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Evaluation on Chemical Analysis and Microbiological Quality of Partially Cooked-Frozen of Malaysian Heritage Food ('Satar')

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

'Satar' is a Malaysian heritage food that made of a blend of 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. Left over 'Satar' is frequently subjected to frozen and re-grilling. Storing the 'Satar' under freezing condition has been a common practice by the owners, but how this practice may affect the quality of this product is yet to be understood. This product may undergo undesirable changes during storage and such deterioration may affect the quality of 'Satar'. The objective of this study was to determine the chemical and microbiological changes of partially cooked-frozen 'Satar' during storage. Initially, 'Satar' was prepared under controlled environment by mixing fish together with onion, shallot, spices, sugar, salt and shredded coconut. The chemical analyses were conducted every two weeks storage at -18ºC in a blast freezer for 2 months period of study. The moisture, carbohydrate, protein, lipid and ash contents of the 'Satar' were 66.89%, 5.39%, 11.71%, 14.06% and 1.87%, respectively. The peroxide value of 'Satar' was significantly increased from 9.23 to 12.75 mEq/kg fat during frozen storage. In terms of microbiological quality of this product, Aerobic Plate Count (APC), Enterobacteriaceae count and Yeast and Mold count were gradually increased throughout 8-weeks of frozen storage, where after 6-weeks of storage showed substantial increase of microbial populations to unacceptable level. In conclusion, storage of partially cooked frozen 'Satar' was acceptable within 4 weeks of storage at -18ºC. After four weeks of storage at -18ºC, lipid oxidation was significantly higher and APC almost reached 1.0 x 10^6 CFU/g which indicate the physicochemical properties and microbiological quality of the product became unacceptable.

KEYWORDS: 'Satar', chemical analysis, microbiological quality, partially cooked-frozen, lipid oxidation

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I. INTRODUCTION

'Satar' is a traditional food which popular in the East Cost of Peninsular Malaysia especially in Terengganu and now commonly served across the states. Based on Tourism of Terengganu (2014), 'Satar' is a blend of succulent boneless fish marinated in spices, wrapped in banana leaves and grilled over a flaming charcoal fire. Its sweet taste is tinged with delicate smell of the banana wrapping, making it a great appetizer and a healthy snack. 'Satar' is prepared by mixing the minced fish flesh with shredded coconut, onion, chili, salt and other ingredients, wrapped in the banana leaves, formed into cone shape, skewed with bamboo stick and grilled until cooked. The frozen 'Satar' is stable until five months storage at -20ºC. Researchers from Malaysian Agricultural Research and Development Institute (MARDI) had reported the proximate analysis of ready-to-cooked 'Satar', where for 100 g of 'Satar' contained 67.0% of moisture content, 14.0% of protein, 7.4% of fat, 8.8% of carbohydrate, 2.0% of ash and 0.9% of fiber (Che Rohani Awang, 2002).

Generally, freezing preserves the taste, texture and nutritional value of foods better than any other preservation methods (George, 1993). Many types of fruits and vegetables are frozen and held in frozen state until just before preparation for consumption purposely for extending the shelf life beyond that of refrigerated storage. Several investigations have reported that frozen storage could affect microbiological quality and physico-chemical characteristic such as oxidation stability and sensory properties (Damen and Steenbekkers, 2007). The common practice among 'Satar' producers is to freeze the left-over 'Satar'. However, frozen 'Satar'

can be easily spoiled once it is grilled. To overcome the problem of frozen 'Satar', partially cooked-frozen 'Satar' has been developed by 'Satar' owner. It is prepared by grilling 'Satar' at 80-90°C for 10 minutes until it is partially cooked, and then, cooled to ambient temperature immediately. This partially cooked 'Satar' is then packed in polypropylene plastic and stored at -18°C in blast freezer. Through observation, this partially cooked frozen 'Satar' is able to retain the freshness of 'Satar' once it is subjected to regrilling, however, no scientific evidence has been reported that this new method of preservation. Therefore, the aim of this study was to investigate the chemical and microbiological quality of partially cooked-frozen Satar.

II. MATERIALS AND METHODS

Preparation of partially cooked 'Satar': 'Satar' was prepared under controlled environment in the Food Service Laboratory, School of Food Science and Technology, UMT by mixing fish together with onion, shallot, spices, sugar, salt and shredded coconut. First, fish was deboned to get the flesh. Then, all of the ingredients were mixed thoroughly. After that, the mixture was wrapped in banana leaf, put into stainless steel skewer and grilled for 10 minutes at 80-90ºC until it was partially cooked. Finally, the partially cooked 'Satar' was quickly removed from the griller to avoid overcooking and cooled to room temperature. Then, this partially cooked 'Satar' was packed in polypropylene plastic and stored at -18ºC in blast freezer for a period of 8-weeks. Prior to chemical and microbiological analyses, the partially frozen 'Satar' that were kept in the freezer were taken out at predetermined intervals of two weeks and thawed in refrigerator (4°C) for 30 minutes before re-grilling using a laboratory griller.

Proximate Analysis: This analysis was carried out on different treatments of 'Satar' which was partially cooked-frozen 'Satar'. The analyses include the determination of moisture, crude fat, crude protein, ash, fiber and carbohydrate content. All the analyses were performed according to AOAC (2000) methods.

Determination of lipid oxidation: In the determination of lipid oxidation content, peroxide value was applied. Firstly, 0.5 g of the ground sample was weighed in the flask and added with acetic acid and chloroform at ratio 3:2. Then, the mixture was swirled until the sample was mixed with the chemical and filter. The filtrate was collected and added with 1 ml saturated potassium iodide and kept in the dark for 10 minutes. The colour of mixture was changed to yellow. 30 ml distilled water and 1 ml of 1% starch were added into mixture and shaked thoroughly. Then, it was titrated slowly with 0.01 N sodium thiosulphate until the dark blue color was disappeared. The percentage of peroxide value was calculated according to Equation 1.

$$\% \text{ Peroxide (mEq/ kg fat)} = \frac{S \times N}{\text{Weight of sample}} \times 1000 \qquad \text{(Equation 1)}$$

where; S = titration volume of the sample
N = normality of Na_2O_2

Microbiological analysis: 25 g of partially cooked frozen 'Satar' stored in the freezer at different storage time were weighed, mixed aseptically into stomacher bag and added with 225 ml 0.1% Buffered Peptone Water. The sample was blended and mixed in the stomacher for 2 minutes. This mixture produced 10^{-1} dilution. The food homogenate was mixed by shaking and 1.0 ml was pipetted into a tube containing 9.0 ml of 0.85% saline and mixed gently the tube. From the first dilution, 1.0 ml was pipetted to second dilution tube containing 0.85% saline. This step was repeated until 10^{-6} dilution. 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, 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. 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 (Merck, 2007).

Statistical analysis: The present study used completely randomized design (CRD) as an experimental design. All the analyses were done in duplicate with three readings for each replication. All the data collected were analyzed using one-way analysis of variance (ANOVA), and the significant difference at ($P<0.05$) between treatments were determined using Tukey's Test. The data obtained were presented as mean ± standard deviation and data analyses were performed using Minitab14 software.

III. RESULTS AND DISCUSSIONS

Proximate analyses and lipid oxidation : The moisture, carbohydrate, protein, lipid and ash contents of the 'Satar' were 66.89%, 5.39%, 11.71%, 14.06% and 1.87%, respectively. The proximate values were not affected by frozen storage for 6 weeks. The only comparison can be made was the proximate analyses carried out by researchers in the Malaysian Agricultural Research and Development Institute (MARDI) as summarized in Table 1. From the Table 1, the lipid and carbohydrate composition from the study by Che Rohani Awang (2002) were different compared to the current study. According to Che Rohani Awang (2002), replacement of shredded coconut to coconut milk powder in the formulation can reduce the fat content of 2%. The lower content of lipid contributes to the higher composition of carbohydrate. Carbohydrate is the remaining percentages of the total proximate analyses. In general, fish are known to have low amounts of carbohydrate in their muscle. Freezing process itself does not destroy nutrients in the food; hence the nutrient of frozen food is likely to be the same with unfrozen food (Kennedy, 2000).

Table 1: Proximate composition of 'Satar' between the study and Che Rohani Awang (2002)

Proximate composition	The result from this study (%)	The result from Che Rohani Awang (2002) (%)
Moisture	66.89±0.781	67.7
Carbohydrate	5.39±1.514	14.2
Protein	11.71±1.440	13.9
Lipid	14.06±0.463	2.0
Ash	1.87±0.310	2.2

Table 2 shows the peroxide value for partially cooked frozen 'Satar' at 6 weeks storage. Peroxide value is widely used as an indicator of the degree of lipid oxidation (Evans, 2008). Lipid oxidation is the reasons for the 'Satar' quality deterioration and contributes to the development of unacceptable organoleptic characteristic. Enzyme hydrolyzed lipids and release free fatty acids which lead to rancid flavour.

Table 2: Lipid oxidation of uncooked, fully cooked and frozen 'Satar'

Storage condition and time	Peroxide value mEq/kg fat
Uncooked, without storage	7.50 ± 0.71
Fully cooked, without storage	11.67 ± 0.467
Week 0 of storage	9.23 ± 0.32
Week 2 of storage	10.54 ± 0.66
Week 4 of storage	12.02 ± 0.05
Week 6 of storage	12.75 ± 0.17

Besides that, this reaction severely limits the shelf life of the product. In the present study, the peroxide value of 'Satar' significantly increased from 9.23 to 12.75 mEq/kg fat during frozen storage. The increasing of lipid oxidation during frozen storage has been demonstrated by Bahar et al. (2006) for fish finger (made from mirror carp fish). This is because the amount of unfrozen water present in the frozen matrix. The unfrozen water is known to be reactive, particularly during frozen storage, rendering the product susceptible to rancid and become off-flavor. Lipid oxidation of uncooked and fully cooked 'Satar' was found to be 7.50 and 11.67mEq/kg fat respectively. The foods and oils are considered fresh when the peroxide value is less than 10 mEq/kg fat (Hraš et al., 2000).

Microbial quality of partially cooked 'Satar' at different storage time

For the determination of the quality of partially cooked 'Satar' before frozen and during the frozen time, aerobic plate count (APC), *Enterobacteriaceae* count and Yeast and Mold count were analyzed. Figures 1 to 3 show the increasing trend of Aerobic plate count, *Enterobacteriaceae* count and Yeast and Mold count during frozen storage in the samples throughout the study.

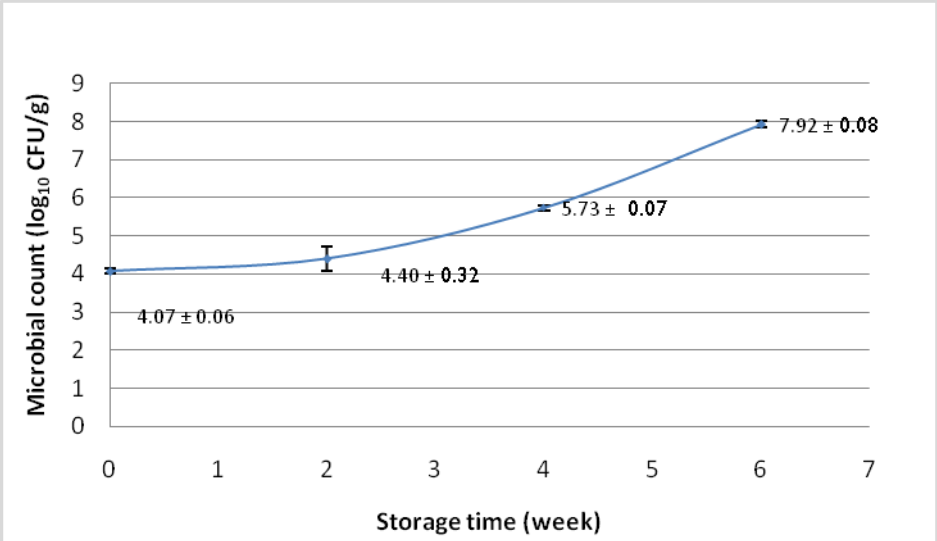

Figure 1: Aerobic plate count of partially cooked 'Satar' during frozen storage

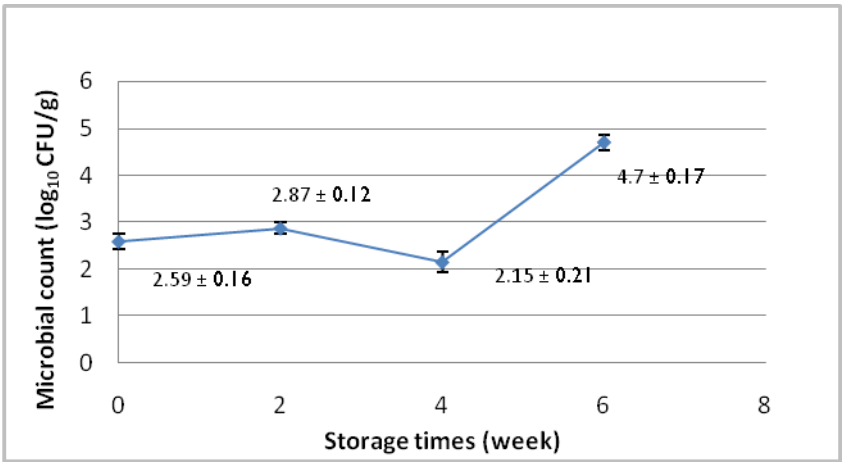

Figure 2: *Enterobacteriaceae* count of partially cooked 'Satar' during frozen storage

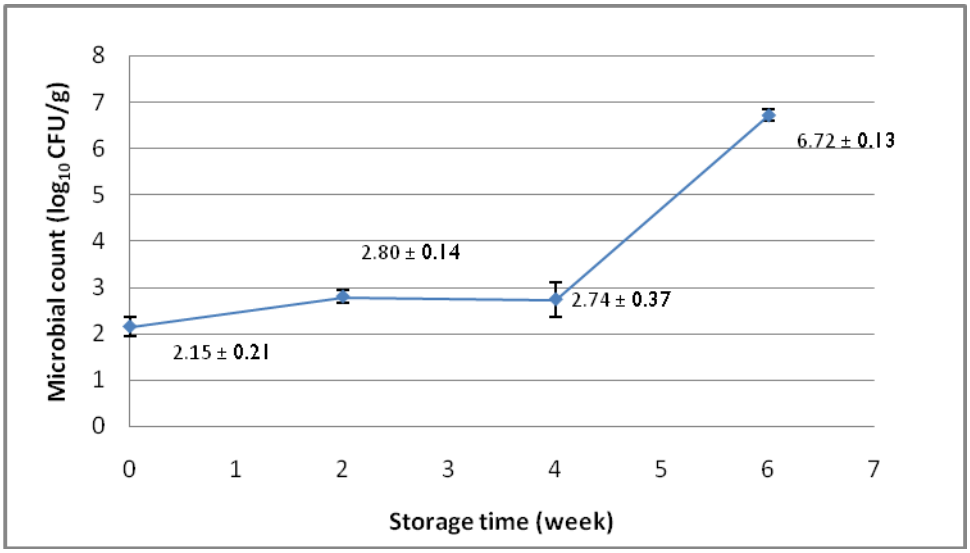

Figure 3: Yeast and Mold count of partially cooked 'Satar' during frozen storage

The aerobic plate count (APC) is an indicator of the overall degree of microbial contamination of foods (ICMSF, 2006). APC does not measure the entire bacterial population but rather the number bacteria growth in the presence of oxygen (aerobically) and at medium range (mesophilic) temperatures. The International Commission on Microbiological Specifications for Food (ICMSF) has recommended that the Aerobic plate Count should not exceed 10^6/g wet weight in ready-to-eat foods. During the 6th weeks of storage, Aerobic Plate count was exceeding the limit that recommended by ICMSF which were 7.92×10^7 CFU/g.

Besides that, at 6th week of frozen storage the trend of APC, *Enterobacteriaceae* count and Yeast and Mold count were significantly increased. A substantial increase of APC, *Enterobacteriaceae* count and Yeast and Mold Count from 4th to 6th week of storage showed that 6-weeks of storage are sufficient for microbial multiplication and become potential hazards for consumption. This is because freezing may affect the structural integrity of food, making it more susceptible to microbial attack.

During thawing at 4ºC for 30 minutes, some of the bacteria in frozen food may become more virulent. Thawed food may have a moist surface due to drip or condensation and may be very good substrate for bacteria. The thawing method that used in this study was thawing in refrigerator. During the thawing process, food deterioration happened at the same rate as in unfrozen products. However, humidity condensation on the surface and release nutrient through drip loss can accelerate microbial multiplication (Evans, 2008).

In this study, 'Satar' was partially cooked for 10 minutes at 80-90ºC and subjected to freezing immediately. These conditions provide cold shock to the microorganisms. Cold adaptation by microorganisms is of particular importance due to the increased use of frozen foods and the increased popularity of fresh or minimal processed food, with little or no preservatives. The mechanisms of cold adaption are membrane composition modifications, to maintain membrane fluidity for nutrient uptake, structural integrity of protein and ribosomes, production of cold shock proteins and uptake of compatible solutes (Phadtare, 2004). Different cold shock treatments prior to freezing have different effects on survival of bacteria after freezing. This might result in a high survival rate of bacteria in frozen products. Furthermore, low temperature adapted bacteria may be relevant to food quality and safety.

Frozen food products and particularly fish product are far from sterile and cannot be considered as microbial safe product (Hui et al., 2004). Many microorganisms are not destroyed by the freezing process and may survive even if they remain inactive during storage. Freezing can produce certain lethality in some microorganisms but this process is very slow and variable, depending on the type of food (Kennedy, 2000). Freezing cannot be regarded as an ultimate method to reduce microbial contamination, therefore hygienic and sanitary conditions prior to processing are very important.

IV. SUMMARY

This study concludes that partially cooked-frozen 'Satar' in term of chemical and microbial qualities were suitable to be stored at -18°C for 4 weeks. The moisture, carbohydrate, protein, lipid and ash contents of the 'Satar' were 66.89%, 5.39%, 11.71%, 14.06% and 1.87%, respectively and not affected even after 6th weeks of frozen storage. The quality of the partially cooked-frozen 'Satar' in term of lipid oxidation was decreased due to storage. The unfrozen water is known to be reactive, particularly during frozen storage, rendering the product susceptible to rancid and become off-flavor. Lipid oxidations of uncooked and fully cooked 'Satar' were 7.50 and 11.67 mEq/kg fat respectively. The microbiological quality of partially cooked frozen 'Satar' that was stored at different storage times increased. Aerobic plate count, *Enterobacteriaceae* count and Yeast and Mold count were gradually increased during frozen storage and within the acceptable range during 4-week storage only. This finding is very useful to help the small-industry of 'Satar' entrepreneurs to produce safe and nutritious foods to consumers.

V. ACKNOWLEDGEMENTS:

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