

Effect of Serving Time on Microbiological Quality of Food Served (Chicken Dish and Rice) During Wedding Banquet

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Abstract: *Ayam masak merah* (chicken in tomato sauce) and *nasi minyak* (flavoured ghee rice) are among the common dish served in Malay wedding banquet. The microbiological quality of these dishes becomes a concern when there was a food poisoning that caused four deaths after attending the wedding banquet. Therefore, the aim of this work is to investigate the microbiological quality of *ayam masak merah* and *nasi minyak* during the serving time (0, 1, 2, 3 and 4 hours) at the wedding banquet. The microbiological analysis were; total plate count (TPC), total coliform, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp., and *Listeria* spp. The results showed that *ayam masak merah* has the highest count for TPC, total coliform, *Escherichia coli*, *Staphylococcus aureus*, but low count in *Listeria* spp. and *Salmonella* spp. was not detected. Highest TPC was observed at the first hour of serving time, 7.33 log CFU/g, while for total coliforms and *Staphylococcus aureus* was at the 4 hours of serving time, 7.44 log CFU/g and 7.27 log CFU/g respectively.

Keywords: *Ayam masak merah*; *nasi minyak*; microbiological quality; serving time.

1. Introduction

Food poisoning cases in Malaysia is increasing; in 2016 the incidence rate is reported 47.34 per 100,000 populations [1]. This food poisoning incidence rate could include the case reported after attending the wedding banquet. In 2013, Salmonellosis outbreak was reported related to the wedding banquet in northern Malaysia, causing four deaths and 270 guests' sick [2]. This outbreak can describe how important of the awareness to prepare safe food. Several factors that lead to the occurrence of food poisoning are mishandling of food, lack of hygiene measures, environmental sanitation and abusing the time-temperature during the food preparation, storage and distribution. The data on the microbiological quality and safety of the food served and hygienic quality of the equipment and utensil used during the wedding banquet is still limited. This data is essential in providing the general idea of the quality and safety status of food that being served during wedding

banquet. The pathogenic microorganisms that always related with chicken dish are *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*. Cooked rice also have the possibility of growing pathogenic microorganisms same as chicken dish but with the addition of *Bacillus cereus*.

Therefore, it is crucial to indicate the microbiological quality and safety of food served during wedding. The aim of this study is to determine the effect of serving time on the microbiological quality of main dishes (chicken dish and rice) and to determine the present of pathogenic microorganism and its concentration in the main dishes (chicken and rice) from Malay wedding banquet.

2. Materials and Methods

Sample. The food samples were conducted at the actual wedding banquet. Food samples which were *ayam masak merah* and *nasi minyak* were collected at 0, 1, 2, 3 and 4 hours, respectively during serving time at the

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wedding event. The bags with food samples were kept in the ice box and transported to the laboratory for further tests.

Microbiological Analysis. Ten gram of each sample was homogenized in a stomacher (Stomacher Lab-Blender 400) with 90 ml of 0.1% peptone water. The homogenized sample was used as the basis for the total plate counts, and enumeration of coliforms, *Staphylococcus aureus* and *Bacillus cereus* (specific for rice sample). The homogenized sample was then pipetted on Plate count agar (PCA) for the total plate count, Mac Conkey agar for total coliform, Violet Red Bile agar (VRBA) for *Escherichia coli*, Baird Parker agar (BPA) for *Staphylococcus aureus* and PEMBA agar for *Bacillus cereus*. For the recovery of *Salmonella* sp. and *Listeria* sp., aliquots of 25g each sample was homogenized in 225ml buffered peptone water and *Listeria* enrichment broth respectively.

Temperature Measurement. Thermocouples (Thermocouple-EFC Fast, Pyrometer CH945) were used in monitoring the food temperature. Then it was sanitized with a tissue soaked with 70% ethanol. The desired temperature according to the compliance level is 60°C and above for hot food, 0 - 5°C for cold food and -10°C and below for frozen food.

Data Analysis. Minitab 16 was used to determine significant differences ($p < 0.05$) of serving time by using a one-way analysis of variance (ANOVA).

3. Results and Discussion

Total Plate Count (TPC). Table 1 shows the total plate count (TPC) of *ayam masak merah* and *nasi minyak* at 0 to 4 hours serving time. For *ayam masak merah*, the TPC detected in 0 hour serving time is 6.41 log CFU/g. While for 1, 2, 3 and 4 hours of serving time indicated no significant increase of TPC with 7.33, 7.04, 7.27 and 7.22 log CFU/g respectively). Whereas for *nasi minyak* also resulting no significant increase during the serving time for TPC. At 0 hour of serving time for *nasi minyak* is 6.07 log CFU/g, followed by 6.33, 6.94, 6.84, and 6.91 log CFU/g after serving time for 1, 2, 3 and 4 hours respectively (Table 1). According to these data, there is no significant increase ($p > 0.05$) of TPC with longer serving time in both of the foods.

Table 1 Total Plate Count (log CFU/g) of *ayam masak merah* and *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

^a Means that do not share a letter are significantly different.

Sample	Serving Time (h)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	6.41± 0.72 ^a	7.33± 0.34 ^a	7.04± 0.26 ^a	7.27± 0.18 ^a	7.22± 0.28 ^a
<i>Nasi Minyak</i>	6.07± 0.39 ^a	6.33± 0.92 ^a	6.94± 0.73 ^a	6.84± 0.57 ^a	6.91± 0.70 ^a

Generally, TPC is the most common method used to indicate the microbiological quality of a food [3]. As per results, both of samples can be assumed that have highly contaminated because the TPC is higher than 6 log CFU/g. It also resulting above the maximum limit ($> 10^6$ cfu/g) or 6 log CFU/g permitted by Food Act 1983 and Food Regulations 1985. This could be due to the foods that have been contaminated and temperature abused or spoilt [3]. In the previous study [4], stated that the contamination can also cause by insufficient heat during cooking process. However, high load of TPC in meat-based preparations might even be due to the existence of spoilage bacteria which is able to grow on a non-selective plate count agar medium [5]. Other important factors that contribute to this high TPC value of *ayam masak merah* and *nasi minyak* could be inappropriate handling and storage of the product [6], and also may due to lack of personal hygiene by staff [7].

Total Coliforms. The total coliform count for *ayam masak merah* and *nasi minyak* is presented in Table 2 There were no significant increase ($p > 0.05$) on the total coliform count during the 4 hours of serving time for *ayam masak merah*. From 0 to 1 hour indicates 6.19 log CFU/g to 6.95 log CFU/g respectively. From 1 hour to 2 hours serving time of *ayam masak merah*, the total coliform count is slightly reduced to 6.85 log CFU/g. Yet, it starts increasing to 6.99 log CFU/g and 7.44 log CFU/g of the total coliform count after 3 and 4 hours serving time respectively. For *nasi minyak*, at 0-hour total coliform count is 7.11 log CFU/g and showed a significant difference ($p < 0.05$) with another hour of serving time. Starting 1 until 4 hours serving time, there is reduction of total coliform of *nasi minyak* to 3.00 log CFU/g

Table 2 Total Coliform (log CFU/g) of *ayam masak merah* and *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (h)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	6.19± 0.73 ^a	6.95± 0.65 ^a	6.85± 0.71 ^a	6.99± 0.60 ^a	7.44± 1.24 ^a
<i>Nasi Minyak</i>	7.11± 1.70 ^a	3.00± 0.00 ^b	3.00± 0.00 ^b	3.00± 0.00 ^b	3.00± 0.00 ^b

^{a,b} Means that do not share a letter are significantly different.

Detection of coliforms is used as an indicator of sanitary quality of water used or as a general indicator of the sanitary condition in the food processing environment [8]. In one study, they stated that the existence of coliforms and *Escherichia coli* in the food samples indicate poor handling practices of food handlers and cross-contamination in the kitchen or serving units [9]. Although *nasi minyak* has higher amount of total coliform at only 0 hour of serving time, it can indicate existing variety of bacteria, and also affected by the primary source of contamination, agricultural production, storage steps and also the condition while preparing *nasi minyak* [10].

The higher numbers of total coliform of *ayam masak merah* could be due to the holding time that can cause bacterial contamination. It is also due to poor handling practices of food handlers and cross contamination in the kitchen [11].

***Staphylococcus aureus* (*S. aureus*) counts.** The *S. aureus* count for *Ayam masak merah*, has no significant increase ($p > 0.05$) during the 4 hours serving time (Table 3). For the *nasi minyak*, the *S. aureus* count is estimated <3 log CFU/g during the 4 hours serving time.

Table 3 *Staphylococcus aureus* (log CFU/g) of *ayam masak merah* and *nasi ayam* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (Hours)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	6.01 ± 0.62 ^a	6.36 ± 0.28 ^a	7.11 ± 0.11 ^a	7.23 ± 0.31 ^a	7.27 ± 0.28 ^a
<i>Nasi Minyak</i>	3.00 ± 0.00 ^a	3.00 ± 0.00 ^a	3.00 ± 0.00 ^a	3.00 ± 0.00 ^a	3.00 ± 0.00 ^a

^a Means that do not share a letter are significantly different.

For *ayam masak merah* at 0 hour, the *S. aureus* count is 6.01 log CFU/g and follows by

6.36, 7.11, 7.23 and 7.27 log CFU/g after 1, 2, 3 and 4 hours of serving time respectively. On the other hand, for *nasi minyak*, *S. aureus* count is estimated less than 3.00 log CFU/g from 0 hour until 4 hours of the serving time. It can indicate that the *nasi minyak* was handled by the waiter or food handler in hygienic condition. The high temperature during cooking processing can reduce the contamination of *S. aureus* in food [12]. The major causes of presences *S. aureus* in the dishes are from human origin and introduced by post-heat treatment and poor handling contamination.

The chicken was the most contaminated food and this may due to inadequate cooking, post-processing contamination, cross contamination through cutting knives or ladles used in the kitchen in addition to raw ingredients and spices during handling the cooked foods [12]. Though *S. aureus* is an abundant organism, the major reservoir of enterotoxin-producing staphylococci is human beings [13]. Therefore, the presence of staphylococci in cooked foods could indicate poor hygiene of food handlers and handling procedures [7]. Other than that, poor temperature control can induce the growth of *S. aureus*, and if the enterotoxin produced, it may remain after heating process and can lead to food poisoning [3].

***Escherichia coli* (*E. coli*) counts.** There is a significant difference ($p < 0.05$) between 0 and 1 hour of serving time *ayam masak merah* with 2, 3 and 4 hours of *E. coli* growth (Table 4). *E. coli* count for 0 and 1 hour serving time for *ayam masak merah* is reduce from 5.86 log CFU/g to 5.18 log CFU/g respectively. For 2, 3, and 4 hours showed *E. coli* reduce to less than 3.00 log CFU/g. Whereas for *nasi minyak*, at 0 hour of serving time (5.48 log CFU/g), then, the *E. coli* count reduce estimated to less than 3.00 log CFU/g. The presence of *E. coli* together with coliforms in food is indicating poor hygienic condition [3], poor handling practices of food handlers and cross-contamination in the kitchen [11].

Table 4 *Escherichia coli* (log CFU/g) of *ayam masak merah* and *nasi ayam* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (hour)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	5.86 ± 0.66 ^a	5.18 ± 0.25 ^a	3.00 ± 0.00 ^b	3.00 ± 0.00 ^b	3.00 ± 0.00 ^b
<i>Nasi Minyak</i>	5.48 ± 0.09 ^a	3.00 ± 0.00 ^b	3.00 ± 0.00 ^b	3.00 ± 0.00 ^b	3.00 ± 0.00 ^b

^{a,b} Means that do not share a letter are significantly different.

The probability of the guest in wedding banquet use hand to take the food may occur and this is an example of route contamination of *E. coli*. The contamination still can occur from human especially in handling salads [14]. It can lead to illness when the bacteria are transmitted from one person to another or from one surface to another by cross-contamination. Foodborne disease can cause by *E. coli* due to the undercooking consumption of ground beef or similar product [15].

***Bacillus cereus* counts.** Table 5 has showed *Bacillus cereus* count in rice with no significant increase ($p > 0.05$) during serving time. The range of means *Bacillus cereus* is from 5.44 log CFU/g at 0 hour to 6.81 log CFU/g at 4 hours. The previous study found that estimation of the infective dose of *Bacillus cereus* that can give food poisoning is vary from 3.00 to 10.00 log CFU/g of food [16]. However, it does not always cause illness when the populations present above 5 log CFU/g in foods [17].

Table 5 *Bacillus cereus* (log CFU/g) of *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (h)				
	0	1	2	3	4
<i>Nasi Minyak</i>	5.44 ± 0.06 ^a	6.27 ± 1.04 ^a	5.89 ± 1.26 ^a	6.41 ± 0.01 ^a	6.81 ± 0.56 ^a

^a Means that do not share a letter are significantly different.

Rice is arguably the most important foodstuff associated with *Bacillus cereus* food-poisoning. There are also common foods can be susceptible to *Bacillus cereus* spoilage such as herbs, spices, seasonings and flavoring [18]. However, a lot of reported cases regarding rice and *Bacillus cereus* caused foodborne illness. It is commonly presumed that the presence of *Bacillus cereus* in the cooked meal is from the multiplication of cells double in every half an

hour, also the consequences of secondary contamination [19].

Bacillus cereus food-poisoning associated with rice is the consequence of holding cooked rice for several hours at room temperatures [20], extended storage at unsatisfactory temperatures and inadequate heat processing [18] which can tolerate *Bacillus cereus* to grow and multiply. In the wedding banquet, *nasi minyak* was cooked several hours in advanced before the guests arrived, and this can be the reason for temperature abuse and improper handling. The foods also can be contaminated by the environment or additional food ingredients while serving until 4 hours [21]. The result off this study indicates a high number of *Bacillus cereus* in *nasi minyak* regardless of serving time, therefore preventive action need to be taken during food handling. [17], suggests minimizing the period of holding time of food between preparation, cooking and consumption, and also storage the cooked rice in proper temperature condition.

***Salmonella* spp. count.** Table 6 presents the results for *Salmonella* spp. It was not detected in *ayam masak merah* or *nasi minyak* during the serving time. *Salmonella* sp. is one of the important factors in foodborne disease. These results are in accord with recent studies indicating that absence of *Salmonella* sp. in their food samples in a total of 288 samples that showed a lack of contamination of raw material and also cross contamination [22].

Table 6 *Salmonella* spp. (log CFU/g) of *ayam masak merah* and *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (h)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	ND	ND	ND	ND	ND
<i>Nasi Minyak</i>	ND	ND	ND	ND	ND

ND = Not Detected

Eggs, poultry, meat and meat products are the most common food vehicles for salmonellosis infection in humans [23]. One of the examples of *Salmonella* origin is from chicken. This salmonellosis can be worse in addition to poor hygiene and sanitation when handling those chickens. From this wedding banquet event, there are no complaints or reported cases from the guests regarding all the symptoms mentioned. Apart from fecal

contamination that also can lead to salmonellosis, cross-contamination of foods by *Salmonella* during food preparation can be an important source of foodborne illness too. Massive efforts need to take into considerations in the areas of human and animal disease control as well as food hygiene in resulting the reduction of foodborne salmonellosis in global [24], or *Salmonella* outbreak will keep occur and give jeopardy to human health.

Listeria spp. counts. Table 7 presents the results obtained from the microbiological analysis of *Listeria* spp. on *ayam masak merah* and *nasi minyak*. It clearly showed for *nasi minyak*, all the samples were not detected in *Listeria* spp. from 0 hour of serving time until 4 hours. Although *Listeria* spp. can survive and multiply at 0°C, it can be destroyed by normal cooking with correct temperature.

Table 7 *Listeria* spp. (log CFU/g) of *ayam masak merah* and *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

Sample	Serving Time (h)				
	0	1	2	3	4
<i>Ayam Masak Merah</i>	3.39 ± 0.10 ^a	3.22 ± 0.08 ^a	3.21 ± 0.32 ^a	3.67 ± 0.05 ^a	3.00 ± 0.00 ^a
<i>Nasi Minyak</i>	ND	ND	ND	ND	ND

ND = Not Detected; ^a ^b Means that do not share a letter are significantly different.

The cases regarding listeriosis on cooked rice in Malaysia is not known because *Listeria* spp. is rarely tested in the food poisoning or infection cases [25]. For *ayam masak merah*, there is no significant increase ($p > 0.05$) of *Listeria* spp. count during 0, 1, 2, 3 hours with 4 hours of serving time.

Temperature. Table 8 shows the recorded temperature of dishes during serving. For *ayam masak merah*, the temperature recorded was within the danger zone (5°C - 60°C). This will favor the growth of bacteria. For *nasi minyak* also the temperature was within danger zone accept for hour 3 which is 65.0 °C. In general, bacteria grow most rapidly in the range of temperatures between 4°C and 60°C, twice replication number as little as 20 minutes. If the temperature is above 30°C, food should not be left out more than 1 hour [26].

Table 8 Temperature of *ayam masak merah* and *nasi minyak* at 0, 1, 2, 3 and 4 hours of serving.

Food Sample	Temperature (°C)				
	Serving Time (Hour)				
	0	1	2	3	4
<i>Ayam masak merah</i>	36.80	34.50	34.60	34.20	33.40
<i>Nasi minyak</i>	45.80	43.70	55.0 0	65.00	48.6 0

4. Conclusion

This study has found that *ayam masak merah* and *nasi minyak* was contaminated with various types of microorganisms. *Ayam masak merah* and *nasi minyak* have the highest concentration in TPC that indicates contamination during serving the foods irrespective of the serving time. However, both foods are free from *Salmonella* sp. Temperature abuse has occurred, both dishes was served at the inappropriate temperature during the serving time.

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