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Microbiological Quality of Malaysian Heritage Food ('Satar') Sold In Marang and Kemaman, Terengganu, Malaysia

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

'Satar' is a Malaysian heritage ready-to-eat (RTE) food, especially in Terengganu and Kelantan. Due to lack of standard hygiene of 'Satar' preparation, microbial load of 'Satar' prior to grilling is considerably high. Therefore, in this study, microbiological methods were used to determine the microbiological quality in raw and cooked 'Satar' at four different stalls in Kemaman and Marang, Terengganu. The samples were analysed for Aerobic plate count (APC), Enterobacteriaceae count, Staphylococcus aureus count, Yeasts and Molds count (YM) and psychrotrophic count. There were significant difference ($P<0.05$) between the microbiological analyses conducted between raw and cooked 'Satar' at four different stalls in Marang and Kemaman. The results showed that grilling had significantly decreased the microbial loads in 'Satar' up to 8-$\log_{10}$ reduction. This study also indicated that the mean of microbial quality of selected 'Satar' premises in Marang and Kemaman were not significantly different ($P>0.05$). This study has provided some scientific evidences on the microbiological quality that reflects the current hygienic practice of 'Satar' premises in Terengganu. The implementation of Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) in 'Satar' production may improve the hygienic status and quality of 'Satar' production.

KEYWORDS : Grilling, Malaysian heritage food, microbiological quality, ready-to-eat food, 'Satar'

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I. INTRODUCTION

'Satar' is one of heritage food in Malaysia and it is normally served in any occasion as an appetizer, in the East Coast of Peninsular Malaysia, especially in Terengganu and Kelantan. Heritage food is part of heritage tourism which is considered as an important segment of tourism industry in order to attract tourists to the destination [1]. 'Satar' is a mixture of boneless fish and spices and wrapped in banana leaf and grilling over the charcoal of fire to make 'Satar' cooked and ready for consumption. The processed fish normally used yellowstripe scad (*Selaroides leptolepis*), crimson jobfish *(Nemipterus* spp., *Pentapodus* spp. and *Scolopsis* spp.*)* and Spanish mackerel (*Scomberomorus* spp.) [2].

Grilling is a choice of preparation of certain RTE foods because it can retain the good sensory characteristics of food compare if it is prepared through direct cooking. It is done on opened grid over a heat source which may be charcoal, a gas-heated element or an electric element and applied to food to improve its microbiological safety by inactivation of pathogenic microorganisms. Besides that, sufficient heat treatment and appropriate grilling method for 'Satar' would help to enhance its flavour and taste as well as increase the shelf life [3].

'Satar' is a 'street food' sold by hawker, where the food is prepared and sold at the streets for immediate consumption at later time without further preparation. Handling, processing, storage and display of 'Satar' may also reflect the microbiological load of RTE foods at the point of sale [4, 5], while the quality of the ingredients contribute to the initial microbiological load of food. Any unhygienic practices during food handling and preparation at eating places may contribute to cause foodborne illness [6]. Recently, Lani and co-workers [7] had reported the microbiological quality of food contact surfaces at selected food premises of

'Satar' in Terengganu. However, the status of microbiological quality of 'Satar' is limited in the literature, therefore, the objective of this present study was to determine the microbiological quality of 'Satar' produced in Marang and Kemaman, Terengganu, as these locations are the major attractions of customers and tourists to buy 'Satar' from local food premises in Terengganu.

II. MATERIALS AND METHODS

Description of samples: Twelve pieces of raw 'Satar' (before grilling) and twelve pieces of cooked 'Satar' (after grilling) were purchased randomly from four different 'Satar' stalls in Marang and Kemaman, Terengganu. The samples were collected in sterile packaging in an icebox (4±1°C) and brought back to Food Microbiology Laboratory in Universiti Malaysia Terengganu and they were analysed immediately upon arrival in the lab.

Microbiological analysis: 25 g of raw and cooked 'Satar' were weighed and transferred into sterile stomacher bag. Then, 225 ml of 0.1% buffered peptone water (Merck, Germany) was added and homogenized for three minutes at normal speed in a stomacher (Bag Mixer, Interscience, France). Serial dilutions were made with 9 ml of 0.1% buffered peptone water until the desired dilutions. Then, 0.1 ml of food homogenate was pipetted out from each dilution of the homogenate into duplicate plates of Plate Count Agar (Merck, Germany) for Aerobic Plate count, Baird Parker Agar (Merck, Germany) for *S. aureus* count, Violet Red Bile Dextrose Agar (Merck, Germany) for *Enterobacteriaceae* count, and Potato Dextrose Agar (PDA) (Merck, Germany) acidified with 10% tartaric acid solution for Yeast and Mold Count [8]. The homogenate was spread well on each plate and all the plates were then inverted and incubated at 35ºC for 24 hours, except PDA plates that were incubated at 25°C for 5-7 days [9]. For psychrotrophic count, Plate Count Agar was used and incubated in the chiller for 7-days before enumeration [8]. Enumeration of microbial count (CFU/g) was carried out using standard microbiological procedures [8].

Statistical analysis: The present study used completely randomized design (CRD) as an experimental design. Mean ± standard deviation of microbial counts of Aerobic Plate Count (APC), *Enterobacteriaceae* count, *S. aureus* count, Yeast and Mould count and psychrotrophic count were analysed using one-way analysis of variance (ANOVA) for different types of microbial count of raw and cooked 'Satar'. The significant differences ($p<0.05$) between treatments were determined using Tukey's Test. Meanwhile, the analysis was continued using Independent sample t-Test for determination of significant difference between mean ± standard deviation of microbial count in raw and cooked 'Satar' in Kemaman and Marang, respectively. Raw and cooked 'Satar' between Marang and Kemaman were analysed using independent sample t-Test. The statistical programme used was Statistical Programme for Social Sciences (SPSS) version 16.

III. RESULTS AND DISCUSSIONS

Table 1 represents microbial analysis of raw 'Satar' in different stalls from Marang. Aerobic plate count was significantly higher from stall C than others while, stall B and C were highly significant than stall A and D for *Enterobacteriaceae* count. *Staphylococcus aureus* count recorded higher microbial count from stall C and the lowest count enumerated from stall A. However, there was no significant different ($P>0.05$) with stall B and D. For yeast and mould count, there were significant different ($P<0.05$) of raw 'Satar' at stall C and B while stall D and A showed significantly lower than stall C. Stall C also recorded significantly difference compared to other in psychrotrophic count.

Table 2 shows there was no significant different ($p>0.05$) from different stalls with different microbial analysis of cooked 'Satar' in different stalls at Marang. For overall result in Marang, stall C was the highest microbial counts in raw 'Satar' followed by stall B, stall A and D. For cooked 'Satar', the microbial counts were substantially decreased about 6-8 $\log_{10}$CFU/g of aerobic plate count, 6-7 $\log_{10}$ CFU/g of psychrotrophic count and *S. aureus* count. Yeast and mould count recorded 4-5 $\log_{10}$ CFU/g decreased.

Table 1: Microbial counts of raw 'Satar' in different stalls at Marang

Sample of raw 'Satar' at Marang	Aerobic plate count	*Enterobacteriaceae* count	*S. aureus* count	Yeast and mould count	Psychrotrophic count
STALL A	7.29 ± 0.06^{c}	7.12 ± 0.10^{b}	6.17 ± 0.13^{b}	4.62 ± 0.28^{bc}	6.44 ± 0.44^{b}
STALL B	7.82 ± 0.31^{b}	7.85 ± 0.07^{a}	6.97 ± 0.49^{ab}	5.36 ± 0.32^{ab}	6.66 ± 0.14^{b}
STALL C	8.38 ± 0.06^{a}	8.11 ± 0.06^{a}	7.21 ± 0.32^{a}	5.65 ± 0.41^{a}	7.65 ± 0.25^{a}
STALL D	6.34 ± 0.10^{d}	6.71 ± 0.24^{c}	6.51 ± 0.15^{ab}	4.46 ± 0.15^{c}	6.32 ± 0.22^{b}

Note: values are Mean± standard deviation (Log_{10} CFU/g) of 3 replicates
(a-c) mean bearing the same superscript within the same column are not significantly different at 5% level ($p<0.05$)

Table 2: Microbial counts of cooked 'Satar' in different stalls at Marang

Sample of cooked 'Satar' at Marang	Aerobic plate count	*Enterobacteriaceae* count	*S. aureus* count	Yeast and mould count	Psychrotrophic count
STALL A	1.59 ± 1.43^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STALL B	1.43 ± 1.25^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STALL C	1.77 ± 1.53^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STALL D	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: values are Mean± standard deviation (Log_{10} CFU/g) of 3 replicates
(a-c) mean bearing the same superscript within the same column are not significantly different at 5% level ($p<0.05$)

Table 3: Microbial counts of raw 'Satar' in different stalls at Kemaman

Sample of raw 'Satar' at Kemaman	Aerobic plate count	*Enterobacteriaceae* count	*S. aureus* count	Yeast and mould count	Psychrotrophic count
STALL A	8.47 ± 0.00^{a}	7.65 ± 0.13^{a}	7.94 ± 0.12^{a}	5.22 ± 0.09^{a}	7.38 ± 0.07^{a}
STALL B	8.40 ± 0.03^{ab}	7.74 ± 0.18^{a}	6.79 ± 0.07^{b}	4.60 ± 0.11^{a}	6.79 ± 0.07^{b}
STALL C	7.15 ± 0.32^{c}	6.72 ± 0.23^{b}	5.41 ± 0.16^{c}	2.67 ± 2.31^{a}	7.43 ± 0.02^{a}
STALL D	7.71 ± 0.42^{bc}	7.45 ± 0.12^{a}	6.87 ± 0.55^{b}	4.65 ± 0.33^{a}	6.46 ± 0.34^{b}

Note: values are Mean± standard deviation (Log_{10}CFU/g) of 3 replicates
(a-c) mean bearing the same superscript within the same column are not significantly different at 5% level ($p<0.05$)

Table 4: Microbial counts of cooked 'Satar' in different stalls at Kemaman

Sample of raw 'Satar' at Kemaman	Aerobic plate count	*Enterobacteriaceae* count	*S. aureus* count	Yeast and mould count	Psychrotrophic count
STALL A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STALL B	0.77 ± 1.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.67 ± 1.15^{a}	0.00 ± 0.00^{a}
STALL C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STALL D	0.83 ± 1.43^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.16 ± 0.67^{a}	0.00 ± 0.00^{a}

Note: values are Mean± standard deviation (Log_{10} CFU/g) of 3 replicates
(a-c) mean bearing the same superscript within the same column are not significantly different at 5% level ($p<0.05$)

Table 5: Significant difference of types of microbial analysis between raw and cooked 'Satar' in Marang

Types of microbial analysis	Microbial count (Log_{10} CFU/g)		Sig. at P<0.05	Conclusion
	Raw	cooked		
Aerobic plate count	7.46 ± 0.79	1.20 ± 0.79	0.00	P<0.05
Enterobacteriaceae count	7.45 ± 0.60	0.00 ± 0.00	0.00	P<0.05
S. aureus count	6.72 ± 0.50	0.00 ± 0.00	0.00	P<0.05
Yeast and mould count	5.02 ± 0.58	0.00 ± 0.00	0.00	P<0.05
Psychrotrophic count	6.77 ± 0.60	0.00 ± 0.00	0.00	P<0.05

Note: Values are average mean ± SD of raw and cooked 'Satar' in Marang

Table 6: Significant difference of types of microbial analysis between raw and cooked 'Satar' in Kemaman

Types of microbial analysis	Microbial count (Log_{10} CFU/g)		Sig. at P<0.05	Conclusion
	Raw	Cooked		
Aerobic plate count	7.93±0.61	0.40±0.93	0.00	P<0.05
Enterobacteriaceae count	7.39±0.44	0.00±0.00	0.00	P<0.05
S .aureus count	6.75±0.94	0.00±0.00	0.00	P<0.05
Yeast and mould count	4.28±1.42	0.33±0.78	0.00	P<0.05
Psychrotrophic count	6.92±0.54	0.00±0.00	0.00	P<0.05

Note: Values are average mean ± SD of raw and cooked 'Satar' in Kemaman.

Table 7: Significant difference of types of microbial analysis in raw 'Satar' at different locations

Types of microbial analysis	Microbial count (Log_{10} CFU/g)		Sig. at P<0.05	Conclusion
	Marang	Kemaman		
Aerobic plate count	7.46±0.79	7.93±0.61	0.12	p>0.05
Enterobacteriaceae count	7.45±0.60	7.39±0.44	0.77	p>0.05
S. aureus count	6.72±0.50	6.75±0.94	0.91	p>0.05
Yeast and mould count	5.02±0.58	4.28±1.42	0.12	p>0.05
Psychrotrophic count	6.77±0.60	6.92±0.54	0.53	p>0.05

Note: Values are average mean ± SD of four stalls at Marang and Kemaman, respectively.

Table 8: Significant difference of types of microbial analysis in cooked 'Satar' at different locations

Types of microbial analysis	Microbial count (Log_{10} CFU/g)		Sig. at P <0.05	Conclusion
	Marang	Kemaman		
Aerobic plate count	1.20±0.79	0.40±0.93	0.93	p>0.05
Enterobacteriaceae count	0.00±0.00	0.00±0.00	-	-
S. aureus count	0.00±0.00	0.00±0.00	-	-
Yeast and mould count	0.00±0.00	0.33±0.78	1.66	p>0.05
Psychrotrophic count	0.00±0.00	0.00±0.00	-	-

Note: Values are average mean ± SD of four stalls at Marang and Kemaman, respectively.

Table 3 represents microbial analysis of raw 'Satar' in different stalls at Kemaman. There was significantly higher aerobic plate count from stall A than stall C and D. For *Enterobacteriaceae* count, stall C was significantly lower one-log_{10} CFU/g than others. While *S. aureus* count recorded highly significant difference ($p<0.05$) from stall A compared to others and there were no significant different ($p>0.05$) between stall B and D. Among the stalls, there was no significant different ($p>0.05$) of yeast and mould count. However, high significant different ($p<0.05$) of psychrotrophic count recorded from stall A and C compared to other. Microbial analysis of cooked 'Satar' at different stall showed no significant ($p>0.05$) from different stalls (Table 4). For overall result in Kemaman, stall A showed the highest microbial counts in raw 'Satar' followed by stall B, stall D and C. For cooked 'Satar', the microbial counts were substantially decreased about 8 log_{10}CFU/g of aerobic plate count, while psychrotrophic count and *Enterobacteriaceae* count were reduced 6-7 log_{10}CFU/g. *S. aureus* count decreased about 5-7 log_{10}CFU/g. Yeast and mould count recorded 4-5 log_{10}CFU/g substantially decreased.

Some factors may contribute to high count in raw 'Satar' such as main sources of product and ingredients, contamination during handling and preparation, packaging and storage condition [4]. Aerobic plate count (APC) is the indicator of overall degree of microbial contamination of foods and also the hygienic status of food premises [10]. After grilling, 'Satar' from different stalls safe to be consumed as the safe limit for APC is less than $\log_{10}$ 5.00 CFU/g [11]. *Enterobacteriaceae* count is indicator organism associated with hygienic status. The presence of *Enterobacteriaceae* in the processed food may come from inadequate treatment or post-process contamination from the environment that may help to indicate the extent of fecal contamination [10].

Yeast and mould easily contaminate raw material and ingredients like fish and grated coconut [12]. The warm and moist environment of food premises can help proliferation of *S. aureus* during preparation especially if cleaning and disinfection procedures were insufficient [13], inadequately cleaned surface after contacted with food [14], which lead to cross contamination as 'Satar' preparation involved a lot of manual handling. Banana leaves covered the 'Satar' had protected them from external source of contamination after grilling such as *S. aureus* with is the major habitat of the pathogen in the nasal membrane and skin of human [15].

Raw 'Satar' showed significantly higher ($p>0.05$) than cooked 'Satar' from Marang (Table 5) and Kemaman (Table 6).This comparison made to evaluate the effectiveness of grilling in ensuring the microbial counts were reduced to the safe levels. Grilling has reduced about 5-7 $\log_{10}$ CFU/g reduction of microbial counts in raw 'Satar' on different types of microbial analysis. The present study proved grilling effect significantly reduced the microbial count in 'Satar'. Grilling is applied to food to improve its hygienic quality by inactivation of pathogenic microorganisms and to enhance its flavour and taste and increase shelf life [3].

Fish product should achieve 145^0 F (62.7^0C) during grilling where is sufficient to destroy foodborne microbes [16]. Ranges of internal temperature (78°C – 92°C) were recorded immediately after 'Satar' was grilled (cooked 'Satar'). The ranges of internal temperatures measured in 'Satar' were sufficient to ensure the level of pathogenic microbes was significantly reduced to safe level for human consumption. In present study, there was no significant different ($p>0.05$) between Marang and Kemaman for raw (Table 7) and cooked 'Satar' (Table 8). Regardless of the different locations and types of 'Satar' premises in Marang and Kemaman, both factors did not significantly ($P>0.05$) affect the microbial quality in 'Satar'. In ensuring and improving the hygienic status of 'Satar' premises, it is suggested the food premises to implement Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP). These quality management systems will reduce the risk of microbial contamination starting from raw materials until the products are served to consumers.

II. CONCLUSION

Microbial counts in raw 'Satar' was the highest in stall C followed by B, A and D at Marang, while at Kemaman, stall A was the highest followed by stall B, D and C. Therefore, Stall D (Marang) and Stall C (Kemaman) were among the most hygienic 'Satar' premises compared to others. After grilling of 'Satar', this food was safe to be consumed as the present study showed the absence of foodborne microorganisms in cooked 'Satar' in different types of microbial analysis. The location and different food handlers of 'Satar' premises in Marang and Kemaman did not influence the microbial quality in 'Satar'.

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