cooked rice spoilage of common rice varieties in Sri Lanka. The study was further extended to compare the effect of clay and aluminium pots on the cooked rice spoilage and to study the potential to put off cooked rice spoilage in the traditionally used botanicals rampe (*Pandans latifolia*), curry leaves (*Murraya koenigii*) and turmeric (*Curcuma domestica*). One Gram positive bacteria was isolated and identified as *Bacillus cereus*. In cooked rice, the total count of Gram positive bacteria increased with time while the total count of Gram negative bacteria decreased. When inoculated separately to sterilized rice, the Gram positive bacteria multiplied to a significantly greater number ($p < 0.05$) than did Gram negative bacteria. The total bacteria count of different rice varieties was not significantly different from each other. Rumpe and curry leaves reduced the total Gram positive bacteria count of rice significantly ($p < 0.05$) while turmeric did not. The total Gram positive bacteria count of rice cooked in aluminium pots was significantly lower ($p < 0.05$) compared to rice cooked in clay pots.

Key words : Rice spoilage, Gram positive bacteria, *Bacillus cereus*, anti-bacterial effect, *Pandanus latifolia*

Introduction

Rice is one of the major cereals of the world and is the staple of more than half of the world's population and for about 2.7 billion people in Asia (Riveros, 2000). Ninety percent of the population in Bangladesh, Burma, Sri Lanka, Vietnam and Kampuchea depend on rice for their major food intake (De Datta, 1981).

Rice, once cooked, is perishable. The spoilage of boiled rice is found to be associated with the presence of Gram-positive *Cocci* and *Bacilli* (Ueda *et. al.,* 1980). Out of them, *Bacillus cereus* is the most prominent bacterium responsible for the cooked rice spoilage (Ueda *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, but to the best of our knowledge, this is the first report on spoilage after cooking of rice varieties commonly consumed in Sri Lanka. We therefore, investigated cooked rice spoilage of some common rice varieties in Sri Lanka (Kora, red raw rice, white raw rice, and samba) and compared the time taken to spoil by the different rice varieties. Some people use aluminium pots to cook rice whereas some use clay pots. Information about the effect of these pots on cooked rice spoilage is not recorded so far. Thus, the present study was further extended to compare the effect of these pots on the cooked rice spoilage. We also studied the potentiality to put off cooked rice spoilage in the traditionally used cooking substances rampe (*Pandanas latifolia*), curry leaves (*Murraya koenigii*) and turmeric (*Curcuma domestica*) as people add these substances into rice in order to make food more appetizing and also for decoration. It has been recorded, especially in ayurvedic medicine that some of these substances have anti-bacterial activity. Curry leaves are considered to be stomachic, appetizing and antiseptic and are useful in treating diarrhea, dysentery and vomiting (Warrier *et al.,* 1995). Turmeric is also used to treat diarrhea and dysentery in ayurvedic medicine (Warrier *et al.,* 1995). Thus, it's important to find if these substances have any effect on delaying cooked rice spoilage.

Most of the consumers who depend on rice as their primary food live in less developed countries (Riveros, 2000). Sri Lanka also is a developing country of which the staple food is rice and it's with difficulty most Sri Lankans earn their daily meal. Also the majority of the people in Sri Lanka do not have refrigeration facilities. Therefore it's important if we can keep cooked rice for a long time. Therefore, the present study will provide valuable information to develop future research on cooked rice spoilage and its prevention.

Materials and Methods

Sample collection and preparation of cooked rice

Rice varieties; Samba, White raw rice, Red raw rice, curry leaves, turmeric and rampe were purchased from the local market. Two hundred grams of rice was washed and cooked in 300 ml of water using a LP gas cooker. To test the effect of curry leaves, turmeric and rampe, 200 g of Samba rice was cooked with adding 0.5 g of curry leaves, ground turmeric and rampe pieces in clay pots seperately. To test the effect of clay and aluminium pots, 200 g of Samba rice was cooked separately in clay and aluminium pots. The resistance of different rice varieties was tested by cooking 200 g of each rice variety in clay pots. To study the spoilage and bacterial growth, samples were taken at 5.5 hour intervals for a period of 2 days. The physical parameters of cooked rice, smell, texture and flavour were recorded at the same time along with the bacteria enumeration.

Enumeration of bacteria

Enumeration of bacteria was carried out by pour plate method (Yamamoto and Suzuki, 1990). At five and half hour intervals 50 g of cooked rice was taken and blended in 450 ml of sterile distilled water. After a serial ten-fold dilution, 1 ml aliquots from each dilution were aseptically pipetted into sterilized Petri plates. Then 15-20 ml of molten nutrient broth (38- 40 °C) was poured into the Petri plates, swirled gently and allowed to solidify and kept upside down in dark for three days at 28 ± 1.4 °C. After three days of incubation Colony Forming Unites (CFU ml^{-1}) were conted assuming a colony arising from a single microbial agent. Throughout the study period humidity was 79% ± 2.2 RH. Triplicate Petri plates were prepared for each dilution. The bacterial colonies appearing in the plates were counted and expressed in $\log_{10}$ well separated ml (CFU ml^{-1}). Bacteria were isolated from colonies and re-isolation was done repeating the pour plate method. Bacteria were tentatively catogarized by colour, shape of the colony and by Grams reaction. Two Gram negative and one Gram positive bacteria were isolated (Table 1). Gram positive bacteria were identified as *Bacillus cereus* (PROB 94% TYPE GP-OXI) using the Biolog System (Microbiology ™ Microbial Identification System, Release 4.0, 1990. Biolog, Inc, Hayward, USA).

Confirmation of spoilage effect of bacteria using the isolated bacteria

To confirm the bacteria that contribute to the rice spoilage, 25 g samples of cooked Samba rice in triplicate were transferred into sterilized Petri plates. The plates were covered with aluminium foil and sterilized at 121 °C for 15 minutes. The sterilized rice was then inoculated with Gram positive and Gram

negative bacteria (initial concentration 2 - 4×10^4cells/ml) isolated from cooked rice. The physical parameters were observed by taking 2 g at 5.5 hour time intervals (0, 5.5, 11, 16.5, 22 hrs) for a period of 24 hours and the bacterial counts of each rice sample and control were enumerated using the pour plate method (Yamamoto & Suzuki, 1990).

Statistical analysis

Two way analysis of variance (2-way ANOVA) and general linear model were used to analyze data. All experiments were carried out in triplicate (n=3). The significance was established at $p < 0.05$.

Results

Identification of bacteria

Three colony types of bacteria were observed. They were the same for all rice varieties and further, the colony types appeared showed a similar pattern of appearance with time for all rice varieties. Out of the three bacteria species, one was Gram positive and two were Gram negative. Gram positive bacteria were identified as *Bacillus cereus*.

Confirmation of the spoilage effect of isolated bacteria revealed that all bacteria types caused spoilage of cooked rice but to different extents. Gram positive bacteria count increased significantly ($p<0.05$) compared to gram negative bacteria (Table 1). Further it was detected that Gram positive bacteria spoiled rice to a greater degree than did Gram negative bacteria.

Table 1: Bacteria counts after 24 hours for Samba inoculated with the three bacteria isolates from cooked rice (log CFU/g).

Bacteria	Bacteria (log CFU/ g) (Cooked rice)
Gram positive -1	9.53 ± 0.16
Gram negative -1	6.67 ± 0.12
Gram negative -2	5.99 ± 0.70

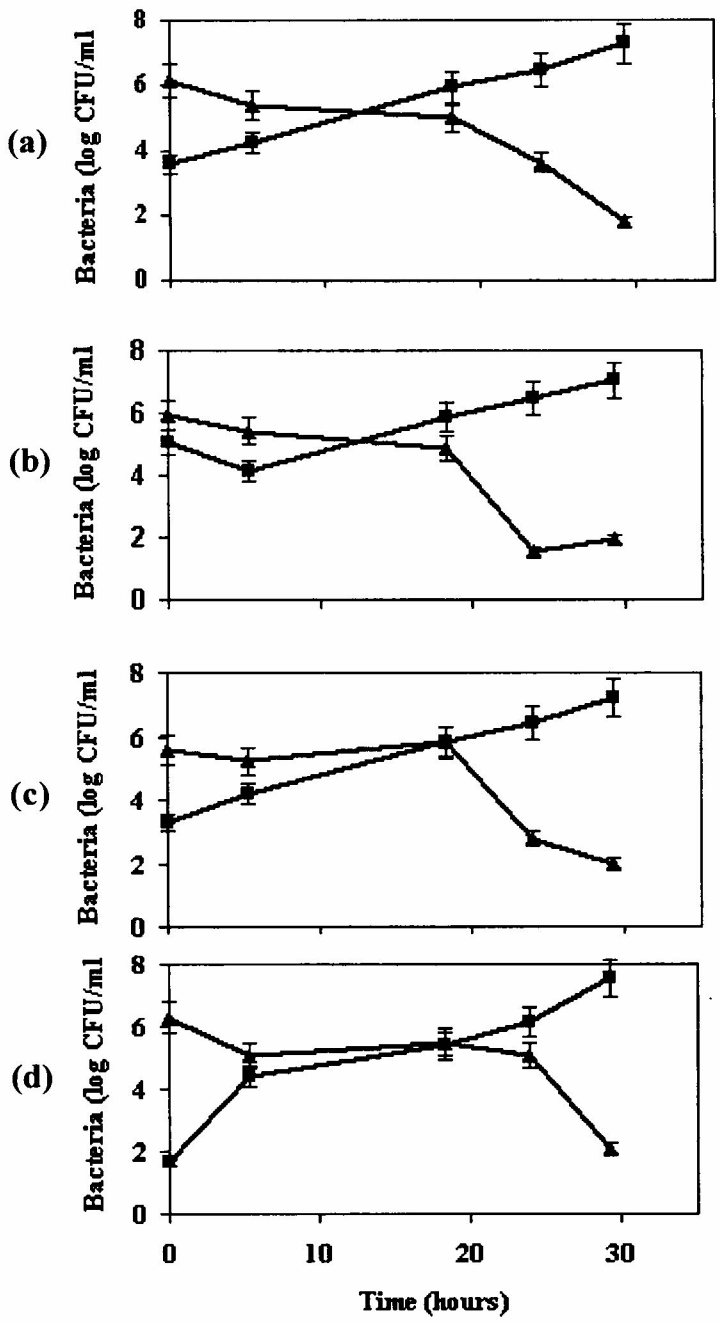

Figure 1: Change of Gram positive and Gram negative bacteria with time for (a) Samba; (b) White raw rice; (c) Kora; (d) Red raw rice (— % — Gram positive bacteria; —²⁰%— Gram negative bacteria). Vertical bars represent standard deviation (n = 3). CFU = Colony Forming Units

Spoilage trend of different rice varieties

In all the four rice varieties, initially the Gram negative bacteria counts were higher than the Gram positive bacteria counts and, thereafter, Gram negative bacteria count decreases gradually while the Gram positive bacteria count increased (Figure 1). The Gram positive bacteria count or the Gram negative bacteria count of different rice varieties at each time was not significantly different ($P > 0.005$) from each other. On average all cooked rice varieties

Table 2: Gram positive and Gram negative bacteria count (log CFU/g) of plain cooked Samba rice at 5.5 hour intervals.

Time (hours)	Gram negative bacteria (log CFU/g)	Gram positive bacteria (log CFU/g)
0.00	6.14 ± 0.49	3.57 ± 3.09
5.50	5.38 ± 0.07	4.21 ± 0.39
18.5	4.97 ± 0.58	5.94 ± 0.73
24.00	3.61 ± 3.12	6.45 ± 0.84
29.50	1.77 ± 3.06	7.27 ± 0.63

Table 3: Gram positive and Gram negative bacteria count (log CFU/g) of plain cooked Samba rice with rampe at 5.5 hour intervals.

Time (hours)	Gram negative bacteria (log CFU/g)	Gram positive bacteria (log CFU/g)
0.00	5.65 ± 0.31	1.02 ± 0.22
5.50	5.30 ± 0.32	1.26 ± 2.18
18.5	5.19 ± 0.07	4.76 ± 0.11
24.00	3.24 ± 2.82	5.42 ± 0.12
29.50	0.00 ± 0.00	6.18 ± 0.37

Table 4: Gram positive and Gram negative bacteria count at 5.5 hour intervals for Samba cooked with curry leaves

Time (hours)	Gram negative bacteria (log CFU/g)	Gram positive bacteria (log CFU/g)
0.00	5.67 ± 0.34	1.43 ± 2.48
5.50	5.43 ± 0.23	2.26 ± 2.13
18.5	3.04 ± 2.92	4.82 ± 1.33
24.00	1.33 ± 2.31	5.89 ± 0.79
29.50	0.00 ± 0.00	6.77 ± 0.88

Table 5: Gram positive and Gram negative bacteria count at 5.5 hour intervals for Samba cooked with turmeric

Time (hours)	Gram negative bacteria (log CFU/g)	Gram positive bacteria (log CFU/g)
0.00	5.67 ± 0.33	2.48 ± 2.19
5.50	5.49 ± 0.24	1.10 ± 1.91
18.5	3.53 ± 3.06	4.80 ± 0.89
24.00	1.812 ± 1.81	6.27 ± 1.52
29.50	0.00 ± 0.00	7.25 ± 0.72

showed first spoilage effects at the same time (Data not shown). All rice varieties were edible up to 25 hours after cooking without any change in smell, texture or flavour. By 29.5 hours after cooking, on average all the tested rice varieties became inedible.

Effect of curry leaves, turmeric and rampe on cooked rice spoilage

The Gram positive bacteria content was significantly lower ($P<0.05$) in rice cooked with rampe (Table 3) and curry leaves (Table 4) than in plain cooked rice (Table 2). But there was no significant difference ($P>0.05$) in the Gram

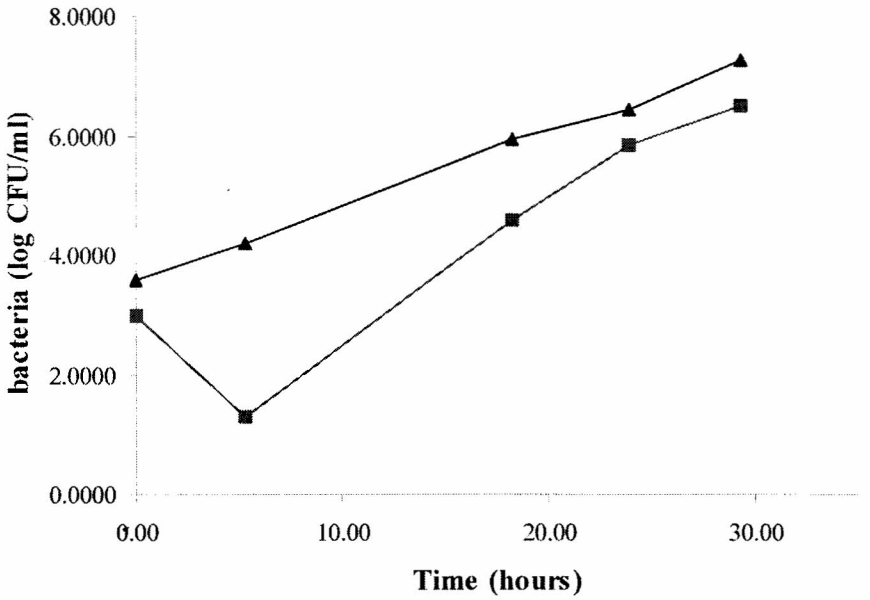

Figure 2: Changes in CFU of Gram positive bacteria with time for Samba (—²%— cooked in clay pot; — %— cooked in aluminium pot). Vertical bars represent standard deviation (n = 3). CFU = Colony Forming Units.

negative bacteria content. Further, there was no significant difference ($p>0.05$) in the bacterial population of plain cooked rice and rice cooked with turmeric (Table 5).

In the examination of physical state, odour, texture, and flavour, rice with every treatment showed no change in the physical state and was edible up to 25 hours after cooking. Rice cooked with curry leaves and rampe was edible by 29.5 hours after cooking and rice cooked with rampe even retained its appetizing smell by that time. But rice cooked with turmeric and plain cooked rice was inedible by 29.5 hours after cooking.

Effect of clay and aluminium pots on spoilage of cooked rice

The Gram positive bacteria content was significantly lower ($p<0.05$) in rice cooked in aluminium pots than in rice cooked in clay pots (Figure 2). But there was no significant difference ($p > 0.05$) in the Gram negative bacteria content. In the examination of physical state, rice cooked both in aluminium and clay pots showed no spoilage up to 25 hours after cooking. But rice cooked in clay pots was inedible by 29.5 hours after cooking while rice cooked in aluminium pots was edible even by that time and became inedible by 31.5 hours after cooking.

Discussion

Rice spoilage is almost always associated with Gram positive bacteria (Brian *et. al.*,1981; Schiemann, 1978; Ueda *et. al.*, 1981) and disease outbreaks associated with cooked rice consumption are also resultant from Gram positive Bacillus type bacteria, especially, *B. cereus* (Granum, 2001). Therefore it's explainable that with time in cooked rice the Gram positive bacteria population should increase. The decrease in Gram negative bacteria population with time can be because of the competition from Gram positive bacteria (Table 2).

The present study was carried out to find if there is a difference in the spoilage of different rice varieties and it was found that the total bacterial population, Gram positive and the Gram negative bacterial population were not significantly different from each other for the four rice varieties tested (Samba, Kora, White raw and Red raw). It may be due to the fact that although there may be slight differences from each other, all rice varieties have the same nutrient composition. People use different amounts of water to cook different rice varieties. For some rice varieties like Kora, they use a higher water content than for others. This may be a contributory factor to the belief that some rice varieties get spoiled quicker than others. In this study, all rice varieties were cooked with the same amount of water.

Although used primarily as flavouring and seasoning agents in foods, many spices posses significant anti microbial activity (Jay, 2000). In ayurvedic

medicine, it is stated that curry leaves are stomachic, appetizing and antiseptic and also it is used to treat diarrhoea, dysentery and vomiting (Warrier *et al.*, 1995). Therefore, there could be some anti bacterial activities in curry leaves. In this study the number of Gram positive bacteria decreased significantly with the treatments rampe and curry leaves indicating the anti-bacterial activity of them. The anti bacterial action of rampe and curry leaves should be especially against Gram positive bacteria since it is the Gram positive bacteria population that decreased significantly when cooked with them. It has been reported that Gram positive bacteria are more sensitive to anti microbial chemicals than Gram negative bacteria (Zeika et al., 1983). Turmeric is also used to treat diarrhoea and dysentery in ayurvedic medicine (Warrier *et al.*, 1995), but not vomiting. In emetic syndrome, initial symptoms are of nausea followed by vomiting and malaise. However, any significant decrease in bacterial number was not observed may be because the small amount of ground turmeric used in rice was too small to exert a significant anti microbial activity. It has been reported that in general spices are less effective in foods than in culture media (Zaika *et al.*, 1983).

Although apparently a medical value of rampe hasn't been reported, from the three treatments employed, rice cooked with rampe was edible by twenty nine and half hours after cooking in all three occasions (where Samba was cooked with rampe) while rice cooked with turmeric and curry leaves was inedible in 1 and 2 occasions respectively.

In Sri Lanka, curry leaves and rampe are freely available and they are not expensive facilitating access to every one. Therefore this anti microbial activity of rampe and curry leaves would be very important.

The common belief is that, rice cooked in clay pots could be kept longer than rice cooked in aluminium pots. According to the results obtained in this study, rice cooked in aluminium pots could be kept longer and in addition the total bacteria count and the Gram positive bacteria count was lower in rice cooked in aluminium pots than in rice cooked in clay pots. Samba cooked in clay pots was inedible by 29.5 hours after cooking while rice cooked in aluminium pots was edible even by that time and become inedible by 31.5 hours. Clay pots have tiny pores on them but in aluminium pots there are no such pores. During spoilage, enzyme activities of bacteria take place in cooked rice and influence changes in physical and chemical conditions of rice (watery condition). Pores on clay pots may facilitate these enzymic activities and thereby result in a higher possibility to get rice spoiled quicker than in aluminium pots, and also result in a higher Gram positive bacteria number.

Conclusions

Gram positive bacteria are responsible for cooked rice spoilage to a greater extent than Gram negative bacteria. There is no significant difference in the

bacterial count or in the time taken to spoil in different rice varieties Samba, Kora, White raw rice and Red raw rice. Curry leaves and rampe reduce the Gram positive bacterial content of cooked rice significantly. The bacterial content is significantly lesser in rice cooked in aluminium pots than in rice cooked in clay pots.

Acknowledgment

This study was supported by University of Sri Jayeawardenepura, Department of Zoology. We thank Prof. Chandrani Wijerathne for her support and advice throughout the study.

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