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The effect of method of cooking and holding conditions on enterotoxin production by *Staphylococcus aureus* in two types of Saudi rice

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Abstract:

Background: *Of most widespread intoxication, which depends largely on sanitary practices is staphylococcal food poisoning. Mandy and Bokhary rice are two common traditional foods consumed in Saudi Arabia.*

Objectives: *This study aims to determine the effect of some factors such as cooking method and holding temperature on growth and enterotoxin production by Staphylococcus aureus in Mandy and Bokhary rice.*

Materials and Methods: *Twenty four Mandy and Bokhary rice samples were obtained from six Makkah restaurants at 12PM and 10PM. Samples were tested for total viable count and their ability to support growth and enterotoxin production by Staphylococcus aureus strains. Autoclaved rice specimens were inoculated with Staphylococcus and sampled every hour. Samples were tested for*

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enterotoxins using RPLA technique and total viable count was determined.

Results: *Rice temperatures were higher at noon (average = 66.4°C) than at night (average = 53.3°C). The 10PM samples showed higher positive bacterial growth percentage (41.6%) than the 12PM samples (16.7%). 70.8% of samples showed no growth. All samples with temperatures ≤ 52°C showed positive bacterial growth while all samples at higher temperatures were negative. S. aureus growth rate was faster in Mandy rice (generation time = 102 minutes) than in Bokhary rice (generation time = 126 minutes). Type of rice and incubation temperature affected enterotoxin production. Enterotoxin production was faster at 45°C than at 23°C. At 45°C the type of rice had no effect on enterotoxin production rate while at 23°C enterotoxins were found to be produced earlier in Bokhary rice than in Mandy rice.*

Conclusion: *Sanitary qualities were best met in Saudi restaurants at noon. The components of rice dishes and cooking method affected the multiplication of S. aureus and toxin production in food. Enterotoxin was detected earlier in Bokhary rice, therefore, Mandy rice can be considered safer for consumption.*

Key words: method of cooking, holding conditions, enterotoxin production, *Staphylococcus aureus,* types of Saudi rice

Introduction

Many inhabitants in Saudi Arabia nowadays depend on prepared cooked foods presented in many restaurants and pantries especially during Hajj and Omra seasons. Hajj is one of the world's biggest religious gatherings, Millions of Muslims gather in Mecca to perform the annual haj pilgrimage. In normal times, food handlers are subjected to medical examination before assignment to work in food stations. However, during high seasons of work, i.e., Hajj those establishments employ temporary workers; mostly lacking training in food handling operations, and sanitary practices, which are not easily enforced during excessive demand than these establishments can afford. This situation can encourage contamination with microorganisms both causing food spoilage and food poisoning. Of most widespread intoxication, which depends largely on sanitary practices is staphylococcal food poisoning (SFP).

Staphylococcal food poisoning results from the ingestion of staphylococcal enterotoxins (SEs) preformed in food by enterotoxigenic strains of *Staphylococcus aureus*.[1] The major antigenic types of staphylococcal enterotoxin have been recognized as SEA to SEJ; however, more recently identified staphylococcal enterotoxins include SEK, SEL, SEM, SEN, SEO, and SEU.[2-5] SEA is the cause of most food poisoning by *S. aureus*, is expressed in the mid-exponential phase, and its gene seems to be transferred by a temperate bacteriophage.[6, 7]

Any food that provides a convenient medium for *S. aureus* growth may be involved in a SFP outbreak. The foods most frequently involved differ widely from one country to another, probably due to differing food habits.[8] For example, in the UK or the United States, meat or meat-based products are the food vehicles mostly involved in SFP.[9] In France, milk-based products are more frequently involved than in other countries.[10]

It is well established that there are significant differences in the behavior of bacteria in the planktonic state and immobilized bacteria found in multicellular communities. Thus, in order to improve the production of microbiologically safe food for human consumption, in situ data on enterotoxin formation in food environments are required to complement existing knowledge on the growth and survivability of *S. aureus*.[11]

The aim of this study is to determine the effect of some factors such as cooking method and holding temperature on the growth and enterotoxin production by *S. aureus* in two of the most popular national dishes in Saudi Arabia.

Materials and Methods

Collection of rice samples

Twenty four rice samples of Mandy were obtained from six different restaurants in Makkah. Restaurants were chosen randomly and designated as R1, R2, R3, R4, R5, and R6. The weight of each sample was approximately 500g. Samples were collected for two days at noon (12:00 PM) when the restaurants begin to open to the customers to serve lunch and also ten hours later at dinner time (10:00 PM). PH and temperature of cooked rice were determined during sampling.

Bacterial total count

The bacterial load was estimated in the rice samples using the pour plate method for the determination of Total Viable Count (TVC).

Serial dilutions up to 10^5 were prepared using peptone water. 0.5 ml of serially diluted sample was poured in each petriplate with 25ml of Plate count agar medium (Oxoid). The petriplates were incubated at 37°C for 24 hrs. Colonies were counted and the microbial load of the rice samples was calculated per gram of sample. The analyses were accomplished in triplicate trials.

Growth curve determination and enterotoxin production in the rice dishes

Two enterotoxin producing strains were selected from staphylococcus isolates in previous study.[12] One was enterotoxin A producing isolate and the other was enterotoxin C producing.

The selected isolates were inoculated in Tryptic Soy broth, and incubated at 35°C for 48h. The culture was then centrifuged at 3000 rpm for 20 minutes, washed twice with Phosphate-buffered saline (PBS), resuspended in saline, and adjusted to about 10^6 cells/ml using a spectrophotometer (LKB Ultrospec 4050 No 40003511. Biochrom. Cambridge) at 600nm. Ten ml of bacterial cell suspension was inoculated into autoclaved rice specimens (100g of autoclaved ready-made Mandy rice or Bokhary rice) and incubated at both 25ºC and 40ºC for 48 hours (the temperature of the incubation was recorded by an automatic recording thermometer, model: No.515P, 7 days, Pacific Transducer Corp. USA).

Mandy or Bokhary rice was sampled every hour. Each sample was tested for toxin presence using reverse passive latex agglutination (RPLA) technique and total viable count was determined by pour plate method using Baird-Park agar medium. Doubling time was calculated according to Sreekrishna.[13]

Detection of enterotoxins in food

For the detection of enterotoxins in food samples, 10g of the food sample was blended with 90mL of Phosphate-Buffered Saline (PBS) for homogenization using Stomacher (Seward Medical UAC House, London, U.K.), followed by centrifugation at 3000 rpm for 20 minutes. The supernatant was used for enterotoxin detection.

Enterotoxin was detected using SET-RPLA kit (Denka Seiken Co. Ltd., Tokyo), a commercial reverse passive latex agglutination test kit for the detection of staphylococcal enterotoxins A, B, C, and D in foods. The test was performed by the procedure recommended by the manufacturer.

Results

Variation in temperature of rice samples

The variability in temperature of rice specimens during ten hours holding time in pots were summarized in **Table 1**. The rice temperatures at noon varied between 50 to 79°C (average =

66.4 and SD = 9.1). However, it was between 45 and 57°C during night (average = 53.3 and SD = 3.9).

Table 1. Average temperature of rice samples in pots

	Restaurant						**Overall Average**
Time	R1	R2	R3	R4	R5	R6	
12:00 PM	59.5ºC	78.5ºC	73.5ºC	59.8ºC	66ºC	64ºC	66.4 (SD = 9.1)
10:00 PM	56.5ºC	56ºC	53ºC	53ºC	54.5ºC	47ºC	53.3 (SD = 3.9)

Total viable count results:

The Bacterial total count (CFU/gm) of Mandy rice samples is shown in **table 2**. 70.8% of samples showed no growth. The 12:00 PM samples showed 16.7% positive growth rate while the 10:00 PM samples showed higher positive growth rate which was 41.6%.

Table 2. The Bacterial total count (CFU/gm) of Mandy rice samples

Restaurant	*Time*	*Temperature*	*CFU at 37°C*	*CFU at 55°C*
R1	12:00 PM	52	30	0
R1	12:00 PM	67	0	0
R1	10:00 PM	57	0	0
R1	10:00 PM	56	0	0
R2	12:00 PM	79	0	0
R2	12:00 PM	75	0	0
R2	10:00 PM	55	0	0
R2	10:00 PM	57	0	0
R3	10:00 PM	51	10	2000
R3	12:00 PM	78	0	0
R3	12:00 PM	69	0	0
R3	10:00 PM	55	0	0
R4	10:00 PM	50	10	0
R4	12:00 PM	50	20	4000
R4	12:00 PM	67	0	0
R4	10:00 PM	56	0	0
R5	10:00 PM	52	10	0
R5	12:00 PM	65	0	0
R5	12:00 PM	67	0	0
R5	10:00 PM	57	0	0
R6	10:00 PM	49	600	10000
R6	10:00 PM	45	200000	15000
R6	12:00 PM	59	0	0

R6	12:00 PM	69	0	0

CFU: Colony forming unit

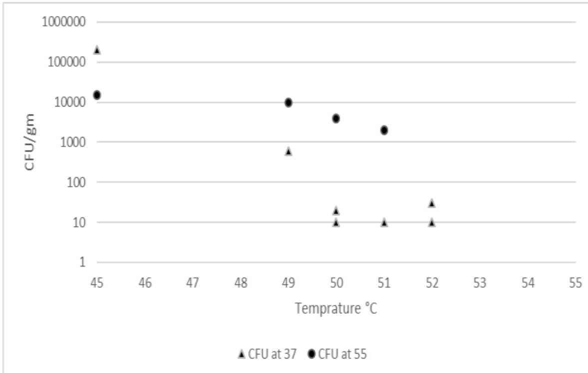

Figure 1. The bacterial count of rice samples in relation to their temperature.

All the samples with temperature below or equal to 52°C had shown bacterial growth and there was no growth in all the samples at higher temperatures. The total viable count was plotted against temperature (Figure 1). The chart shows that the lower the temperature of rice sample the higher the bacterial count. CFU count exceeding 10^5 CFU/ml was obtained from samples that had 45°C temperature.

Aside from temperature, the pH of the rice exerts a limited influence on the growth of *S. aureus*. The average of the pH of the rice samples was 4.7 for Bokhary rice and for Mandy rice it was 5.5. It seems from the results that the average of Bokhary rice was relatively lower than the average of Mandy rice.

Growth curves of *S. aureus* in rice dishes

The organism grew in both kinds of rice at 45ºC. Determination of the growth curve for *S. aureus* in Mandy rice showed that the population increased from 1.67×10^6 to 3.01×10^{10} within 24 hours (average generation time was 102 minutes) whereas the population increased in Bokhary rice from 1.44×10^6 to 3.92×10^9

within 24 hours (average generation time was 126 minutes). The growth curves of *S. aureus* in Mandy and Bokhary rice is shown in Fig (2) which demonstrate that the growth was faster in Mandy rice than in Bokhary rice.

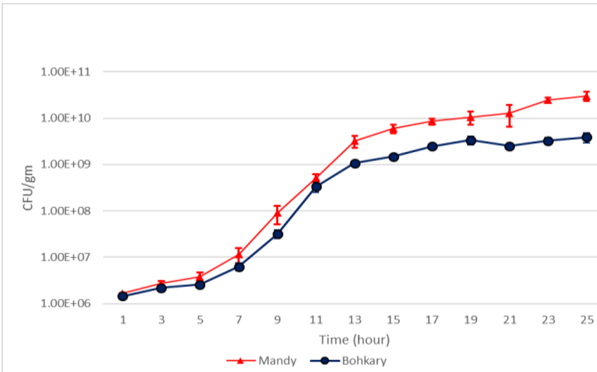

Figure 2. Growth curve of *S. aureus* in Mandy and Bokhary rice at 45°C. The mean of the quadruplicate data was taken and standard error bars presented.

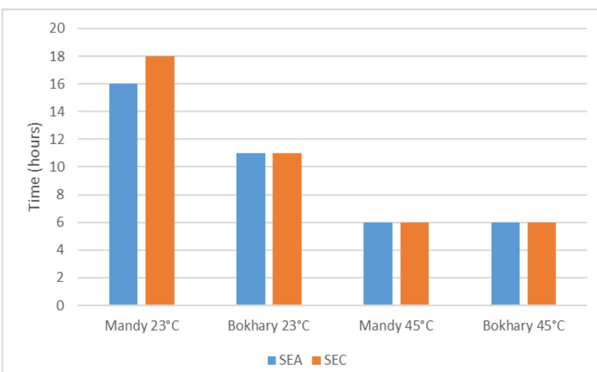

Figure (3): Effect of different temperatures on SEA and SEC enterotoxin production in Bokhary and Mandy Rice

Enterotoxin production

From the results presented in figure 3, SEA was first detected in Mandy rice after 16 hours and 6 hours when the incubation temperature was 23°C and 45°C respectively, while the SEC was detected after 18 hours and 6 hours when the incubation's temperature was 23ºC and 45ºC respectively. In Bokhary rice, SEA was detected after 10 hours at 23ºC incubation temperature, and after 6 hours at 45ºC, while the SEC was detected after 11 hours at 23ºC, and after 6hr at 45ºC incubation temperature. In both isolates the concentration of the enterotoxin increased as the growth curve progresses.

Discussion

The control of the holding temperature of foods after processing and before consumption is crucial for safe food production. General recommended holding temperatures are < 8°C for cold foods and > 63 8°C for hot food.[14]

Determination of variability in rice temperature of specimens during ten hours of incubation in pots were summarized in **Table 1**. The temperature of the rice samples varied during the day with higher temperatures were scored at the 12:00 PM noon samples (average 66.4, SD = 9.1) and lower temperatures obtained at the 10 hours later night samples (average 53.3, SD = 3.9). Two samples from restaurants R1 and R4 respectively, which have been taken at 12:00 AM had relatively low temperatures; this could be due to reheating of left over rice. Because most of the fresh cooked rice temperatures were higher than 60 °C even if more than one hour has already been passed after cooking.

Chart (1) shows that variation in rice temperatures had allowed bacterial growth particularly at lower temperatures. Higher bacterial count was obtained from samples with lower temperatures. The highest CFU count (2 x 10^5) was obtained from a sample that had a temperature of 45°C. These lower temperatures allow the multiplication of *S. aureus* which can grow at temperatures between 7 and 48◦C.[15]

It appears that sanitary qualities were best at noon, when the pots were not opened too much and the temperatures were relatively high. The average of night samples enter what is called “the microbiological temperature danger zone”.[14] All the samples with temperature below or equal to 52°C had shown bacterial growth and there was no growth in all the samples at higher temperatures. This confirm that restaurant should maintain the temperature of the served food outside the “microbiological temperature danger zone”.

However, 70.8 % of the total samples showed no growth, and 16.7 % showed CFU count > 2 x 10^3 cell/gm. This is lower than the results obtained by Jaad[16] who found that 67% of samples were rejected because they contain more than 1× 10^5 cell/gm, and higher than the results obtained by Ghazoli[17], who reported that 2.3% of samples were rejected. The type of the rice and the difference of total number and various kinds of dishes presented by restaurants could explain the variation of the results. The restaurant that sells only one type of food may concentrate more than that presents different kinds of dishes in many respects relating to efforts concerning preparation and sanitation.

The method of rice cooking had had an effect on the growth of *S. aureus* and enterotoxin production. Figure 2 demonstrates the growth curve of *S. aureus* in Mandy and Bokhary rice. It could be noticed that the growth was definitely faster in Mandy rice than in Bokhary rice. The difference could be attributed to: (1) The pH of the Mandy rice dish was 5.5 while Bokhary rice was 4.7 due to the addition of tomato past and lemon juice, which is in agreement with Genigeorgis and Sadler [18] who found that aerobic growth was prevented when the initial pH 4.8 in the medium. (2) Spices content of Bokhary rice, may cause inhibitory or interfering effect with the growth of *S. aureus* and other microorganism.

The components of rice dishes and method of cooking as well as the temperature of holding, all have an effect on the production of *S. aureus* toxin in the food. When the incubation temperature was 45°C, both SEA and SEB enterotoxins were detected after 6 hours in both types of rice. When the incubation temperature was 23ºC SEA and SEB enterotoxins both were detected after 11 hours in Bokhary rice and after 16 (SEA) hours and 18 hours (SEB) in Bokhary rice.

The minimum temperatures for SE production varies quite irregularly over a broad range between 15 and 38 °C and the maximum temperatures from 35 to 45 °C.[1] In the present study enterotoxin production was faster at 45 ºC than at 23ºC in both types of rice. It appears that the rate of toxin production was higher at the maximum temperature of enterotoxin production. At 45 ºC the type of rice had no effect on the rate of enterotoxin production as the two enterotoxins were detected at equal times in both types of rice dishes. However, at 23ºC enterotoxin was found to be produced earlier in Bokhary rice than in Mandy rice.

Although the growth rate was faster in Mandy rice than in Bokhary rice, enterotoxin was detected earlier in Bokhary rice. Staphylococcal food poisoning is a direct result of enterotoxin production and therefore enterotoxin production is more important than bacterial population per se. There are many factors that affect enterotoxin production, for example glucose, pH, NaCl concentration, temperature, water activity and redox potential.[11] In fact, in many countries, low degree contaminations by *S. aureus* are tolerated in most foodstuff (up to 10^3 cfu/g in raw milk cheeses, in France), as they are not considered a risk for public health.[8]

Conclusions

The components of rice dishes and method of cooking have an effect on the multiplication of *S. aureus and* toxin production in the food. Sanitary qualities were best and hence consumption of food from restaurant is KSA is safer at noon in the lunch time than in dinner time. The cooking method of rice had had an effect on the growth and enterotoxin production by *S. aureus*. Although the growth rate was faster in Mandy rice than in

Bokhary rice, enterotoxin was detected earlier in Bokhary rice. Entertoxin production rather than *S. aureus* population is the direct cause of staphylococcal food poisoning, therefore, from microbiological point of view, Mandy rice can be considered safer than Bokhary rice as regards food poisoning. The sanitary conditions in restaurant should be tightly monitored especially at Hajj season and education programs about proper methods of holding cooked rice and maintaining holding temperature and disposal of left-over food should be performed.

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