MICROBIOLOGICAL QUALITY AND SHELF LIFE STUDY OF READY TO EAT (RTE) *HALAL* BURGERS SOLD AT STREET STALLS AROUND KUANTAN CITY, PAHANG

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ABSTRACT: This study aimed to evaluate RTE *halal* burgers' microbiological quality and determine their shelf life. A total of 68 samples were randomly purchased from street stalls around Kuantan city as convenience selected samples and examined for Total Plate Count (TPC), presumptive *S. aureus* count and presumptive *E. coli* count. For the shelf-life study, 14 samples were purchased at one time from one street stall and the analyses of TPC and presumptive *S. aureus* count were performed at seven intervals of holding times. The results showed that all samples have low to medium risks with TPC $<3.0x10^3$ cfu/g and presumptive *S. aureus* count range between $0.1x10^2 – 3.3 x10^3$ cfu/g. In addition, Presumptive *E. coli* were not detected in all samples. *Therefore, halal* RTE street burgers have low to medium risks even though they were left at ambient temperature up to 24 hours of shelf life.

KEYWORDS: *Ready-to-eat Halal street burger, Total plate count, S. aureus, E. coli and Shelf-life study*

1. INTRODUCTION

Halalan toyyiban food means food that is permissible for consumption according to *Shariah* law and suitable for human consumption. *Toyyiban* aspects include cleanliness and safety where the food supposedly not cause any negative implication to human health. The most crucial element of *toyyiban* is an assurance that the food is free from any contaminants or hazards. According to Fahmi Abu Al-Rub *et al*. (2019), there are three categories of hazards in food which are physical, chemical and biological hazards. Biological hazards, especially microbiological hazards, are the most critical because pathogenic microorganisms and toxins can suddenly affect the consumers' health, including the cause of food poisoning and foodborne epidemic. Food microbiological analyses are then essential to be evaluated to ensure the safety aspect of *toyyiban* food, (Saeed, M.

& Baig, A. K., 2013). Laur Manea *et al*. (2017) said that microbiological analysis is also essential to determine the shelf life of food products.

The burger is one of the popular RTE food in Malaysia, which is claimed *halalan toyyiban.* RTE burger is appreciated well by young generations because of its delicious taste, convenient for immediate cooking and consumption and sold at an affordable cost, especially the one sold at street stalls. Compare to *Halal* RTE burgers sold at fast food chain restaurants. *Halal* RTE street burgers are more vulnerable which are seen exposing to clouds of dust and other street contaminants. Many burger stalls are located near roadsides and have no proper wall or protective barrier protecting them from street contaminants. Improper location of street burger stalls plus improper cooking standard can jeopardize *toyyiban* status of *Halal* RTE street burgers. Wong, W. C. *et al.* (2012) found that *L. monocytogenes* exist in 33.3% of raw frozen chicken burger patties, 22.9% of raw frozen beef patties and 10.5% of raw frozen fish patties that sold at retail markets in Malaysia. Babji *et al*. (2000) showed that TPC analysis of local brand frozen beef burgers was very low (in the range of 1x10 to 8x10 cfu/g). Similarly, the local brand frozen chicken burgers with a TPC range from 1x10 to 4x10 cfu/g. Safa *et al.* (2014) revealed that the total viable count of *halal* meat burger was $4.1x10^4$ cfu/g, while Al-Dughaym *et al.* (2010) stated that the mean bacterial count for raw frozen burgers sold in the Saudi Arabia market was ranged from $3.3x10^7$ cfu/g.

It was said that burger act as a potential source of *Listeriosis* spp. Suppose the contaminated burger patty is consumed without adequate cooking. Thus, raw frozen burgers must be defrosted and appropriately cooked to kill all the microorganisms before eating as RTE food. Many researchers performed studies on the cleanliness and safety of street foods. Shafizi, A. W. *et al*. (2016) and Kim, T. *et al*. (2016) found that *Staphylococcus aureus* exists in selected RTE food sold at the street market in Malaysia. Other studies related to the safety of RTE street foods in Malaysia also conducted by Alimi, B. A. *et al.* (2016), Ismail, F. H. *et al.* (2016) and Jores, D. *et al*. (2018) but specific knowledge about the microbiological contamination of RTE street burgers in Malaysia is still limited. According to Hussein, M. H. *et al.* (2011), burgers have limited stability, mainly because of microbiological activity and lipid oxidation. There were high initial counts of viable psychotropic and mesophilic microorganisms found in the samples, (Karpińska-Tymoszczyk, M., 2010). If the raw burger patties do not undergo a good cooking process, it will cause microbiological contamination and shorten their shelf life.

The purposes of this study were to evaluate the microbiological quality of *Halalan Toyyiban* RTE burgers sold at street stalls around Kuantan town and to determine their shelf life.

2. MATERIALS AND METHODS

2.1 RTE Street Burger Samples

About 68 *Halal* RTE street burgers (34 chicken burgers and 34 beef burgers) were conveniently randomly purchased from street stalls around Kuantan town in July 2019 as convenience randomly selected samples. Whole *Halal* RTE burger, which is sold at street markets in Kuantan town, comprises of fried chicken or beef burger patty, toasted rounded bun, cleaned and cut raw vegetables (few slices or small cuts of cucumber, onion, tomato and salad leaves or cabbage) and fill with condiment sauces (chilli, tomato and or black paper sauces). The whole *Halal* RTE burger was wrapped with plastic laminated paper or put in a polystyrene box. The purchased samples were kept in a sterile plastic bag and were placed in an icebox at $<4^oC$. The samples were then transported immediately to the microbiological laboratory at The Department

of Nutrition Sciences, International Islamic University Malaysia, Kuantan campus and were continuously kept at <4^{o}C by putting them in the chiller. Microbiological analyses were performed in that laboratory within 24 hours.

2.2 Microbiological Analysis

2.2.1 Total Plate Count (TPC)

All the samples were treated individually. First, the whole *Halal* RTE burger sample was mixed by hand, pressing in its sample plastic. The plastic of the sample was then opened aseptically and about 25±1g of mixed samples were transferred into a sterile stomacher bag which contained 225ml of sterile peptone water, (Oxoid, Basingstoke, UK) and was homogenized using Stomacher Lab-blender 400, (Seward Medical, London, UK) for 60 seconds. The homogenized sample was considered as the first sample dilution (10^{-1} sample dilution factor). Next, another dilution series was made by pipetting 1ml of homogenate from the first sample dilution into a test tube containing 9ml of sterile peptone water (this dilution was then considered second dilution or 10^{2} sample dilution factor). Finally, the third sample dilution was prepared by pipetting 1ml of homogenate from the second sample dilution into a test tube containing 9ml of sterile peptone water (this dilution was determined as third dilution or 10^{-3} sample dilution factor).

About 0.1 ml homogenate samples from each dilution were pipetted onto two Plate Count Agar (PCA), (Oxoid, Basingstoke, UK) plates (as duplicate) and were spread using a sterile bent glass rod. All the plates were then incubated inversely in an oven for 48±2 hours at 37±1 °C. After 48-hours of incubation time, the agar plates with 25 - 250 visible colonies were selected according to the dilution factors. The number of visible colonies (white colour) at each selected plate were counted using a colony counter. The total colony forming unit (cfu) per g of the samples tested were calculated using a specific formula. The sample, which has a total colony forming unit less than $1.0x10^{3}$ cfu/g, was considered low contaminated with TPC, while the amount between $1.0x10^{3}$ – $1.0x10^{6}$ cfu/g was deemed to be medium contaminated with TPC. The sample with more than 1.0x106 cfu/g was considered highly contaminated with TPC, (Roberts and Greenwood, 2008).

2.2.2 S. aureus Count

A series of test sample dilutions (10^{-1} - 10^{-3} dilution factors) were prepared using a similar method in TPC analysis. About 0.1 ml homogenate samples from each dilution series were pipetted and spread onto two Baird Parker Agar (BPA), (Oxoid, Basingstoke, UK) agar plates (as duplicate) using a sterile bent glass rod. All the plates were then incubated inversely in an oven for 48±2 hours at 37±1°C. After 48-hours of incubation time, visible colonies of presumptive *S. aureus* appeared as black colonies surrounded by a light region. The agar plates, which have 25-250 visible colonies, were selected according to the dilution factors. The total colony forming unit (cfu) per g of the samples tested were calculated using a specific formula. The sample with total colony forming unit < $1.0x10^{2}$ cfu/g was considered low contaminated with presumptive *S. aureus*, whereas the amount between $1.0x10^{2}$ – $1.0x10^{4}$ cfu/g was deemed to be medium contaminated with presumptive *S. aureus*. The sample with

more than 1.0x104 cfu/g was considered highly contaminated with presumptive *S. aureus*, (Roberts and Greenwood, 2008).

2.2.3 Detection and Enumeration of E. coli spp.

Analysis of *E. coli* spp. was started with the study of Coliform and F. coliform. The Most Probable Number (MPN) technique was employed where a statistical approach for estimating the number of microorganisms in a test sample was used. A series of test samples dilutions were prepared using the same procedures as in 2.2.1 and 2.2.2. About 1 ml of homogenate sample from each of the dilution tube was pipetted into 9 test tubes (3-3-3 formation) that contained Lauryl Tryptose Broth (LTB), (Oxoid, Basingstoke, UK). In each LTB tube, there was a Durham tube in which its function was to collect the gas production. LTB tubes were then incubated at 37±1 °C in an oven for 24±1 hours and after the incubation, the LTB tubes were assessed for the growth reaction giving a pattern of positive and negative results. A positive result was shown by the turbidity of LTB broth and gas production in the Durham tube. The negative LTB tubes were incubated back at 37±1 °C in an oven for an additional 24±1 hours and examined positive and negative results. A confirmation test on all presumptive positive (gas) tubes was done were from each gasses LTB tube, a loopful of suspension was streaked to Eosine Methyline Blue (EMB), (Oxoid, Basingstoke, UK) agar and then was incubated at 37±1 °C for 24±1 hours. The confirmed presence of coliforms in the sample was characterized by the growth colonies on EMB agar which are green shiny metallic colour with the dark purple area in the centre. The most probable number (MPN) of coliforms was calculated based on the proportion of confirmed LTB tubes using the pattern or profile of positive and negative results. The profile was converted to a most probable number by referring to published MPN tables, (Roberts and Greenwood, 2008).

From each of gasses LTB tube from the presumptive coliform test, a loopful of suspension was transferred to a tube of Triptone Broth (TB), (Oxoid, Basingstoke, UK) and to a tube of Brilliant Green Lactose Broth (BGLB), (Oxoid, Basingstoke, UK) which also contained a Durham tube. The BGLB tubes were then incubated in a water incubator for about 48±2 hours at 44±1°C. Positive gasses of TB tubes were incubated in an air oven for 24±1 hours at 37±1°C. The presence of F. coliform was shown by the turbidity of the BGLB tubes and the production of gas in the Durham tube after 48±2 hours. The confirmation test for F. coliform was conducted by adding Kovacs' reagent into Triptone broth. The appearance of a distinct red colour in the upper layer of the broth represented a positive result for the confirmation test of F. coliform. The number of F. coliform in the sample was calculated using the MPN method like coliform calculation, (Feng et al., 2002).

Analysis of *E. coli* spp. was performed by gently agitating each of the BGLB gasses tubes. A loopful of the BGLB suspension was then streaked on an Eosine Methylene Blue (EMB) agar plate. The EMB agar plate was then incubated for 24±1 hours at 37±1 °C in the oven. Suspicious colonies of *E. coli* spp. were seen flat and dark in the centre with or without metallic sheen. Up to 5 suspicious *E. coli* spp. Colonies from each EMB plate were transferred to PCA slants and incubated for 24±1 hours at 37±1 °C. Colonies of presumptive *E. coli* spp. was shiny green metallic with a dark purple in the centre. The sample that contained E. coli was considered high risk (Feng *et al*., 2002).

2.3 Shelf Life Study

About 14 samples of *Halal* RTE burgers were purchased at the same time from one street burger stall in Kuantan town as purpose selective samples. Each *Halal* RTE burger samples have a fried chicken burger patty, toasted burger bun, vegetables and condiment sauces and wrapped with plastic laminated paper by the seller. Each wrapped *Halal* RTE burger sample was put in a sterile plastic bag and kept in an icebox at <4^{o}C. All the samples were transported immediately to the microbiological laboratory at The Department of Nutrition Sciences, International Islamic University Malaysia, Kuantan campus. All the samples were then divided into seven groups, where each group contains two burgers (duplicate). The seven groups represented seven interval holding hours (<4h, 4h, 8h, 12h, 16h, 20h, 24h). The wrapped burger samples were then kept at room temperature (about 23±1^{o}C) in the laboratory according to dedicated holding hours. Analyses of TPC and *S. aureus* count were performed for each group of samples at each interval holding hours. TPC and *S. aureus* counts indicate general microbiological contamination and contamination by poor cleanliness of food handler, respectively (Halkman, H.B.D and Halkman, A. K., 2014).

3. RESULTS AND DISCUSSION

3.1 Microbiological Quality of RTE Street Burgers

The results of microbiological analyses of *Halal* RTE burgers sold at street stalls around Kuantan town is shown in Table 1.

Table 1: Total Amount of Indicator and Presumptive Pathogenic Bacteria in RTE *Halal* Street Burger Samples.

Bacteria	Min	Max
TPC	6x10 cfu/g	3.0 x10^{3} cfu/g
Presumptive *S. aureus*	1.0 x10 cfu/g	3.3 x10^{3} cfu/g
Presumptive *E. coli*	Not detected	Not detected

Table 1 shows that the minimum total amount of indicator bacteria (TPC) in RTE *Halal* street burger samples was 6.0x10 cfu/g while the maximum total amount was 3.0x10^{3} cfu/g. According to Roberts and Greenwood (2008), if the amount of TPC in RTE food less than 103 cfu/g shows low contamination and if the amount of TPC in RTE food between 10^{3} to 10^{6} cfu/g, it shows medium contamination. In this study, the amount of TPC in RTE street burger samples was between 6.0x10 - 3.0x10^{3}. This means the samples have low to medium contamination of TPC or very minimum bacteria contamination in the study samples. According to Mendonca *et al.* (2020), analysis of TPC is used to estimate the bacterial population in a food sample, which estimates the numbers of microorganisms that can grow aerobically at mesophilic temperatures. Therefore, it can judge sanitary quality, sensory acceptability and conformance with good manufacturing practices (GMPs). The low count of TPC in street RTE burgers may vary due to several reasons, such as the raw meat patty was well defrosted and fried using high temperature, the burger bun was well toasted, all vegetables used were clean, all condiments used were not contaminated and the whole set of the burger was immediately wrapped and served to the customer. The whole *Halal*

RTE street burger was wrapped properly using plastic laminated paper and not exposed to the surrounding environment.

This study also shows that the count of presumptive *S. aureus* in the *Halal* RTE street burger samples was between 10 to 3.3 $x10^3$ cfu/g. It shows that the samples contained a low to moderate count of presumptive *S. aureus.* The amount was not exceeded 10^4 cfu/g as suggested by Roberts and Greenwood (2008). Thus, the *Halal* RTE burgers at street markets in Kuantan town are still safe for consumption. According to the European Commission Regulation on the microbiological criteria for foodstuff, (European Commission, 2005), the food is considered high microbiological risk if it contains presumptive *S. aureus* more than 10^4 cfu/g. When the result of this study is compared to Australia and New Zealand Guidelines for the level of presumptive *S. aureus* in ready to eat food, (Food Standards Australia New Zealand, 2012), the amount of the bacteria between 10^2 -10^3 cfu/g is considered at the marginal range or at borderline which means it still safe for consumption but may indicate possible hygiene problem in the preparation of food. The contamination may come from the hand of food handlers or other personal hygiene. Taylor, T. A. and Unakal, C. G. (2021) said that *S. aureus* is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals. Noor-Azira, A. M. *et al.* (2012) found that the prevalence of *S. aureus* carriage is high among our food handlers in Kuala Pilah, Malaysia and suggested proper training on food safety among them to improve food handler's sanitation.

As for the *E. coli* determination, the results show negative detection. No single colony of *E. coli* was detected in all the samples. It also shows no faecal contamination and no presence of enteric pathogens such as *S. Typhi.* Therefore, the results confirmed the safety level of *Halal* RTE burgers sold at the street market in Kuantan town.

3.2 Shelf Life Study of RTE Street Burgers

Figure 1 shows the microbiological growth of TPC and *S. aureus* in RTE burger samples at different intervals hours of holding time. Figure 1 shows that *Halal* RTE street burgers contained some amount of TPC ($1.2x10^2$ cfu/g) at an initial time and it was considered a low amount ($<10^3$ cfu/g). The bacteria might come from raw vegetable or condiment sauces. It is pretty hard to say that the initial bacteria come from the burger patty and toasted bun since the patty and the bun were appropriately cooked and still hot when the *Halal* RTE burger was wrapped. The TPC growth was seen declining to $0.6x10^2$ cfu/g (lowest amount) after 4 hours of holding time. This situation can be considered the lag phase of the bacteria growth, where some of the bacteria were killed during the adaptation process to the new environment. According to Yates, G. T. and Smotzer, T. (2007), the lag phase is thought to be due to the physiological adaptation of the cell to the culture conditions. In this study, the lag phase occurred for about 4 hours, and it indicates the safest time to eat the *Halal* RTE burger. After four hours of holding time, the bacteria growth was seen increasing again to $1.7x10^2$ cfu/g at eight hours of holding time. This situation might be the log phase of the bacteria growth where the new bacteria proliferate, or duplication process of the bacteria are actively happening due to less competition of life. According to Todar, K. (2020) log phase or exponential phase is a balance bacteria growth pattern where all the cells are dividing regularly by binary fission and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and incubation conditions.

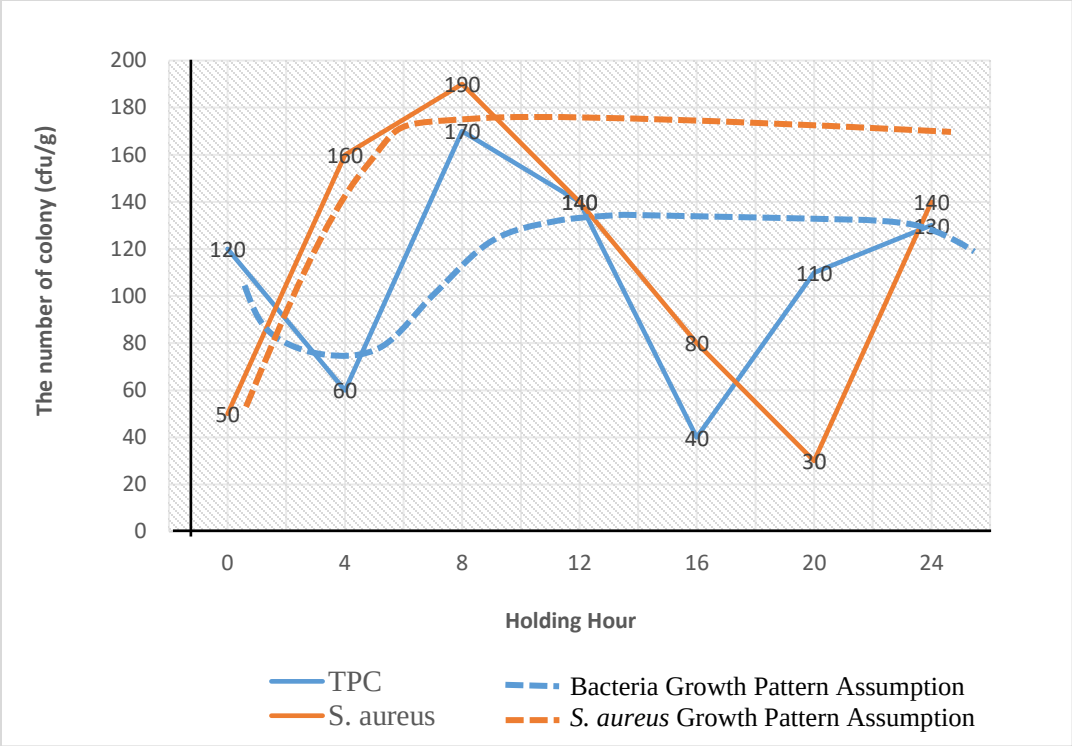

Figure 1: The Microbiological Growth of TPC and *S. aureus* at Different Holding Times.

In this study, even though the number of bacteria was increased after four hours to eight hours, the amount was still $<10^3$ cfu/g (low risk). The TPC growth was seen decreasing to $1.4x10^2$ cfu/g and $0.4x10^2$ cfu/g after 12 hours and 16 hours, respectively. The TPC growth was seen increasing back after 16 hours and the growth was kept increasing after 20 hours and 24 hours but not exceeding $1.3x10^2$ cfu/g. At 24 hours of holding time, the number of bacteria still not exceeding 10^3 cfu/g, which means the burger was still safe for consumption. We assumed that the growth of bacteria in this study from the 8th hour to the 24th hour is at the stationary phase even though the growth fluctuated every four hours (at 12th, 16th, 20th and 24th hour). The blue dotted line indicates the assumption growth pattern of TPC in Figure 1. If this assumption is valid, the bacteria growth pattern in this study is nearly similar to the bacteria growth curve, as suggested by Todar, K (2020). This study was ended at 24 hours of holding time. It was considered the limitation of this study and need further investigation, which the stationary phase may be prolonged, thus increasing the shelf life of *Halal* RTE street burgers.

For the growth of *S. aureus* in this study samples, a similar fluctuation pattern was seen as of TPC growth. The initial amount of *S. aureus* was at $0.5x10^2$ cfu/g (medium risk). The existence of *S. aureus* in the samples might come from the un-hygienic hands of food handlers. The bacteria might affect the burger when handling vegetables or sauces or when the burger was wrapped. The growth pattern of *S. aureus* in this study is seen as no lag phase, or the lag phase is too short to be identified. This may be because the amount of S. aureus at the initial growth point is higher than other bacteria or at the dominant stage. Within four hours of holding time, the amount of *S. aureus* increased drastically to $1.6x10^2$ cfu/g. The amount of *S. aureus* kept increasing to $1.9x10^2$ cfu/g after 8 hours of holding time. The increasing pattern of *S. aureus* from 0 to 8 hours is considered as the log phase. After 8 hours of holding time, the amount of *S. aureus* decreased to $1.4x10^2$ at 12

hours of holding time and $0.8x10^2$ cfu/g at 16 hours of holding time, respectively. Finally, *S. aureus* was seen increasing again to $0.3x10^2$ cfu/g and $1.9x10^2$ cfu/g at 20 hours and 24 hours, respectively. Similarly to TPC growth, we assumed that the growth of *S. aureus* in this study from the 8th hour to the 24th hour is at stationary phase even though the growth was fluctuated every four hours (at 12th, 16th, 20th and 24th hour). The red dotted line indicates the assumption growth pattern of S. aureus in Figure 1. If this assumption is valid, the *S. aureus* growth pattern in this study is nearly similar to the bacteria growth curve suggested by Todar, K (2020). According to Todar, K (2020), bacteria exponential growth cannot be continued forever. Bacteria growth is limited by exhaustion of available nutrients and accumulation of inhibitory metabolites or end products. Wang, J. (2017) reported that Staphylococci are susceptible to nutrient depletion.

The growth of *S. aureus* in this study was seen fluctuating between $0.5x10^2$-$1.9x10^2$ cfu/g (medium risk) within 24 hours of holding time. According to European Commission (2005), the amount of *S. aureus* $<10^4$ cfu/g is considered safe for consumption. This means that *Halal* RTE street burgers which sold at street market at Kuantan city are safe for consumption within 24 hours of shelf life even though it contains medium risk. The contamination of *S. aureus* in *Halal* RTE street burgers may cause poor personnel hygiene, inadequate handwashing practices, and inappropriate handling, preparation, and burger wrapping.

4. CONCLUSION

Halal RTE burgers sold at street markets in Kuantan city are safe for consumption even though they have low to medium risks where TPC was enumerated at <3.0x103 cfu/g and S. aureus <3.3 x 10^3 cfu/g. Pathogenic *E. coli* were also not detected in all *Halal* RTE street burger samples. Microbiological growth study shows that *Halal* RTE street burgers are still safe for consumption even though after left at ambient temperature for up to 24 hours.

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