MICROBIOLOGICAL QUALITY EVALUATION OF FRIED RICE SOLD AT FOOD PREMISES IN KUANTAN CITY, PAHANG

IBRAHIM ABU BAKAR, PhD (CORRESPONDING AUTHOR)
DEPARTMENT OF NUTRITION SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN, MALAYSIA.

iab@iium.edu.my

AININ AZWANI ABDUL RAFA
DEPARTMENT OF NUTRITION SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN, MALAYSIA.

Ainaz0308@gmail.com

NORRAKIAH ABDULLAH SANI
SCHOOL OF CHEMICAL SCIENCES AND FOOD TECHNOLOGY, DEPARTMENT OF FOOD SCIENCE, FACULTY OF SCIENCE AND TECHNOLOGY, UNIVERSITI KEBANGSAAN MALAYSIA, BANGI.

norrasani@ukm.edu.my

ABSTRACT

Food contamination is a crucial health problem as it could result in food-borne illness. This research aimed to evaluate the microbiological quality of ready-to-eat (RTE) fried rice dishes sold at different type of food premises in Kuantan city, Pahang. Total Plate Count (TPC), *Staphylococcus aureus, Bacillus cereus* and *Aeromonas* spp. bacteria were used as microbiological contamination indicators. About 52 samples were collected stratified randomly from four types of food premises (restaurant, cafeteria, food stall and night market) where about 13 samples were respectively collected from each type of the food premises. The results showed that TPC had medium mean count ($6.30x10^5 \pm 1.47x10^5$ cfu/g), *S. aureus* and *B. cereus* had high mean counts ($7.70x10^4 \pm 2.22x10^5$ cfu/g and $3.85x10^5 \pm 1.67x10^6$ cfu/g respectively), while *Aeromonas* spp. had medium mean count ($7.13x10^4 \pm 2.42x10^5$ cfu/g). The mean counts of TPC in the samples collected from cafeteria were highest compare to other food premises.

KEYWORDS: Ready-to-eat fried rice, Total Plate Count, *Staphylococcus aureus, Bacillus cereus*

INTRODUCTION

Changing life style including consumption of more convenience foods on the-go had consequence a broad range of emerging food safety issues. Consumers now a day tend to choose food which is ready-to-eat (RTE) as they have less time to prepare it. Thus eating outside become more convenience way. RTE foods require no further preparation or processing prior to consumption. Fried rice is one of RTE dishes where boiled or steamed rice is stir-fried with cooking oil, soy sauce, chili sauce and tomato sauce. Typically it includes beaten egg, chopped meat, chicken, seafood and vegetables. Fried rice is well known all over the world since rice is a staple food for over half of the world's population. Fried rice is also popular food menu in Malaysia. It contains variety of food ingredients and fulfill nutrition requirement per serving size.

RTE fried rice dishes are sold at various food premises in Malaysia as breakfast menu. It also can be bought at street market especially at night market as dinner menu. Since the preparation of fried rice is normally too early before breakfast time, its safety status is questionable. The safety status of food sold at night market is also questionable. Therefore this study is designed to evaluate the safety status of RTE fried rice dishes sold at various food premises using microbiological contamination indicators that are TPC, *B. cereus, S. aureus* and *Aeromonas* spp. bacteria.

MATERIALS AND METHODS

Sample collection

A total of 52 fried rice samples were stratified randomly collected from four types of food premises (restaurant, cafeteria, food stall and night market) where about 13 samples were respectively collected from each type of the food premises in Kuantan city, Pahang. Samples were collected using conveniently random method to ensure the representativeness of the sample selection (Meldrum, *et al*., 2003). All fried rice samples were purchased and transported to the food analysis laboratory at Department of Nutrition Sciences, International Islamic University Malaysia, Kuantan campus in sterile plastic bag within 4 hours after sampling under controlled temperature conditions.

Sample preparation

The sample plastics were opened aseptically and about 25±1g samples were transferred into a sterile stomacher bag and homogenized for 60s with 225ml of sterile peptone water (Oxoid, Basingstoke, UK) using Stomacher Lab-blender 400 (Seward Medical, London, UK). Three serial dilutions were made using 1ml of the homogenate mixed with 9ml of sterile peptone water.

Total Plate Count (TPC)

A series of test sample dilutions were prepared using the same procedure as in 2.2. About 0.1 ml samples from each dilution series were pipetted on the Plate Count Agar (PCA) plates and were spread using bent glass rod (hockey stick). The plates were then incubated for 48±2 hours at 37±1 °C in an incubator. After incubation, the colonies of all the bacteria were observed to have appeared as white colonies. The plates which have 25-250 visible colonies were counted.

The total number of bacteria was calculated as mean colonies counted on each plate multiply with the dilution factor and then divided by the amount of diluted sample which was pipetted on the agar. The amount of total bacteria that was less than $1.0x10^3$ cfu/g was considered low while the amount between $1.0x10^3$ – $1.0x10^6$ cfu/g was considered medium and those which was more than $1.0x10^6$ cfu/g was considered high (Roberts and Greenwood, 2003).

***B.cereus* count**

About 0.1 ml samples from each dilution series were pipetted on Mannitol Yolk Polymyxin (MYP) agar plates and were spread using glass hockey stick. The plates were then incubated for 24±1 hours at 30±1 °C in an incubator. If colonies were not clearly visible then the plates were incubated again at 30±1 ^oC for another 24±1 hours. Colonies of presumptive *B. cereus* appeared as pink coloured colonies on the MYP agar and were usually surrounded by a zone of opacity. The plates which had 25-250 visible colonies were counted. The total number of *B. cereus* was calculated as mean colonies counted on each plate was multiplied with the dilution factor and then divided by the amount of diluted sample which was pipetted on the agar. The amount of *B. cereus* which was less than $1.0x10^2$ cfu/g was considered low whereas the amount between $1.0x10^2$ – 10^4 cfu/g was considered as medium and more than 10^4 cfu/g was considered high (Chang *et al.*, 2011).

***S. aureus* count**

A series of test sample dilutions were prepared using the same procedure as in 2.2. About 0.1 ml samples from each dilution series were pipetted on a Baird Parker Agar (BPA) plate and were spread using glass hockey stick. The plates were then incubated for 48±2 hours at 37±1 °C in an incubator. After incubation, the colonies of presumptive *S. aureus* appeared as black colonies surrounded by a light region. The plates which have 25-250 visible colonies were counted. The amount of *S. aureus* which was less than $1.0x10^2$ cfu/g was considered low whereas the amount between $1.0x10^2$ – $1.0x10^4$ cfu/g was considered as medium level and more than $1.0x10^4$ cfu/g was considered as high.

***Aeromonas* spp. count**

Analysis of *Aeromonas* spp. was started by preparing the test samples. A series of test sample dilutions were prepared using the same procedure as in 2.2 except that the peptone water was change with alkaline peptone water. About 0.1 ml samples from each dilution series were pipetted on selected agar (Ryan's agar) and were spread using glass hockey stick. The plates were then incubated for 18-24 hours at 30±1°C in an incubator. The colonies of presumptive *Aeromonas* spp. appeared as opaque dark green with darker centres. The plates which had 25-250 visible colonies were counted. The total number of *Aeromonas* spp. was calculated as mean colonies counted on each plate multiply with dilution factor and then divided by the amount of diluted sample which was pipetted on the agar. The amount of *Aeromonas* spp. which was less than 10^3 cfu/g was considered low while the amount between 10^4 – 10^5 cfu/g was considered as medium and a reading of more than 10^6 cfu/g was considered high.

RESULTS

Table 1 shows the total amount of indicator bacteria in the RTE fried rice samples and their statistical *p*-values.

TPC showed medium mean count ($6.30x10^5 \pm 1.47x10^5$ cfu/g) where the count was less than $1.0x10^6$ cfu/g. *S. aureus* and *B. cereus* showed high mean counts ($7.70x10^4 \pm 2.22x10^5$ cfu/g and $3.85x10^5 \pm 1.67x10^6$ cfu/g respectively), while *Aeromonas* spp. showed medium mean count ($7.13x10^4 \pm 2.42x10^5$ cfu/g). *B. cereus* had the highest mean count in total samples compare to the other bacteria.

Table 1. Total amount of indicator and presumptive pathogenic bacteria in RTE fried rice samples and their P-value

Indicator bacteria	Min	Max	Mean ± S.D.	p-value
TPC	$<1.00x10^2$ cfu/g	$7.40x10^6$ cfu/g	$6.30x10^5 \pm 1.47x10^5$ cfu/g	0.00
S.aureus	$<1.00x10^2$ cfu/g	$1.13x10^6$ cfu/g	$7.70x10^4 \pm 2.22x10^5$ cfu/g	0.10
B.cereus	$<1.00x10^2$ cfu/g	$1.00x10^6$ cfu/g	$3.85x10^5 \pm 1.67x10^6$ cfu/g	0.17
***Aeromonas* spp.**	$<1.00x10^2$ cfu/g	$1.61x10^6$ cfu/g	$7.13x10^4 \pm 2.42x10^5$ cfu/g	0.12

Table 1 also showed statistical comparison (*p*-value) between mean amounts of bacteria in samples which were collected at different type of food premises. Only TPC has a significant different value ($p<0.05$) compare to the other indicators bacteria. It means that there is at least one significant different of mean amount of TPC in samples which collected from different type of food premises. The difference is shown in Table 2.

Table 2 shows that only RTE fried rice samples which were taken from night market and cafeteria have significant different of their mean±S.D counts. The mean count of TPC in fried rice samples which were taken from cafeteria was higher than the samples from night market. All other indicator bacteria have no significant difference ($p>0.05$) in term of their mean±S.D count according to the type of food premises even though their amount were quite high (between 10^4-10^6 cfu/g).

Table 2. Total amount of indicator bacteria in RTE fried rice samples collected from food premises

Type of Food Premises	Mean ± S.D. (cfu/g)			
	TPC	***S. aureus***	***B. cereus***	***Aeromonas spp.***
Restaurant	$3.84x10^5 \pm 6.36x10^{5\ ab}$	$8.29x10^4 \pm 2.61x10^{5\ a}$	$1.18x10^5 \pm 1.59x10^{5\ b}$	$9.66x10^3 \pm 2.38x10^{4\ c}$

Cafeteria	$1.94x10^6$ ± $2.71x10^{6\ a}$	$1.54x10^5$± $3.31x10^{5\ a}$	$8.71x10^5$± $3.23x10^{6\ b}$	$1.81x10^5$± $4.16x10^{5\ c}$
Stall	$5.72x10^5$ ± $1.04x10^{6\ ab}$	$4.30x10^4$± $1.11x10^{5\ a}$	$5.38x10^5$± $6.91x10^{5\ b}$	$5.13x10^4$± $1.15x10^{5\ c}$
Night market	$1.36x10^5$ ± $4.28x10^{5\ b}$	$2.17x10^4$± $4.95x10^{4\ a}$	$1.23x10^4$± $2.53x10^{4\ b}$	$4.31x10^4$± $1.88x10^{5\ c}$

Note: Same symbol $^{a, b \text{ or } c}$ within the same column shows no significant difference ($p > 0.05$). S.D means Standard Deviation.

DISCUSSION

Total Plate Count (TPC) indicates microbiological quality of food. High amount of TPC means a contamination had occurred which presented a potential health hazard. This study shows RTE fried rice samples had medium mean count of TPC ($6.30x10^5 \pm 1.47x10^5$ cfu/g). The count was less than $1.0x10^6$ cfu/g which practically used by Ministry of Health Malaysia as microbiological level of satisfactory. Thus RTE fried rice dishes which was sold at food premises in Kuantan town had medium health risk. Contamination of TPC indicates poor food preparation and handling practices. The contamination may started from raw materials and longer holding hours of finished product at ambient temperature before being served. Fried rice dishes normally prepared by hand and cross contamination during the food handling may also occur. High counts of TPC alone do not make the food hazardous, but they show poor general hygiene (Smittle, 2000).

In this study, the RTE fried rice samples which were collected from cafeteria have highest TPC count. Cafeterias, which normally located at office complexes or education institutions, serve ready to eat food to many customers at one time. The cooks usually prepare a variety of foods in a big mass volume and food handling done in hurry. The possible contamination may also come from preparation area which normally quite small for food preparation, handling and storage (De Cesare *et al*., 2003). The mean count of TPC in fried rice samples which were taken from cafeteria was higher than the samples from night market. Fried rice dishes sold at night markets are normally still fresh (just cooked) or still at hot temperature. That is because of high demand from customers which caused the cooks to prepare the fried rice more frequently even though it was sold in a big mass volume.

S. aureus is a common bacteria that cause food poisoning. This study also shows *S. aureus* counts were highest in samples which were collected from cafeterias compare to other food premises. The presence of *S. aureus* in food may originate from unclean food handlers and their bad habits such as through coughing or sneezing. Hatakka *et al*. (2000) reported that *S. aureus* were detected in nose, throat, hand, and nail of food handlers.

B. cereus were also highest in RTE fried rice sample which were collected from cafeteria compared to other food premises. This result was supported by study conducted by Rusul and Yaacob (1995) where they found *B. cereus* in cooked and uncooked rice greater than 10^3 cfu/g. They also found that 28 % of *B. cereus* were detected in cooked food purchased from

University Putra Malaysia cafeterias. Kramer and Gilbert (1989) said *B. cereus* can lead to emetic food poisoning started with consumption of rice-based products and starchy foods.

Subsequently, *Aeromonas spp.* also showed high count at cafeteria. *Aeromonas hydrophilla* is a widespread representative of *Aeromonas* that can be found in foods such as raw meat, fish, shellfish, poultry vegetable and miscellaneous foods (Daskalov, 2006), although the organisms are considered as natural inhabitants in the aquatic environment. Contamination by *Aeromonas spp.* might have been due to method of raw material storage as it is a psychrotroph bacteria, *A.hydrophilla* grows in foods during refrigeration and currently has the status of a food-borne pathogen of emerging importance (Adams and Moss, 2000). Suspected foods were probably either inadequately cooked before consumption or were consumed raw or with minimal cooking (Daskalov, 2006). The usage of vegetables as one of the fried rice condiments probably led to *Aeromonas* spp. contamination. Based on McMahon and Wilson (2001), *Aeromonas* spp. were isolated from 34 % out of 86 organic vegetables samples and *Aeromonas schubertii* was the most commonly isolated species.

CONCLUSION

RTE fried rice dishes which are sold at the market in Kuantan city, Pahang especially at cafeteria have medium to high health risks.

REFERENCES

Adams, M. R., & Moss, M. O. (2000). *Food Microbiology* (Second.). The Royal Society of Chemistry.

Chang, H., Lee, J., Han, B., Kwak, T., & Kim, J. (2011). Prevalence of the levels of Bacillus cereus in fried rice dishes and its exposure assessment from Chinese-style restaurants. *Food Science and Biotechnology*, *20*(5), 1351–1359.

Daskalov, H. (2006). The importance of *Aeromonas* hydrophila in food safety. *Food Control*, *17*(6), 474–483.

De Cesare, A., Sheldon, B. W., Smith, K. S., & Jaykus, L. A. (2003). Survival and persistence of Campylobacter and *Salmonella* species under various organic loads on food contact surfaces. *Journal of Food Protection*, *66*(9), 1587–1594.

Hatakka, M., Björkroth, K. J., Asplund, K., Mäki-Petäys, N., & Korkeala, H. J. (2000). Genotypes and enterotoxicity of *Staphylococcus aureus* isolated from the hands and nasal cavities of flight-catering employees. *Journal of Food Protection*, *63*(11), 1487–1491.

Kramer, J. M., & Gilbert, R. J. (1989). *Bacillus cereus* and other Bacillus species. *Food-borne Bacterial Pathogens*, *19*, 21–70.

McMahon, M. A. S., & Wilson, I. G. (2001). The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *International Journal of Food Microbiology*, *70*(1–2), 155–162.

Meldrum, R. J., Smith, R. M. M., Ellis, P., & Garside, J. (2006). Microbiological quality of randomly selected ready to eat foods sampled between 2003 and 2005 in Wales, UK. *International Journal of Food Microbiology*, *108*(3), 397–400.

Rusul, G., & Yaacob, N. H. (1995). Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. *International Journal of Food Microbiology*, *25*(2), 131–9.

Roberts, D., & Greenwood, M. (2003). *Practical Food Microbiology* (Third.). United Kingdom: Blackwell Publishing.

Smittle, R. B. (2000). Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *Journal of Food Protection*, *63*(8), 1144–1153.