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Microbiological Quality of Food Contact Surfaces At Selected Food Premises of Malaysian Heritage Food ('Satar') in Terengganu, Malaysia

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----------ABSTRACT----------

Satar' is a blend of succulent boneless fish marinated in spices, wrapped in banana leaves and grilled over flaming charcoal. It is a very popular ready-to-eat food sold in the East Coast of Peninsular Malaysia. The vehicle and routes of 'Satar' contamination could come from raw materials and food contact surfaces during preparation and handling of 'Satar'. However, this study only focused on the possibility of contaminations which came from food contact surfaces. This study was carried out to determine the Aerobic Plate Count (APC), Enterobacteriaceae count, Staphylococcus aureus count, Pseudomonas count and the presence of Salmonella sp. *in swab samples from ten selected food contact surfaces in two popular 'Satar' premises in Terengganu. Results showed that all food contact surfaces used in the Premise A which were cutting board, knife, table of preparation, mixer, food handler's hand, container, spoon, banana leaves, skewer and surface of griller were highly contaminated with indicator microorganisms (aerobic mesophilic organisms, Enterobacteriaceae and Pseudomonas) compared to food contact surfaces of premise B. This findings highlight the possibility of microbial contamination in 'Satar' that could come from contaminated food contact surfaces. Further study should be carried out in improving the hygienic status of 'Satar' premises and local RTE foods.*

KEYWORDS: *Microbiological qualities, 'Satar', Food contact surfaces, indicator organisms, RTE foods*

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I. INTRODUCTION

'Satar' is a ready-to-eat (RTE) food and it is a special dish in any occasion and popular among tourists in the East Coast of Peninsular Malaysia. 'Satar' is a blend of succulent boneless fish marinated in spices, wrapped in banana leaves, put into skewers and grilled over a flaming charcoal fire until the filling is dry and firm. The slightly burnt banana leaves give a lingering aroma to the product, which is sweet, fragrant and rich in flavours (Anonymous, 2014). This product has become popular as a great appetizer and a healthy traditional snack and its demand and consumption is increasing due to its unique taste and its special recognition as one of 'heritage food' in Malaysia. Presently, safety and quality of 'Satar' is variable as the microbiological safety and quality specification for this product has not been fully established. Each premise has its own family recipe for preparation of 'Satar' that has been inherited by generations. Due to its ingredients contain perishable items, such as fish, grated coconut and spices, this product has limited shelf life and only can be sold in small industry to ensure its freshness and tastefulness.

Possible sources of microbial contamination may come from poor quality of raw materials, time and temperature abuse during grilling and storing (Roberts, 1990), insanitary equipments and utensils, poor hygienic practices of food handler's hand (Gilbert et al., 2007) and recontamination (Reij et al., 2004). 'Satar' preparation involved a lot of contact surface of utensils such as cutting board, knife, blenders, table of preparation, containers, surfaces of grillers, food handlers hand, skewer, spoon and banana leaf. Various studies have reported that complete elimination of pathogens from food processing environment and utensils are difficult, as many foodborne pathogens are known to be able to attach on food contact surfaces (Gounadaki et al., 2008). Examination of foods for microbial indicator organisms, such as Aerobic Plate Count, *Enterobacteriaceae*

count, and *Pseudomonas* count has become normal practice to monitor food safety and quality control of food, besides evaluating the overall food sanitation system applied to the food operation. In addition, *S. aureus* count was used to determine the possibility of contamination come from food handlers. There is limited research has been reported on traditional Malaysian ready-to-eat (RTE) foods where few studies have been published include microbiological quality of 'Keropok lekor' (Nor-Khaizura, et al., 2009). Hence, this study provides the evidence that food contact surfaces play an important role as the vehicle for contamination in 'Satar' operation. These findings would be useful for improving the processing procedure and maintaining the quality of 'Satar' during and after preparation until it is sold. The objective of this study is to evaluate the microbiological quality of food contact surfaces that have been used in the production of 'Satar' premises using swab method.

II. MATERIALS AND METHODS

Sampling procedure: A total of 18 sets of 'Satar' before grilling and 18 set of 'Satar' after grilling were purchased over a period of three months (from May to July 2010) with 82 selected food contact surfaces from two most popular 'Satar' producer in Kuala Terengganu. Kuala Terengganu was chosen because it is a capital city of Terengganu and has received many tourists attraction. Samples were collected from two selected 'Satar' premises in Kuala Terengganu. Surface samples were collected from ten control points of the processing environment and equipment from each premise; cutting board, knife, mixer, table of preparation, container, surface of griller, food handlers' hand, skewer, spoon and banana leaves. The whole experiments were repeated three times for both premises in order to obtain the average data for statistical analysis.

Microbiological analysis: Food contact surfaces sampling was performed by swabbing a delimited area (100 cm^2) according to Yousef and Carlstrom (2003). Swab head rubbed slowly and thoroughly over an area of about 50 cm^2 of sampled area as mentioned earlier. Then, rinsed swab head into sterile 10 ml of 0.1% buffered peptone water (MERCK) and 10 ml of Lactose Broth (MERCK) as pre-enrichment for *Salmonella* and pressed out the excess. It was repeated for other 50 cm^2 area using the same swab head. The swab was broken off, while the head was remained.

The total surface swab for each food contact surfaces in this study was 100 cm^2. All swab samples were placed in an ice-cooled box and transported immediately to the laboratory for microbial analysis. Upon arrival in the laboratory, the swab samples were transferred into 10 ml of buffered peptone water (MERCK) making 10^{-0} dilution factor. One milliliter of the homogenized 10^{-0} diluted sample was transferred and mixed with 9 ml of peptone water solution to provide 10^{-1} dilution. The step was repeated until the desired dilution was taken. These serial dilutions containing samples were further analyzed. In order to enumerate the microflora, 0.1 ml portions of appropriate dilution were poured or spread onto duplicate plates of the appropriate culture medium as follows: Aerobic plate count (APC) were obtained from the following plate count agar (MERCK), incubated at 35^0C for 24 h; *Enterobacteriaceae* count on the Violet Red Bile Dextrose Agar (MERCK), incubated at 35^0C for 24 h; *Pseudomonas* on the *Pseudomonas* agar base (OXOID) incubated at 35^0C for 24 h; *Staphylococcus aureus* on Baird-Parker agar supplemented with Tellurite Yolk Egg (MERCK), incubated at 35^0C for 48 h (Gibbons et al., 2006). Enumeration of agar plates as CFU/g for all microbial analyses followed the counting rules by American Public Health Association (Downes and Itoh, 2001). Whenever necessary, biochemical tests were carried out for further confirmation according to the standard protocols (Bacteriological Analytical Manual, 1998).

Isolation and identification of *Salmonella* sp.: Detection of *Salmonella* was carried out according to the Bacteriological Analytical Manual, established by FDA, USA (1998). Initially, the swab samples were initially transferred into Lactose Broth (MERCK) and incubated at 35°C for 24 hours. Then, 0.1 ml mixture was transferred to 10 ml Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml Tetrathionate (TT) broth. The mixtures were vortexes gently. The swab samples were incubated in RV medium at 42 ± 0.2°C for 24 ± 2 hours and TT broth was 35 ± 2.0°C at 24 ± 2 hours. After incubation, 1 loopful of TT broth and RV medium were transferred into three selective media from MERCK; Bismuth Sulphite (BS) agar, Xylose Lysine Desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. All plates then were incubated at 35°C for 24 ± 2 hours. For XLD agar, the typical *Salmonella* colonies were red-pink colonies commonly have a black center while BSA, the typical *Salmonella* colonies were black to green without a dark halo and metallic sheen and for HE agar a typically few *Salmonella* cultures produced yellow colonies with or without black centres (Merck, 2007). Lastly, biochemical identifications were continued using Triple Sugar Iron (TSI) agar and Lysine Iron (LI) agar. Using a sterile inoculating needle, the selected colony was touched and stabbed with needle into the butt of TSI and LIA, stopping 1-2 cm from the base of the tube and incubated at 35^oC for 24 hours. A *Salmonella* positive result was an alkaline (red) slant and acid (yellow) butt, probably, with black precipitates present. Sometimes gas also was produced (Bacteriological Analytical Manual, 1998).

Statistical analysis: Mean and ± standard deviation of microbial counts were analyzed using Minitab Version 14. Microsoft Excel 2007 was used to enter raw data of microbial count from different microbial analysis into $\log_{10}$ CFU/cm^2. While in Minitab, the significance was determined using Paired samples *t*-test. Independent factor (food contact surfaces) was performed on response parameters (microbial counts for microbiological analysis).

III. RESULTS AND DISCUSSION

Microbiological analysis of food contact surfaces : Figure 1 and 2 show the average means of three visits for the microbial counts of Aerobic Plate Count, *S. aureus* count, *Pseudomonas* count and *Enterobacteriaceae* count in the ten selected food contact surfaces obtained from Premise A and Premise B, respectively. Table 1 shows the significant different ($P<0.05$) between both premises using t-test. These microbial analyses were commonly used as indicator for the hygienic status in the food premises. Environmental contamination in 'Satar' premise may enter into food through direct contact and cross-contamination during the handling and preparation of 'Satar'.

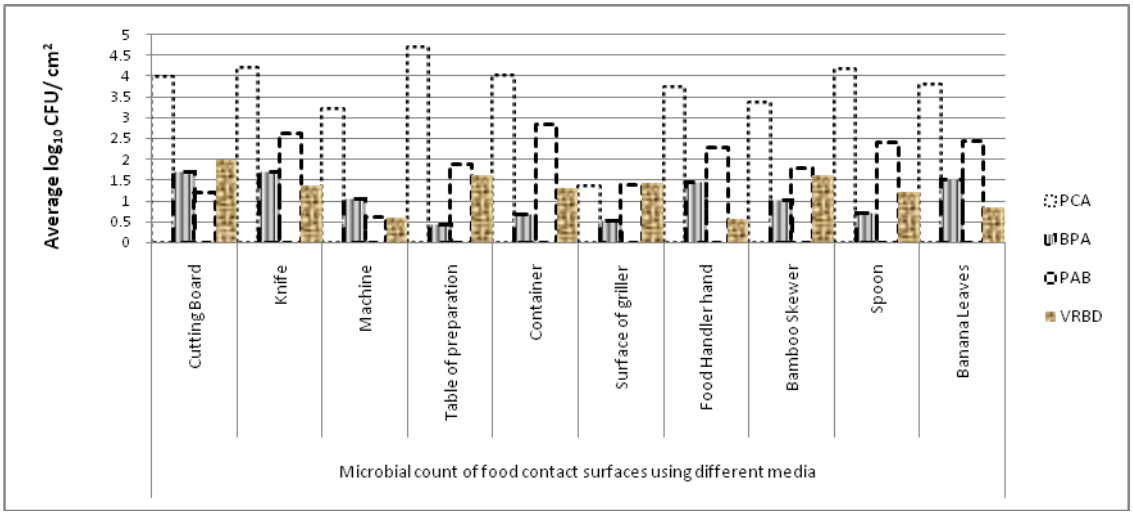

Figure 1 : Average means of various microbial counts (CFU/cm^2) of food contact surfaces in the Premise A

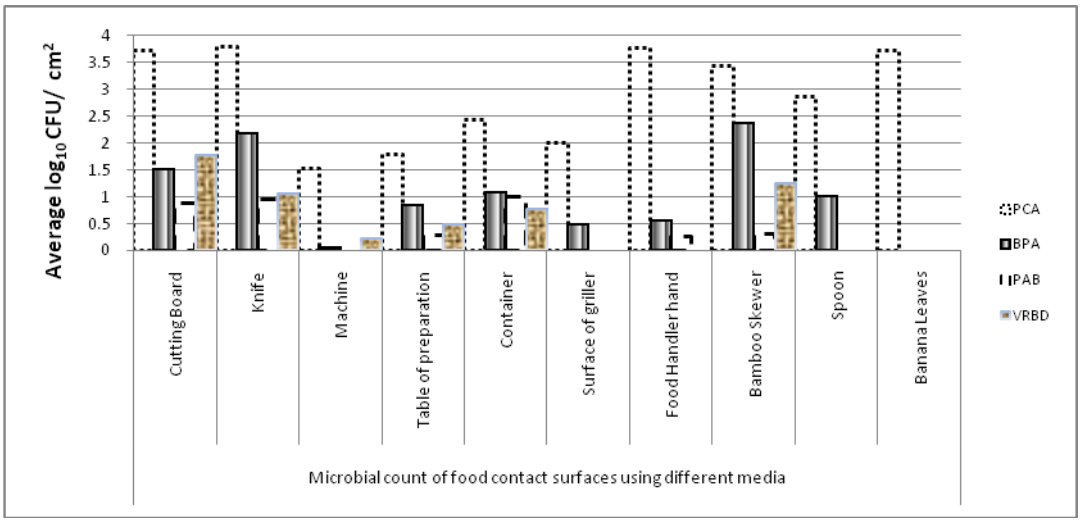

Figure 2 : Average means of various microbial counts (CFU/cm^2) of food contact surfaces in the Premise B

For the microbial count in food contact surfaces, the ranking of decreasing order of microbial counts in Premise A was APC > *Pseudomonas* count > *Enterobacteriaceae* count > *S. aureus* count. Whereas, the ranking of decreasing order of microbial counts in Premise B was APC > *S. aureus* count > *Enterobacteriaceae* count > *Pseudomonas* count.

Table 1: Mean and standard deviation of three visits of microbiological quality analysis on food contact surfaces in selected food premises

		Average log_{10} CFU/cm^2									
		Cutting board	Knife	Table of Preparation	Mixer	Food Handler Hand	Container	Spoon	Skewer	Banana Leaves	Surface Griller
		Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Aerobic Plate Count	Premise A	3.99 [a] ± 0.62	4.19 [a] ± 0.20	4.68 [a] ± 0.43	3.21 [a] ± 0.54	3.73 [a] ± 0.60	4.02 [b] ± 0.17	4.16 [a] ± 0.91	3.37 [a] ± 0.55	3.80 [a] ± 0.92	1.37 [a] ± 1.45
	Premise B	3.71 [a] ± 0.35	3.79 [a] ± 0.66	1.79 [a] ± 0.63	1.53 [b] ± 0.55	3.77 [a] ± 1.09	2.42 [a] ± 0.55	2.86 [a] ± 0.78	3.44 [a] ± 0.42	3.72 [a] ± 0.38	2.01 [a] ± 0.65
S. aureus	Premise A	1.72 [a] ± 0.21	1.13 [b] ± 0.98	0.46 [a] ± 0.71	1.08 [a] ± 1.31	1.48 [a] ± 0.17	0.69 [a] ± 0.66	0.73 [a] ± 0.14	1.03 [a] ± 0.89	1.52 [a] ± 1.36	0.53 [a] ± 0.91
	Premise B	1.50 [a] ± 1.36	2.17 [a] ± 0.09	0.84 [a] ± 0.75	0.04 [a] ± 0.06	0.56 [a] ± 0.96	1.07 [a] ± 0.94	1.01 [a] ± 0.88	2.37 [a] ± 2.19	0.00 [b] ± 0.00	0.49 [a] ± 0.84
Pseudomonas	Premise A	2.04 [a] ± 1.07	2.61 [a] ± 0.07	1.89 [a] ± 0.92	0.63 [a] ± 0.55	2.29 [a] ± 0.50	2.84 [a] ± 0.78	2.41 [a] ± 0.31	1.80 [a] ± 1.56	2.44 ± 0.78	1.40 [a] ± 1.26
	Premise B	0.88 [a] ± 1.53	0.96 [b] ± 1.01	0.29 [b] ± 0.50	0.00 [b] ± 0.00	0.26 [b] ± 0.44	1.00 [b] ± 0.94	0.00 [b] ± 0.00	0.31 [b] ± 0.54	0.00 ± 0.00	0.00 [b] ± 0.00
Enterobacteri-aceae	Premise A	1.99 [a] ± 0.31	1.39 [a] ± 1.22	1.63 [a] ± 1.02	0.59 [a] ± 0.51	3.37 [a] ± 0.55	1.30 [a] ± 0.39	1.22 [a] ± 1.04	1.63 [a] ± 1.52	0.85 [a] ± 1.08	1.43 [a] ± 1.47
	Premise B	1.78 [a] ± 1.94	1.05 [a] ± 0.92	0.48 [b] ± 0.83	0.21 [a] ± 0.36	3.44 [a] ± 0.55	0.78 [a] ± 0.39	0.00 [b] ± 0.00	1.25 [a] ± 1.42	0.00 [a] ± 0.00	0.00 [b] ± 0.00

Note: (a- b) mean bearing the same superscript within the same columns are not significantly different at 5% level ($P<0.05$)

The current study revealed that the majority of the tested food contact surfaces (FCS) in the Premise A were highly contaminated with Aerobic Plate Count and *Pseudomonas* count, however, food contact surfaces in the Premise B were highly contaminated with Aerobic Plate Count and *S. aureus* count. Result clearly showed that microbial counts in all food contact surfaces in the Premise A (cutting board, knife, table of preparation, mixer, food handler hand, container, spoon, banana leaves, skewer and surface of griller) were higher than in the Premise B. The variability of microbial flora contaminating the surfaces and the equipments has been reported in small-scale processing units of traditional dry fermented sausages in Mediterranean countries and Slovakia (Talon et al., 2007). They had elaborated that the different cleaning, disinfecting and manufacturing practices of the small-scale processing units could be responsible for this variability.

The recovery of *Pseudomonas*, *Enterobacteriaceae* and APC from food contact surfaces in both premises as found in this study was expected. The APC and *Enterobacteriaceae* are widely used to provide indication of hygiene and the likelihood of post-processing contamination as well as the presence of pathogens (Food Safety Authority of Ireland, 2000). Thus, the results had proven that both premises had poor hygienic practice. The exact source for the presence of the spoilage flora could not be determined because microbial ecology in food is a complex interaction between the intrinsic and extrinsic factors, that require consideration of many variables that difficult to be controlled. However, this study confirms the role of food contact surfaces as the 'house' of these microorganisms. The *S. aureus* was recovered from all food contact surfaces in both premises except in banana leaves in Premise B. Therefore, the distribution of *S. aureus* within the house-flora of the facilities in the 'Satar' premises may be the outcome of cross-contamination of the environment or food handlers. *Pseudomonas* on food contact surfaces can cross-contaminate foods because this organism is able to grow and multiply rapidly in foods stored at 4°C (Toule and Murphy, 1978), which could serve as a source of contamination for other foods. In other study by Gounadaki et al. (2008), they had reported that *Pseudomonas* is the most predominant contaminant in small-scale facilities producing traditional sausages in Greece.

Detection and isolation of *Salmonella sp.* from food contact surfaces: The detection and isolation of *Salmonella* from food contact surfaces were only carried out for the single visit as shown in Table 2. It was found that no *Salmonella* was detected in any of ten selected food contact surfaces. The results strongly suggested that *Salmonella* contamination in the 'Satar' premise was not associated with any food contact surfaces. This study confirms that if *Salmonella* were present in 'Satar', its presence is associated with other route of contamination, such as raw materials and ingredients.

Table 2: Identification of positive isolate of *Salmonella* sp. on food contact surfaces (100 cm^2) in selected 'Satar' premises

Type of surface	No of sample collected		No. (%) positive for *Salmonella*
	Premise A	Premise B	
Cutting Board	1	1	0/2 (0%)
Knife	1	1	0/2 (0%)
Mixer	1	1	0/2 (0%)
Table of preparation	1	1	0/2 (0%)
Container	1	1	0/2 (0%)
Surface of griller	1	1	0/2 (0%)
Food handlers' Hand	2	2	0/4 (0%)
Skewer	1	1	0/2 (0%)
Spoon	1	1	0/2 (0%)
Banana Leaves	1	1	0/2 (0%)
Total	**11**	**11**	0/22 (0 %)

IV. CONCLUSION

The current investigation revealed that hygienic status of food contact surfaces play an important role in the microbial quality and safety of 'Satar'. Results showed that all food contact surfaces from the premise A were highly contaminated with indicator microorganisms (APC, *Enterobacteriaceae* and *Pseudomonas*) compare to food contact surfaces of the premise B. At the same time, no presence of *Salmonella* was found in all food contact surfaces of food premises. It should be stressed that contamination by pathogens, such as *Salmonella*, if any, demonstrated that very poor hygiene practice and impose a serious health hazard for consumers. Hence, hygienic processing and preparation of food are vital as a basic requirement and the first line of defense against pathogenic microorganisms. To obtain zero contamination of microbes is impossible; therefore, good hygiene, cleaning and sanitation are necessary to secure low levels of microorganisms on the final product (Huss, 1997). Good hygiene practice (GHP) should be enforced in each step of 'Satar' operation in order to eliminate the post-processing contamination.

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