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Microbiological and Physicochemical Analysis of *Keropok lekor* at Three Storage Temperatures

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ABSTRACT

Keropok lekor is a one of a popular traditional food in east coast of peninsular Malaysia specifically in Terengganu. However, it is known to be highly perishable when stored at ambient temperature. This study aimed to investigate the microbiological and physicochemical quality of *keropok lekor* stored at ambient and refrigerated temperature. Samples of *keropok lekor* were collected from a local premise located in Besut, Terengganu and were packaged either ordinary sealed or vacuumed. The samples were stored at ambient (25℃-28℃), and refrigeration temperature (0℃-4℃) for up to 7 days. Microbiological analysis was performed using spread plate method and physicochemical analysis such as moisture, colour, pH and texture. The *keropok lekor* stored at ambient temperature showed significant increase in microbial count after one day for both vacuum and ordinary sealed pack at 6.18 log cfu/g and 7.85 log cfu/g, respectively. Refrigerated samples also showed significant increase after 7 days. The samples did not exhibit significant changes with regards to moisture, colour and hardness but pH was significant decrease during the storage which shows sign of spoilage. However, the *keropok lekor* chewiness significantly increased during storage. In conclusion, ambient temperature shows rapid spoilage of *keropok lekor* regardless of the packaging and refrigerated condition can extent the shelf life to 2 days for ordinary sealed samples and 3 days for vacuum samples.

Keywords: *keropok lekor,* microbiological quality, physicochemical quality, storage temperature

INTRODUCTION

Keropok lekor is a one of a popular traditional food in a region of Malaysia called Terengganu. It is widely sold in the local market because it is one of the attractions for tourist since it considered as tourism icon in Terengganu. *Keropok lekor* usually being sold at street stalls, night market or kiosk (Nor-Khaizura et al., 2009). The *keropok lekor* dough is prepared by mixing several ingredients such as fish flesh with sago starch, salt, crushed ice and monosodium glutamate (MSG). Then, the dough rolled and shaped like sausage before boiled to serve. It can be eaten either steamed or deep fried. According to Nor-Khaizura (2010), *keropok lekor* was originally produced from backyard manufacturing process but now the production is upgraded to small or medium scale (SME) enterprise (Wan Hamat et al., 2020) due to the higher demand of this food product locallly. *Ikan parang* (*Chirocentrus dorab*), *ikan selayang* (*Decapterus russelli*), *ikan tamban beluru* (*Clupea leiogaster*), and

other common varieties of fish used in *keropok lekor* processing include *ikan tamban bular* (*Dussumieria hasselti*), *ikan tamban sisek* (*Sardinella fimbriata*), *ikan biji nangka* (*Sciaena* spp.) (Nor-Khaizura et al., 2010).

Based on the study from Wan Hamat et al., (2019) reported that the shelf life of *keropok lekor* is short if stored at ambient temperature and only last for 24 hours because fish flesh contains high nutrients that can trigger the microbial growth. Despite the high demand in Malaysia, the distribution process can be difficult because *keropok lekor* is highly perishable product. Microbial growth also can occur through contamination from raw materials, food handlers, equipment, and environment. Nowadays the production of *keropok lekor* has improved to commercialize the product in form of frozen product in order to extend the shelf life and limit the bacterial growth.

According to study conducted by Ismail et al. (2021), *keropok lekor* needs the proper storage temperature to keep the food product in a good condition and avoid any damage. *Keropok lekor* in Terengganu usually has higher percentage of fish content compare to flour (Rasat el al., 2017).

Nowadays, *keropok lekor* has been commercialised in different types in market such as fresh *keropok lekor* and frozen *keropok lekor*. Both types have their advantage and disadvantage. Fresh *keropok lekor* has better texture and taste but shorter shelf life than *keropok lekor* in frozen state and it is inconvenient for transportation (Yusnita et al., 2017). Frozen *keropok lekor* is slightly greyish in colour and has fishy odor (Ying & Anuar, 2019). Using fresh fish usually causes less microbial contamination than refrigerated or frozen fish, and different types of fish may have varying initial microbial loads (Lani et al., 2017).

The problem to be addressed through this study is the spoilage of *keropok lekor* and the effect of different storage temperatures on the shelf life. It is widely known that *keropok lekor* is a perishable product since the product deteriorate easily when store at room or ambient temperature (Wan Hamat et al., 2019). Short shelf life of food product might affect the production of *keropok lekor* in a large quantity and decrease the quality of product.

MATERIALS AND METHODS

Sample collection

Samples of *keropok lekor* was collected from selected processing premises in Besut, Terengganu. The samples then stored in three different storage temperatures for 7 days. The samples were packed using normal sealed plastic or vacuum pack. These samples were stored at ambient temperature (25℃-28℃) for 3 days and refrigeration temperature (0℃-4℃) were stored for 7 days.

Microbiological Analysis

Microbiological analysis was performed using total plate count method for three replicates of experiment. For samples stored at room temperature, the analysis was carried out on day 0, 1, 2 and 3. Meanwhile, for samples stored at chilled and refrigerated temperature were analysed daily from day 0 to day 7. A 10 g of *keropok lekor* was transferred to a sterile stomacher bag with 90 ml of sterile peptone water and mixed in a stomacher homogenizer (Seaward Stomacher 400, BA-7021) (Nor-Khaizura et al., 2009a).Approximately 50 µl of sample was transferred onto nutrient agar (Merck, Darmstadt Germany) plate and spread plate method was performed before the plate being incubated for 24 h at 37℃. After incubation, colonies formed on the agar was counted using colony counter.

Physicochemical analysis

The analysis includes in physicochemical analysis were moisture content, colour, texture, and pH. All measurements were performed for three replicates experiment.

Moisture content
Three grams of sample was weighed onto the plate that placed in the moisture analyzer machine (Sartorius Moisture Analyzer MA150). The results shown on the machine were recorded.

Texture analysis
The sample was cut into pieces (1.0cm x 1.0cm x 1.0cm). The texture analyzer (TA.HDplusC Stable Micro system) was tested using 35mm cylindrical probe according to machine instruction. The probe was set on 5.0mm/s and hardness, chewiness and gumminess calculate from a texture profile analysis graph.

pH
One gram of sample was blended with 10 ml of distilled water. pH of the homogenized sample was measured with a pH-meter (ThermoFisher Scientific A-5150).

Colour
Colour of sample was measured with a chroma meter (Konica Minolta Chroma MeterCR-410). The *colour* representing by Hunter's *colour* values of L*=lightness (0=black,100= white), a* (a*=greenness, +a*=redness) and b* (b*=blueness, +b*= yellowness).

Statistical analysis
All data were recorded in Microsoft excel include result for replication. In a Statistical Package for the Social Sciences (SPSS) version 24, the significance different was analysed by using One-Way analysis of Variance (ANOVA) and post-hoc test.

RESULTS AND DISCUSSION

Effect of storage temperature time on microbial growth of *keropok lekor*

Table 1 shows the microbial growth on *keropok lekor* at different storage conditions. There are no significant differences ($p>0.05$) in microbial growth at 0 day in three storage temperatures for both types of packaging; sealed pack and vacuum pack. The total plate count (log cfu/ml) of *keropok lekor* in sealed pack at ambient, and refrigeration temperature were 3.33 ± 4.70 and 2.57 ± 0.99, respectively. The total plate count in vacuum pack samples were 2.71 ± 3.83 and 2.64 ± 1.14, respectively. The microorganism increased significantly after one day at ambient temperature for both sealed and vacuum pack samples. When stored at refrigerated condition, microorganism increased significantly after day 3 and day 4 for sealed and vacuum samples, respectively. *Keropok lekor* provide suitable conditions for microbial growth are based on specific internal and external factors such as water activity, pH, microbial interaction, and nutrient content (Nor-Khaizura et al., 2009a). Rapid spoilage of samples stored at ambient temperature were expected and in line with finding by Wan Hamat et al. (2020) in which the samples became unacceptable for consumer to consume after storing at ambient temperature for more than a day. Refrigeration slows the spoilage significantly and vacuum packaging further extend the shelf life for additional one more day. The vacuum packaging limit the growth of aerobic bacteria in the absence of oxygen.

Table 1 Effect of storage conditions on microbial growth of sealed and vaccum pack *keropok lekor*.

Day(s)	Microbial Growth (log cfu/ml)			
	Sealed		Vacuum	
	Ambient	Refrigeration	Ambient	Refrigeration
0	3.33 ± 0.28^{Aa}	2.57 ± 0.99^{Aa}	2.71 ± 0.62^{Aa}	2.64 ± 1.14^{Aa}
1	7.85 ± 0.76^{Ba}	2.68 ± 0.45^{ABb}	6.18 ± 0.22^{ABa}	3.36 ± 0.62^{Ab}
2	8.31 ± 0.20^{Ba}	3.13 ± 1.05^{ABb}	7.34 ± 0.05^{BCa}	3.22 ± 1.79^{Ab}
3	8.38 ± 0.61^{Ba}	7.63 ± 0.54^{Ba}	8.06 ± 0.06^{Ca}	3.86 ± 1.26^{Ab}
4	ND	6.85 ± 0.25^{AB}	ND	6.64 ± 0.04^{A}
5	ND	7.33 ± 0.89^{AB}	ND	6.72 ± 0.87^{A}
6	ND	7.75 ± 0.17^{B}	ND	6.70 ± 0.18^{A}
7	ND	4.41 ± 1.31^{B}	ND	6.89 ± 0.15^{A}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done

Moisture content of *keropok lekor* are shown in Table 2 for sealed and vacuum pack. The moisture content in samples stored at ambient temperature increased significantly after 2 days can be due to the spoilage that produces sliminess and off-odour (Nor-Khaizura et al., 2009a). Moisture content increment was significantly lower than samples stored at ambient temperature due to moisture evaporation in the refrigerator (Gandotra et al., 2012). Thus, the sliminess from spoilage was evaporated which lead to lower moisture content.

Table 2 Effect of storage conditions on moisture content of sealed and vacuum pack *keropok lekor*.

Day(s)	Moisture Content (%)			
	Sealed		Vacuum	
	Ambient	Refrigeration	Ambient	Refrigeration
0	59.95 ± 0.28^{Aa}	59.35 ± 0.46^{ABa}	59.28 ± 0.87^{Aa}	59.73 ± 0.14^{ABa}
1	59.84 ± 1.01^{Aa}	58.91 ± 0.16^{Aa}	62.63 ± 0.73^{ABa}	58.78 ± 0.33^{Ab}
2	64.30 ± 0.04^{Bc}	60.26 ± 0.11^{BCb}	64.09 ± 1.15^{Ba}	62.11 ± 0.21^{Da}
3	61.40 ± 0.65^{Aa}	62.72 ± 0.13^{Da}	60.03 ± 0.94^{Aa}	62.20 ± 0.23^{Da}
4	ND	61.04 ± 0.08^{C}	ND	61.28 ± 0.08^{CD}
5	ND	60.72 ± 0.22^{C}	ND	61.09 ± 0.43^{CD}
6	ND	60.54 ± 0.28^{C}	ND	60.36 ± 0.20^{BC}
7	ND	60.36 ± 0.27^{C}	ND	60.68 ± 0.50^{BC}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

Texture profile analysis (TPA) is a valuable approach for assessing food quality during product research and development. Table 3 show the changes of hardness and chewiness level when the *keropok lekor* for sealed pack. Table 4 showed the changes of hardness and chewiness of *keropok lekor* for vacuum pack. Both sealed and vacuum pack samples yield similar results. From the data shown, the *keropok lekor* stored at refrigeration temperature have the hardest texture compared to ambient temperature.

There are no significant differences for hardness of *keropok lekor* throughout the storage period. The refrigerated samples were significantly harder than samples stored at ambient temperature. Previous study found that fresh Malaysian commercial fish sausage (*keropok lekor*) has a hardness range of 3.3-5.7 kg (Santana et al., 2015). Samples hardness have been linked to water loss (purge) during storage at refrigeration

temperature (Cardoso et al., 2008). Amiza and Ng (2013) found that increasing the starch levels can increase the hardness of fish sausages.

The energy required to chew a solid sample to a stable state of swallowing is referred to as chewiness. Murad et al. (2017) stated that protein content is the most important component influencing chewiness. As a result, because the mixing process stimulates the interaction of the protein network in *keropok lekor*, the mixing duration may be one of the elements that encourage chewiness. The samples mixing time and process were not known as the samples were bought from the suppliers. There are high chances that the time and process of mixing of *keropok lekor* was inconsistent.

Table 3 Effect of storage conditions on texture of sealed pack *keropok lekor*.

Day(s)	Hardness		Chewiness	
	Ambient	**Refrigeration**	**Ambient**	**Refrigeration**
0	5.71 ± 0.97^{Aa}	7.74 ± 0.19^{Ab}	3.31 ± 0.18^{Ba}	3.27 ± 0.21^{Aa}
1	5.78 ± 1.28^{Aa}	7.63 ± 0.20^{Ab}	3.14 ± 0.10^{ABa}	3.53 ± 0.11^{Ab}
2	5.92 ± 1.43^{Aa}	7.69 ± 0.05^{Ab}	2.87 ± 0.17^{ABa}	3.50 ± 0.25^{Ab}
3	5.75 ± 1.51^{Aa}	8.66 ± 0.92^{Ab}	2.59 ± 0.12^{Aa}	3.67 ± 0.11^{ABb}
4	ND	7.60 ± 0.52^{A}	ND	3.12 ± 0.18^{A}
5	ND	7.28 ± 0.05^{A}	ND	4.26 ± 0.12^{BC}
6	ND	8.69 ± 0.04^{A}	ND	3.28 ± 0.22^{A}
7	ND	8.61 ± 0.19^{A}	ND	4.48 ± 0.14^{C}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

Table 4 Effect of storage conditions on texture of vacuum pack *keropok lekor*.

Day(s)	Hardness		Chewiness	
	Ambient	**Refrigeration**	**Ambient**	**Refrigeration**
0	5.77 ± 0.11^{Aa}	6.34 ± 0.21^{Aa}	5.04 ± 0.08^{Ca}	4.08 ± 0.16^{ABCb}
1	5.06 ± 0.22^{Aa}	6.47 ± 0.09^{Ab}	3.46 ± 0.27^{Ba}	3.78 ± 0.25^{ABa}
2	5.21 ± 0.41^{Aa}	7.15 ± 0.16^{Bb}	2.34 ± 0.30^{Aa}	3.72 ± 0.09^{Ab}
3	4.97 ± 0.10^{Aa}	7.32 ± 0.04^{Bb}	2.46 ± 0.21^{Aa}	5.00 ± 0.03^{Db}
4	ND	8.19 ± 0.23^{CD}	ND	3.60 ± 0.09^{A}
5	ND	7.71 ± 0.21^{BC}	ND	3.52 ± 0.20^{A}
6	ND	8.66 ± 0.14^{D}	ND	4.47 ± 0.21^{CD}
7	ND	8.68 ± 0.14^{D}	ND	4.40 ± 0.18^{BCD}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

Based on Table 5, it was observed that there was significant reduction in the pH values of the *keropok lekor* stored at ambient temperature from 7.89 to 6.96 for sealed pack and 7.90 to 6.39 for vacuum pack. pH value correlates with the microorganism growth since most of bacteria are neutrophiles. Refrigerated samples

decreased to pH 6.45 and 6.33 for sealed and vacuum samples, respectively. The optimum pH for the growth is at a pH within one or two pH units of the neutral pH of 7 (Parker et al., 2012). The pH range of *keropok lekor* is favourable for microorganism to grow rapidly (Nor-Khaizura et al., 2010). A decreasing trend in pH was observed after 5 days and above, which may indicate that some form of fermentation had taken place (Bakar et al., 2020).

Table 5 Effect of storage conditions on pH of sealed and vacuum pack *keropok lekor*.

Day(s)	pH			
	Sealed		Vacuum	
	Ambient	Refrigeration	Ambient	Refrigeration
0	7.89 ± 0.02^{Cb}	7.09 ± 0.01^{CDa}	7.90 ± 0.02^{Db}	7.07 ± 0.01^{Ca}
1	7.86 ± 0.01^{Cb}	7.79 ± 0.08^{Eb}	7.81 ± 0.01^{Cb}	7.11 ± 0.01^{Ca}
2	7.05 ± 0.03^{Bb}	7.00 ± 0.01^{Ca}	6.95 ± 0.03^{Ba}	7.05 ± 0.00^{Cb}
3	6.96 ± 0.05^{Aa}	7.07 ± 0.00^{CDb}	6.39 ± 0.03^{Aa}	7.08 ± 0.02^{Cb}
4	ND	7.11 ± 0.01^{D}	ND	7.09 ± 0.01^{C}
5	ND	7.11 ± 0.01^{D}	ND	7.07 ± 0.02^{C}
6	ND	6.80 ± 0.00^{B}	ND	6.83 ± 0.00^{B}
7	ND	6.45 ± 0.00^{A}	ND	6.33 ± 0.01^{A}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

The lightness of *keropok lekor* is presented in Table 6 and 7 shows the L*, a* and b* values of sealed and vacuum pack *keropok lekor* during stored at ambient and refrigerated temperature. The lightness, redness and yellowness increased for *keropok lekor* stored at ambient temperature but no significant different for samples stored at refrigerated temperature. A previous study on the Malaysian commercial fish sausages (*keropok lekor*) reported the L*, a* and b* were from 58.73-79.56, -0.58-17.43 and 12.69-22.96, respectively. The value is affected by the ingredients used during preparation (Murad et al., 2017). This product's spoiling effect include sliminess and the appearance of spots on the surface caused by bacterial growth. Spoilage bacteria produce enzymes capable of breaking down starch present in *keropok lekor* which cause the production of slim and the samples absorb moisture. Absorption of moisture by *keropok lekor* cause the increase of lightness (Donmez et al., 2021). The increase in redness and yellowness which indicated darkening in colour might be due to the formation of metmyoglobin (Chaijan and Panpipat, 2009).

Table 6 Effect of storage conditions on colour of sealed pack *keropok lekor*.

Time (Day/s)	Colour					
	L*		a*		b*	
	Ambient	Refrigeration	Ambient	Refrigeration	Ambient	Refrigeration
0	54.41 ± 2.04^{Aa}	52.96 ± 1.03^{Aa}	2.13 ± 0.51^{Ba}	2.66 ± 0.20^{Ba}	9.23 ± 0.65^{Aa}	9.39 ± 0.36^{Ba}
1	56.12 ± 1.29^{Aa}	55.58 ± 0.99^{Ba}	$1.64 \pm 0.58A^{Ba}$	3.52 ± 0.04^{Cb}	9.77 ± 0.81^{Aa}	10.50 ± 0.47^{Ca}
2	55.77 ± 3.72^{Aa}	53.83 ± 1.92^{Aa}	1.31 ± 0.38^{Aa}	2.38 ± 0.94^{ABa}	9.45 ± 1.66^{Aa}	8.98 ± 1.10^{Aa}
3	60.51 ± 0.97^{Bb}	54.29 ± 0.57^{Aa}	2.14 ± 0.42^{Ba}	2.76 ± 0.96^{Ba}	11.62 ± 0.27^{Ba}	$9.90 \pm 0.96B^{Ca}$
4	ND	54.76 ± 0.40^{AB}	ND	2.67 ± 0.47^{B}	ND	9.86 ± 0.36^{B}
5	ND	55.29 ± 0.98^{B}	ND	1.30 ± 0.35^{A}	ND	8.14 ± 0.85^{A}
6	ND	52.98 ± 0.91^{A}	ND	1.27 ± 0.09^{A}	ND	9.37 ± 1.75^{AB}
7	ND	55.51 ± 0.61^{B}	ND	2.06 ± 0.67^{B}	ND	8.52 ± 0.31^{A}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

Table 7 Effect of storage conditions on colour of vacuum pack *keropok lekor*.

Time (Day/s)	Colour					
	L*		a*		b*	
	Ambient	Refrigeration	Ambient	Refrigeration	Ambient	Refrigeration
0	51.53 ± 0.53^{Aa}	52.62 ± 2.00^{Aa}	2.55 ± 0.74^{Aa}	3.10 ± 0.26^{BCb}	8.20 ± 1.15^{Aa}	9.33 ± 0.77^{Bb}
1	53.05 ± 1.28^{Aa}	55.11 ± 0.71^{Bb}	2.62 ± 0.53^{Aa}	3.20 ± 0.22^{Cb}	9.21 ± 1.06^{Aa}	9.12 ± 0.91^{Ba}
2	59.42 ± 0.95^{Bb}	56.69 ± 1.96^{Ba}	2.85 ± 0.36^{Aa}	2.78 ± 0.72^{Ba}	11.07 ± 0.94^{Bb}	9.39 ± 3.61^{Ba}
3	59.77 ± 2.38^{Bb}	53.77 ± 0.62^{ABa}	3.76 ± 0.59^{Ba}	3.37 ± 0.75^{Ca}	11.51 ± 1.03^{Bb}	9.95 ± 0.42^{Ba}
4	ND	53.68 ± 2.91^{A}	ND	3.05 ± 0.27^{BC}	ND	9.69 ± 0.82^{B}
5	ND	54.89 ± 0.97^{AB}	ND	1.75 ± 0.35^{A}	ND	7.80 ± 0.46^{A}
6	ND	52.07 ± 1.37^{A}	ND	2.67 ± 0.69^{B}	ND	9.60 ± 0.40^{B}
7	ND	54.84 ± 1.29^{AB}	ND	2.56 ± 0.65^{B}	ND	7.71 ± 1.67^{A}

$^{A, B, C}$ Data in the same column with different letter is significantly different ($p<0.05$)
$^{a, b, c}$ Data in the same row with different letter is significantly different ($p<0.05$)
ND - Not Done.

CONCLUSION

Rapid deterioration was found in *keropok lekor* stored at ambient temperature with significant changes in the microbial growth and physiochemical qualities in both vacuum and sealed packaging. Refrigeration significantly reduce the microbial spoilage of *keropok lekor* to 3 days with no significant changes. Vacuum packaging capable of prevent the microbial growth up to 4 days but no significant differences for physicochemical properties compared to sealed packaging. Thus, storage temperature plays an important factor in ensuring the stability of *keropok lekor* during storage.

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