Asia Pacific Environmental and Occupational Health Journal (ISSN 2462 -2214), Vol 10 (7): 1-13, 2024
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Mutagenic Potential of Fried and Boiled "Keropok Lekor": A Study Using the Ames Test

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ABSTRACT

Introduction: "Keropok lekor", a traditional Malaysian fish sausage, is widely consumed, yet its mutagenic potential has not been thoroughly investigated. **Objective:** This study evaluated the mutagenic activity of keropok lekor samples. **Methodology:** Samples were analysed using the Ames test with *Salmonella typhimurium* strains TA98 and TA100, with and without S9 metabolic activation. Boiled "keropok lekor" and fried samples variants were tested at concentrations of 1.0, 2.0, and 4.0 mg/mL. **Results**: The fried samples demonstrated significant mutagenicity in the absence of metabolic activation, with revertant colony counts exceeding the two-fold threshold of solvent controls at higher concentrations. In contrast, the boiled samples showed lower mutagenic activity, suggesting that frying may increase mutagenicity. Furthermore, while the addition of S9 metabolic activation reduced mutagenic responses, a dose-response relationship was noted in both variants, indicating a concentration-dependent effect. These findings highlight the potential health risks associated with frequent consumption of fried "keropok lekor" and emphasize the need to evaluate traditional foods for genotoxic risks.

Keywords: Mutagenicity, keropok lekor, Ames test, *Salmonella typhimurium*, mutation

1. Introduction

Processed fish products, widely consumed in Southeast Asia, are increasingly scrutinized for potential health risks, particularly due to the formation of mutagenic and genotoxic compounds during production (De-la-Torre, 2020). Traditional processing methods for popular foods, such as keropok lekor, often involve high temperatures and chemical additives that may unintentionally introduce harmful substances, raising consumer safety concerns (Zare et al., 2015). Studies have documented those biogenic amines, including histamine, can accumulate in processed fish products, occasionally exceeding safe limits, especially in dried fish (Eldaly et al., 2015). Histamine levels above 500 mg/kg pose significant health risks,

highlighting the importance of vigilant monitoring in fish processing (Zare et al., 2015). Furthermore, fish processed through traditional methods can be vulnerable to contamination by pathogens such as *Listeria monocytogenes*, intensifying public health risks in regions where fish-based diets are prevalent (Eldaly et al., 2015). As consumer demand for fish-based products increases, particularly in Southeast Asia, thorough safety assessments are essential to ensure these products do not compromise public health (Alfio et al., 2021; Bogard et al., 2019).

Keropok lekor, a traditional Malaysian fish snack, is a distinctive cultural food; however, limited research has examined its potential mutagenic properties. Studies on similar processed fish products have highlighted safety concerns, suggesting the need for safety evaluations of culturally significant foods like keropok lekor (Hussain et al., 2019). Research on biogenic amines in fish-based snacks, including keropok lekor, reported low total amine concentrations but emphasized the importance of regular testing for potential contaminants (Hussain et al., 2019). Additionally, the growing demand for processed seafood necessitates comprehensive assessments to verify safety, particularly for traditional products with high local consumption (Bogard et al., 2019). Although the Ames test is a well-established method for detecting mutagenicity in food extracts, its application to keropok lekor remains limited, indicating a gap in safety evaluations for this popular snack (Zeiger, 2013). Addressing this gap could provide important insights into the safety of keropok lekor, especially given the potential formation of mutagens during traditional production processes (Rubio et al., 2019).

Environmental contaminants, particularly along Malaysia's east coast, affect the safety of small-scale fishery products such as keropok lekor. Studies have identified heavy metals, including mercury and lead, in marine fish, with some concentrations exceeding safety limits (Azmi et al., 2019). Malaysia's high per capita fish consumption, estimated at 55.9 kg which is over three times the global average highlights the urgency for rigorous safety standards (Venggadasamy et al., 2021). Furthermore, microplastics have been detected in commercial fish species, complicating food safety as these contaminants enter the food chain and accumulate in human diets (Ma et al., 2020). This contamination poses public health risks, emphasizing the need for comprehensive assessments of local fish products to address the risks of cumulative exposure (Venggadasamy et al., 2021).

Mutagenic risks in fish products are heavily influenced by processing variables, including cooking methods, temperatures, and ingredient combinations. High-temperature techniques, such as frying, are known to produce polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HAAs), compounds with established mutagenic properties (Omoruyi et al., 2020). For instance, research has shown that PAH levels in smoked fish increase with prolonged processing times, indicating a clear link between production practices and potential health risks (Abiona, 2020). Additionally, traditional and industrial methods produce varying outcomes; for example, liquid smoke generally results in lower levels of harmful compounds than traditional smoking (Özpolat & Patir, 2016). These findings underscore the need for optimized processing techniques to reduce mutagenicity in fish products, highlighting the essential impact of cooking conditions on health outcomes.

Keropok lekor, a staple rooted in Terengganu's cultural and culinary heritage, serves as both a dietary essential and a popular attraction in regional tourism. Made primarily from local fish, sago flour, and seasonings, it reflects the coastal traditions of local communities (Jalis, 2018). Its widespread presence in local markets and street food venues highlights its role in local diets, providing both nutritional value and social significance (Afifi et al., 2023). The growing popularity of keropok lekor and other fish-based snacks among Malaysians, especially youth, aligns with dietary trends favoring convenience foods (Asma' et al., 2019). However, traditional preparation methods, often involving deep frying, raise health concerns due to high fat and sodium levels, underscoring the need for comprehensive safety assessments for this culturally valued food (Haron et al., 2022; Tamsir et al., 2021).

Given the contaminants from increased coastal human activity in Malaysia, there is an urgent need to evaluate the mutagenic risks associated with keropok lekor. The Ames test offers a reliable method for detecting potential mutagens in this widely consumed fish snack, which has not been extensively studied in this context (Rubio et al., 2019; Zeiger, 2013). This study aims to implement comprehensive mutagenicity testing on keropok lekor, providing valuable insights

into its safety profile and addressing a critical gap in the understanding of processed seafood safety in Malaysia.

2. Materials and Method

2.1. Study design and sampling method

This research was a cross-sectional study designed to identify the intake of keropok lekor and evaluate the potential mutagenicity of keropok lekor manufactured in Kemaman, Terengganu. The sampling for this study involved two parts which were convenience sampling to select the respondents for the Food Frequency Questionnaire (FFQ) and sampling on keropok lekor samples. For the FFQ, the population that lives in Kemaman were selected as respondents to answer the questionnaire. Keropok lekor samples were obtained from local stores in Geliga, Kemaman, and handled according to the Protocol for Sampling and Methods of Analysis for Malaysian Food Composition Database (FCD) that is established by the Institute for Public Health (IKU) and the Institute for Medical Research (IMR). The mutagenic activities of keropok lekor were identified by using the Ames test incorporating *S. typhimurium* strains (Ames et al., 1973).

2.2. Food frequency questionnaire

A modified version of the Food Frequency Questionnaires (Ethical Clearance Number: JKEUPM-2023-444) was utilized to gather information on keropok lekor intake from Kemaman, Terengganu. The questionnaire was developed based on the Malaysian Adult Nutrition Survey (Aris et al., 2014) which consists of three parts including socio-demographic information of respondents, health symptoms related to heavy metals exposure, and keropok lekor intake. The survey data were utilized to identify the preferred types of keropok lekor samples within the community and determine the locations where individuals typically purchase keropok lekor.

Only the respondents aged from 18 to 60 years old who fulfilled the inclusion criteria were provided with the FFQ in which the inclusion criteria included respondents who lived in Kemaman for at least a year and consumed keropok lekor in their diet. More importantly, the study included respondents who can recall their dietary habits. The exclusion criteria were respondents who reported a history of heavy metal exposure and respondents who are currently taking medication or supplements.

The first part of the self-administered questionnaire was the socio-demographic information of respondents consists of nine items including gender, age, ethnicity, religion, marital status, education level, employment, monthly household income and number of households. Next, the second part of the questionnaire is the acute and chronic health symptoms related to heavy metals exposure. The final section of the questionnaire addresses keropok lekor intake. The initial item assesses the frequency of weekly keropok lekor consumption, followed by the number of times it is consumed in a day. A consumption scoring system ranges from "one time a day" to "seven times a day." Subsequently, respondents report the total servings for each consumption which the total serving referred to the number of slices of keropok lekor consumed (1-5 slices, 6-10 slices, 11-15 slices, 16-20 slices and 21-25 slices). Concluding the questionnaire, participants were asked about the location of their keropok lekor purchases and their preferences regarding the types of keropok lekor.

2.3. Sampling of keropok lekor

This study focused on comparing two predominant types of keropok lekor, namely, keropok lekor rebus and keropok lekor goreng, which are widely consumed by the community in Kemaman as described in the survey result. 250 g of each sample were purchased from four randomly selected sampling points along the Geliga area (Wan-Hamat et al., 2020). Samples were placed in zip lock bags, then wrapped in dark plastic bags before being stored in a cooled box with ice packs and ice cubes (0-4℃) during transportation to the laboratory within 24 hours. After reaching the laboratory, the samples were kept in the freezer at -20℃ before sample extraction (Hussain et al., 2019).

2.4. Extraction of keropok lekor samples

About 100 g of samples were weighed and then blended using a heavy-duty blender. Then, 50 g of homogenized samples were blended again with 50 mL of methanol and chloroform with a 1:1 (v/v) ratio. The mixtures were shaken for five hours using an orbital shaker and subsequently stored at -20°C for 24 hours. Following this, the mixture underwent filtration using Whatman No.1 filter paper, and the resulting filtrates were evaporated at 50 °C – 60 °C under low pressure using a rotary evaporator. Finally, the crude extract was scrapped from the flask and dissolved with DMSO solution to a concentration of 4 mg/ml and kept at -20 °C before the experiment.

2.5. Ames test experiment

The Ames test experiment was conducted according to the Standard Operation Procedure of Biocompatibility and Toxicology Lab provided by the Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia (UKM). The positive controls for the experiment were prepared, with 2-Nitrofluorene and Sodium Azide chosen for TA98 and TA100, respectively. Meanwhile, for the assays employing S9-mix for the metabolic activation, 2-Aminoanthracene was used as the positive control. Dimethylsulfoxide (DMSO) was chosen as the solvent control for this study. The exposure concentrations used in this experiment were 4 mg/mL, 2 mg/mL, and 1 mg/mL. Each solution was diluted from the stock solution (4 mg/mL) of extracted keropok lekor with a specific DMSO volume to obtain the desired concentration.

Pre-incubation of bacteria strains was conducted one day prior to the experiment day in which 12 µl of bacteria from frozen stock was added into 12 mL of nutrient broth in a conical flask. The bacterial culture of TA98 and TA100 were then incubated at 4 °C for 7 hours. Then, the conical flasks were transferred into the water bath at 37 °C to be shaken and incubated for 12-16 hours. After 12-16 hours, the bacterial cultures were ready to be used for the Ames test. 0.5 mL of 0.1 M sodium phosphate buffer or S9-mix, 0.1 mL of test article (positive controls, negative controls, and samples), and 0.1 mL of bacterial suspensions were added together into a culture tube. Then, the culture tubes were incubated in the water bath shaker for 20 mins at 37 °C. After 20 minutes, 2 ml of melted top agar supplemented with 0.6% histidine and biotin was added to each mixture in the culture tube. Subsequently, the culture tubes were gently swirled to ensure proper mixing before being poured onto Glucose Minimal (GM) agar. Lastly, the GM agar plates were incubated at 37 °C for 42 hours before the revertant colonies were counted.

2.6. Quality Control

Quality control was a focal point during the Ames test experiment. All glassware utilized in the experiment were autoclaved to ensure sterilization before use. Additionally, glass wares were cleaned with Decon 90, soaked overnight post-experiment to get rid of any bacterial contamination. Furthermore, the experiment was executed under sterile conditions within a fume hood located near a flame to minimize the risk of contamination. Furthermore, when conducting the experiment with S9 metabolic activation, it was carried out in a dark environment, and the S9-mix was maintained under icy conditions. This precaution was essential to prevent the activation of substances during the experiment. Lastly, positive controls experiments were conducted together in all experiments to ensure the test's sensitivity and the reliability of the experiments.

3. Results

3.1. Socio-demographic data

A total of 260 respondents participated, comprising 85 males and 175 females, as detailed in Table 1. The largest group of respondents fell within the age range of 26 to 35 years, representing 35.4% of the total sample, with a specific count of 92 individuals. Following this, the second-highest number of respondents, 78 in total, belonged to the 35 to 45 years age group. The third-highest participation was observed among individuals aged 18 to 25 years, accounting for 20.8% of the respondents, totalling 54 people. The two age groups with the lowest participation included 46 to 55 years (29 respondents) and 56 to 59 years (7 respondents), respectively.

The study predominantly involved Malay participants, comprising 239 individuals, which accounts for 91.9% of the total respondents. Additionally, there were 15 Chinese respondents, consisting of 5.8% of the participants, and 6 Indian respondents, representing 2.3% of the total. Most respondents in this study hold a bachelor's degree, comprising 50.8% of the total respondents (n = 132), followed by the Pre-University category with 21.5% (n = 56). The largest group of respondents was employed in the private sector, with 79 individuals, followed by semi-government officers (n = 59). Half of the respondents (50%, n = 130) have a monthly household income between RM 2,001 to RM 5,500 and 112 have a monthly income of less than RM 2,000.

3.2. Keropok lekor consumption

Most respondents were reported consuming keropok lekor only one times a day (n = 192, 73.8%) respondents. In addition, 54 (20.8%) respondents reported to be consuming keropok lekor two times a day. Meanwhile, 3.5% respondents consumed keropok lekor 3 times a day, 1.5% respondents consumed 4 times a day and 0.4% respondents consumed 7 times a day.

Figure 1 presents the total servings of keropok lekor consumed by the respondents. It was shown that majority of respondents consisting of 141 (54.1%) individuals consumed six to ten slices of keropok lekor every time they eat it. Addtionally, the study recorded 101 individuals, accounting for 39% of the total respondents, consumed one to five slices of keropok lekor per serving. Furthermore, Figure 1 revealed that 17 individuals reported consuming 11 to 15 slices perserving, while only one person reported consuming 16 to 20 slices of keropok lekor each time.

Table 1. Socio-demographics of respondents (n=260)

Socio-demographic	Information	Frequency (n)	Percentage (%)
Gender	Male	85	32.7
	Female	175	67.3
Age	18 to 25	54	20.8
	26 to 35	92	35.4
	36 to 45	78	30.0
	46 to 55	29	11.2
	56 to 60	7	2.7
Ethnicity	Malay	239	91.9
	Chinese	15	5.8
	Indian	6	2.3
Religion	Islam	240	92.3
	Hindu	6	2.3
	Christian	11	4.2
	Buddhist	3	1.2
Marital Status	Single	90	34.6
	Married	165	63.5
	Divorced	5	1.9
Education Level	Primary level	1	0.4
	Secondary level	17	6.5
	Vocational	49	18.8
	Pre-university	56	21.5
	Bachelor's degree	132	50.8
	Postgraduate	5	1.9
Employment	Government	29	11.2
	Semi-government	59	22.7
	Private sector	79	30.4
	Retiree	6	2.3
	Housewives	19	7.3
	Self employed	34	13.1
	Student	34	13.1
Monthly Household Income	< RM 2000	112	43.1
	RM2001–RM 5500	130	50.0
	>RM5501	18	16.9

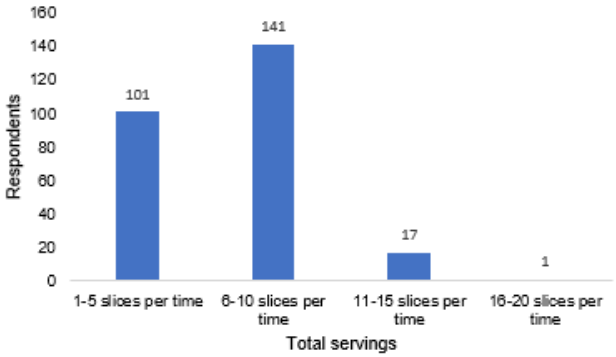

Figure 1: Total servings of keropok lekor consumed

3.3. Mutagenic activities

3.3.1. Mutagenic activities of keropok lekor samples

Table 2 showed the count of revertant colonies formed on S. typhimurium strains TA98 and TA100, both in the presence and absence of S9 metabolic activation. All samples were treated with three extracts concentration which are 1.0, 2.0 and 4.0 mg/ml. Keropok lekor rebus, tested at a concentration of 1.0 mg/ml on TA98, resulted in the formation of 10 colonies without S9 metabolic activation and 18 colonies with S9. The numbers of revertant colonies increased at a concentration of 2.0 mg/ml, with 20 colonies formed without S9 and 22 colonies with the presence of S9. At the highest concentration (4.0 mg/ml), the colonies formed without S9 were 43 revertant colonies, decreasing to 32 colonies when treated with S9 metabolic activation.

Table 3 Number of revertant colonies of keropok lekor extracts treated with and without S9 metabolic activation

Sample	Conc. (mg/ml)	No. of revertant colonies[a]			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Solvent	0	35	21	33	25
Positive[b]	5 µg/L	122	52	207	60
Keropok lekor rebus	1	10	18	12	18
	2	20	22	37	29
	4	43	32	54	34
Keropok lekor goreng	1	18	11	13	19
	2	49	14	41	22
	4	57	36	70[c]	29

[a]mean (from 3 replicates)

[b]experiment without S9 used positive controls which are 2–Nitrofluorene (TA98) and Sodium azide (TA100); meanwhile, experiment with S9 used 2-Aminoanthracene.

[C]exceed the two-fold of solvent control

The number of colonies formed when it was treated at 1.0 mg/ml sample concentration was 12 colonies in the absence of S9 and 18 colonies in the presence of S9. Meanwhile, the sample concentration at 2.0 mg/ml formed 37 and 29 colonies when tested without and with S9 respectively. The colonies count relatively increased to 54 (without S9) and 34 (with S9) colonies when tested with 4.0 mg/ml sample concentration.

Furthermore, keropok lekor goreng samples, with the same concentrations, were also tested on TA98 and TA100 in the presence and absence of S9 metabolic activation. At a concentration of 1.0 mg/ml, keropok lekor goreng formed 18 colonies on TA98 strains when tested without S9, while 11 colonies were observed with S9. The colony counts increased at the concentration of 2.0 mg/ml, with 49 colonies without S9 and 14 colonies with S9. At the highest concentration (4.0 mg/ml), the observed colonies were 57 without S9 and 36 with S9.

The results for the number of revertant colonies formed on TA 100 for keropok lekor goreng samples. At the lowest concentration of 1.0 mg/ml, the results show 13 colonies formed when tested without S9 and 19 colonies with S9. Moving to the 2.0 mg/ml sample concentration, the numbers of colonies increased to 41 col-onies (without S9) and 22 colonies (with S9). Lastly, at the highest concentration of 4.0 mg/ml, 70 colonies were counted in the absence of S9, and 29 colonies were observed with the presence of S9 metabolic activation.

3.3.2. Mutagenic activities of keropok lekor samples tested on strain TA98 and TA100 in the absence of S9 metabolic activation

Figure 2 shows the comparison of mutagenic activities of keropok lekor rebus and keropok lekor goreng in the absence of S9 metabolic activation for both strains TA98 and TA100. It was found that the revertant colonies counted for keropok lekor goreng was higher compared to keropok lekor rebus for all concentration. From Figure 2(a), the results showed that out of all concentration of samples tested with TA98, 4.0 mg/ml produced the highest number of revertant colonies (57 colonies) compared to 2.0 mg/ml and 1.0 mg/ml. Furthermore, it is observed in Figure 2(a) that all concentrations tested with TA98 without S9 does not exhibit mutagenic activities as the number of revertant colonies does not exceed the double fold of solvent controls at 70 colonies.

Meanwhile, Figure 2(b) presented that the highest revertant colonies when tested with strain TA100 was recorded at the concentration of 4.0 mg/mL (70 colonies) compared to other two concentrations. It was observed that the colonies counted for all samples concentration does not exceed the double fold of solvent control at 66, except for sample at the concentration of 4.0 mg/ml. It was recorded that number of revertant colonies at 4.0 mg/ml slightly exceed the double fold of solvent control with 70 colonies when tested with TA100 without S9 indicating a direct acting mutagen with a significant difference ($p<0.05$). Furthermore, based on Figure 2(a,b), it can be observed that the number of revertant colonies increased relatively with the increasing sample concentration for both types of keropok lekor, indicating a positive dose-response relationship.

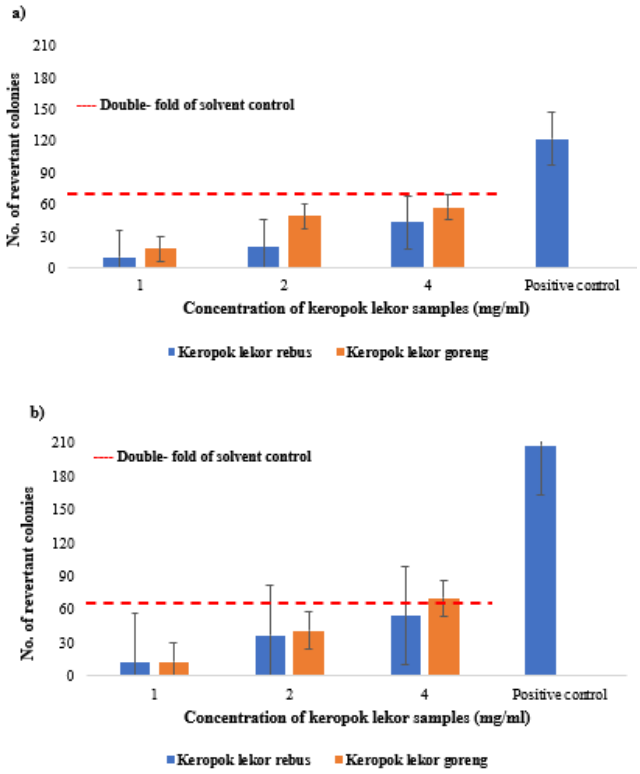

Figure 2: Mutagenic activities of keropok lekor samples in the absence of S9 metabolic activation a) Tested with TA98, b) Tested with TA100

3.3.3. Mutagenic activities of keropok lekor samples tested on strain TA98 and TA100 in the presence of S9 metabolic activation

Figure 3 presents the mutagenic activities of keropok lekor samples in the presence of S9 metabolic activation, tested on strains TA98 and TA100. It is noticeable that the highest numbers of revertant colonies for both keropok lekor rebus and keropok lekor goreng samples were recorded at the concentration of 4 mg/ml when tested on both strains TA98 and TA100. As tabulated in Figure 3, it is shown that the number of revertant colonies for both tests with TA98 and TA100 in the presence of S9 does not exceed double the fold of the negative control at 42 and 50, respectively. This indicates that both keropok lekor samples do not exhibit mutagenic activities.

Furthermore, Figure 3(a) indicates that the revertant colonies for keropok lekor rebus extracts at 1.0 and 2.0 mg/ml were higher than the keropok lekor goreng extracts when tested with TA98. Similarly, in the test with strain TA100, as depicted in Figure 3(b), the revertant colonies recorded were higher for keropok lekor rebus at the concentrations of 2.0 (29) and 4.0 (34) mg/ml. However, it is noticable from both Figure 3(a,b) that the count of revertant colonies increased along with the upward trend of the extract concentrations.

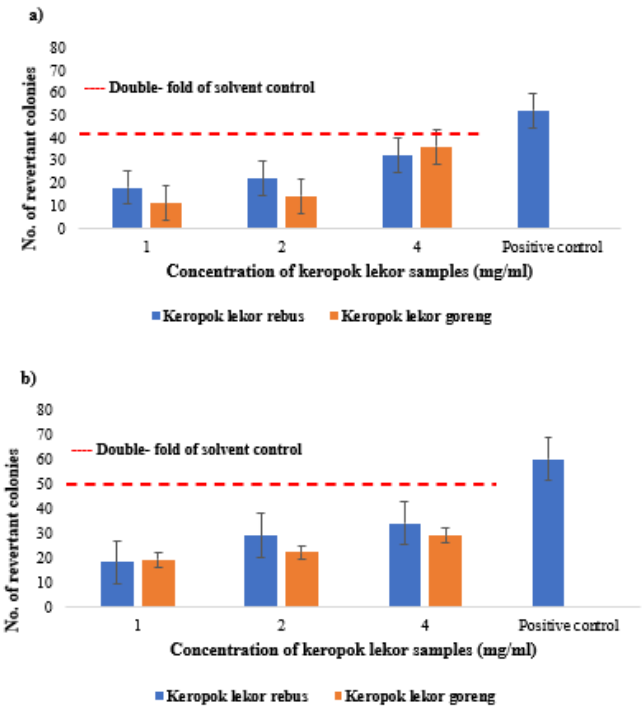

Figure 3: Mutagenic activities of keropok lekor samples in the presence of S9 metabolic activation a) Tested with TA98, b) Tested with TA100

3.3.4. Comparison of mutagenic activities of keropok lekor samples tested on TA98 and TA100 between the absence and presence of S9 metabolic activation

The mutagenic activities of both boiled keropok lekor and fried keropok lekor tested on both strain TA98 and TA100 with and without the S9 metabolic activation were compared below in Figure 4. Based on Figure 4(a), the observed revertant colonies for boiled keropok lekor samples tested at 1.0 (10 colonies) and 2.0 (20 colonies) mg/ml without the presence of S9 are lower compared to the experiment with S9, where 18 and 22 colonies were recorded, respectively.

However, the revertant colonies for boiled keropok lekor tested with strain TA98 at the concentration of 4.0 mg/ml showed a higher colony count (43) when tested without S9 compared to the count when tested with S9 (32). Meanwhile, for fried keropok lekor, the revertant colonies for all sample concentrations tested with strain TA98 shows a higher count when conducted without the presence of S9 compared to with S9. In addition, according to the paired sample t-test analysis the revertant colonies of fried keropok lekor extract at the concentration of 2.0 mg/ml showed a significant difference ($p < 0.05$) when tested without and with S9.

Furthermore, Figure 4(b) showed the comparison of revertant colonies formed when tested on TA100 between the condition with and without S9. The result presented that the experiment without S9 produced higher count of colonies for both types of keropok lekor at all concentration except for the extracts at the concentration of 1.0 mg/ml. Boiled Keropok lekor extracts at 1.0 mg/ml produced lower colonies when tested without S9 (12) compared to when it is treated with S9 (18). Similarly, colonies counted for fried keropok lekor extracts with the concentration of 1.0 mg/ml tested without S9 (13) is relatively lower than results with S9 (19).

In addition, from Figure 4(b), it can be observed that the highest revertant colonies for boiled keropok lekor and fried keropok lekor tested without S9 were recorded at the concentration of 4.0 mg/ml with 54 and 70 colonies, respectively. The samples extract treated with S9 were also recorded the highest number of revertant colonies when tested at the highest concentration of 4.0 mg/ml. Furthermore, the paired sample t-test analysis reveals a significant difference in the colonies formed when boiled keropok lekor at 1.0 mg/ml and fried keropok lekor at 4.0 mg/ml are treated with and without S9.

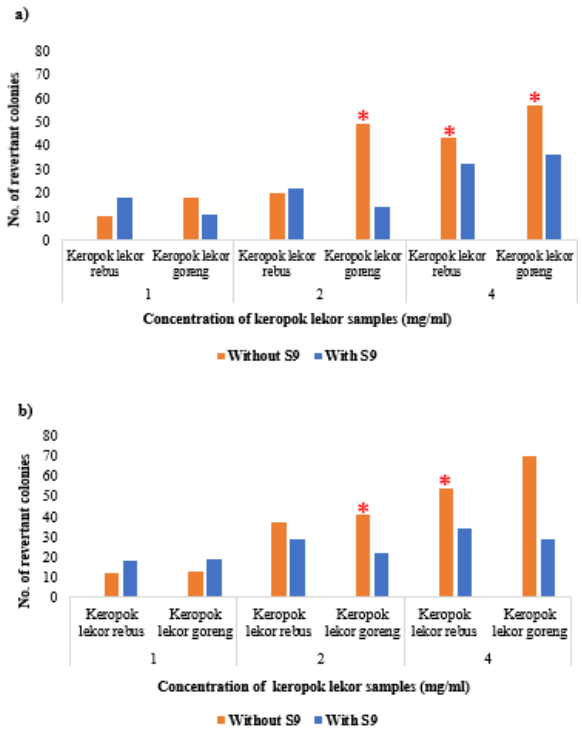

Figure 4: Comparison of mutagenic activities of keropok lekor samples in the presence and absence of S9 metabolic activation a) Tested with TA98, b) Tested with TA100

4. Discussion

This study has provided substantial evidence of mutagenic activity in keropok lekor samples, with notable variations based on preparation method. The fried variant, fried keropok lekor, demonstrated significantly higher revertant colony counts in both TA98 and TA100 strains compared to the boiled version, boiled keropok lekor. Notably, mutagenic activity in fried keropok lekor was particularly elevated without S9 metabolic activation, suggesting the presence of direct-acting mutagens within the sample (Hussain et al., 2019; Inagaki & Hirai, 2016).

These findings are consistent with prior studies on fried foods prepared at high temperatures, which report the formation of heterocyclic aromatic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) which are the mutagenic compounds associated with cancer risks (Gibis, 2016; Rose et al., 2015). Additionally, a dose-response pattern was observed across keropok lekor samples, with revertant colony counts increasing with sample concentration, underscoring the need for thorough assessments of health risks associated with high consumption levels of fried keropok lekor, especially given its popularity among Malaysian consumers (Jeevanaraj et al., 2020).

The mutagenic potential observed in keropok lekor, particularly in the fried variant, aligns with existing research on the risks associated with high-temperature cooking methods. Studies indicate that frying generates HCAs and PAHs, compounds linked to greater mutagenic potential, whereas boiling or steaming—using lower temperatures and moisture—tends to minimize these risks (Chiavarini et al., 2017; Inagaki & Hirai, 2016). This finding is consistent with research on other regional fish-based snacks, where boiling has demonstrated reduced mutagenicity likely due to the absence of direct high heat. For keropok lekor, this distinction is significant, as frying reaches temperatures that facilitate the formation of mutagenic compounds (Wang et al., 2019). Future research could explore whether air-frying or other low-oil cooking methods might reduce mutagenic compound formation while maintaining the desired flavor and texture of fried keropok lekor.

Without S9 activation, the fried keropok lekor samples demonstrated significant mutagenicity, especially at higher concentrations, suggesting the presence of compounds capable of causing mutagenic effects without metabolic activation. This finding is consistent with research on foods cooked at high temperatures, where the Maillard reaction and amino acid breakdown produce mutagenic byproducts such as HCAs and PAHs (Crudo et al., 2023; Sheng et al., 2020). Additionally, the amino acid and fatty acid content in keropok lekor may contribute to enhanced mutagenic responses, a pattern frequently observed in fried foods (Savinova & Yerzhanova, 2021). This direct mutagenicity in keropok lekor goreng highlights the need to assess traditional fried foods for mutagenic risk, particularly for frequent consumers who may face cumulative exposure risks (Reng et al., 2022).

The potential release of histidine from the protein-rich keropok lekor could lead to false positives in mutagenicity tests. To mitigate this risk, the treat-and-wash assay was applied, allowing for a more accurate distinction between true mutagenicity and histidine-induced responses. This approach was essential for precisely assessing the mutagenic potential of keropok lekor, as research has shown that protein-rich foods can produce misleading results if histidine interference is not managed (Makhafola et al., 2016; Vo et al., 2021). These findings underscore the importance of

employing alternative assays, such as treat-and-wash, to reduce the likelihood of overestimating mutagenic risks due to histidine release.

When evaluating the health risks associated with keropok lekor consumption, both mutagenic activity and sodium content warrant careful consideration. Studies on fried foods and processed seafood highlight potential health risks from cumulative consumption, with dietary sodium intake posing concerns, especially among regular consumers of fried fish-based snacks (Haron et al., 2022; Qin et al., 2021). Additionally, while biogenic amine levels in keropok lekor remain below harmful thresholds, further assessment is needed to explore potential synergistic effects with other compounds formed during frying (Hussain et al., 2019). Future research should examine whether modifying ingredients or using alternative cooking methods could reduce both mutagenicity and sodium-related health risks.

Significant variability in mutagenic activity was observed between batches of keropok lekor, likely influenced by differences in frying temperature, cooking duration, and raw material composition. This finding is consistent with similar studies, where variations in preparation conditions led to fluctuations in mutagenic activity levels (Dong et al., 2020; Nadeem et al., 2021). Standardizing preparation methods could help reduce mutagenic risk in keropok lekor. Additionally, public health efforts might focus on educating consumers about cooking practices and consumption patterns that minimize exposure to mutagenic compounds. Research into alternative methods, such as air-frying or lower-temperature frying, could also support safer keropok lekor production.

The findings of this study hold significant public health implications, particularly given the widespread consumption of keropok lekor in Malaysia. The detection of mutagenic activity in fried keropok lekor samples, especially without S9 metabolic activation indicates that consumers may be exposed to compounds with potential genotoxic effects. This underscores the need to consider the impact of cooking methods on mutagenicity, as high-temperature frying appears to elevate mutagenic risk relative to boiling. The study thus provides foundational evidence for Malaysian regulatory bodies to evaluate and, if needed, establish guidelines promoting safer preparation practices or alternative cooking methods to reduce mutagenic compound formation in popular food items.

Additionally, the observed correlation between keropok lekor concentration and mutagenic activity raises concerns about the health risks associated with frequent or high-volume consumption. The increased revertant colony counts at higher concentrations of fried keropok lekor suggest a dose-dependent mutagenic response, highlighting the importance of public awareness regarding safe intake levels. This dose-response relationship is particularly relevant for habitual consumers and warrants further research into safe consumption limits, which could help shape public health recommendations. By employing the Ames test to assess keropok lekor's mutagenicity, this study also establishes a methodological framework applicable to other traditional Malaysian foods, enabling future research to deepen our understanding of mutagenic risks linked to local dietary practices.

This study has several limitations that should be considered when interpreting the findings. Firstly, the cross-sectional design captures only a single snapshot of the mutagenic potential in keropok lekor samples from a specific region in Malaysia. This design limits the ability to draw causal inferences or generalize findings beyond the sampled products. Moreover, regional variations in keropok lekor preparation methods could result in differing levels of mutagenic activity not reflected in this study. Sampling was restricted to products from Kemaman, Terengganu, without accounting for possible differences in ingredients, cooking techniques, or environmental conditions in other Malaysian areas.

A further limitation lies in the mutagenicity testing method. While the Ames test using TA98 and TA100 strains is well-regarded for detecting mutagenic activity, it primarily identifies specific mutation types. Thus, it may not detect other genotoxic effects, such as chromosomal alterations or DNA strand breaks, which assays like the comet assay might capture. Additionally, although S9 metabolic activation was used to simulate human metabolic conditions, this system does not fully replicate the human body’s complex enzymatic processes, potentially limiting the direct relevance of these findings to actual human health risks. Future research should include additional mutagenicity assays and more detailed metabolic simulations to corroborate these results.

These limitations highlight the need for broader, longitudinal studies involving keropok lekor samples from various regions, multiple genotoxicity assays, and an analysis of specific ingredient contributions to mutagenicity. Addressing these limitations could yield a more comprehensive understanding of the potential health risks of keropok lekor consumption and support

the development of evidence-based dietary guidelines for traditional foods.

Future research on the mutagenicity of keropok lekor could benefit from examining other common varieties and preparation methods to provide a comprehensive evaluation of genotoxic risks associated with traditional Malaysian fish-based snacks. While this study focused on samples from Kemaman, analyzing keropok lekor produced in diverse Malaysian regions could reveal potential regional variations in mutagenic potential. Additionally, incorporating a broader range of mutagenicity assays, such as the comet assay or micronucleus test, could enhance the Ames test findings by detecting genotoxic effects on chromosomal integrity and DNA strand stability, thus offering a more detailed understanding of potential health risks.

Further investigation into alternative cooking methods, such as air-frying or baking, could assess whether these techniques reduce the formation of mutagenic compounds commonly associated with high-temperature frying. Comparing the mutagenic profiles of these alternatives with traditional frying may identify safer preparation options that maintain keropok lekor's cultural significance. Longitudinal studies examining the health effects of regular keropok lekor consumption, especially among populations with high intake, could also provide valuable insights into the long-term health implications of exposure to mutagenic compounds in this food. Such studies would support the development of evidence-based dietary guidelines and inform public health strategies, encouraging safer consumption practices for traditional foods.

5. Conclusion

In conclusion, this study provides valuable insights into the mutagenic potential of keropok lekor, particularly in its fried variant, which displayed higher mutagenic activity in the absence of S9 metabolic activation. The findings indicate that traditional high-temperature cooking methods may contribute to the formation of mutagenic compounds, with significant dose-response effects observed as concentrations increased. While the mutagenicity detected without metabolic activation highlights the need for caution in frequent consumption of fried keropok lekor, the use of S9 activation suggests that these risks may be mitigated under conditions simulating human metabolism. Given the popularity of keropok lekor and its cultural significance, further studies assessing various preparation methods and ingredient variations could enhance understanding of potential health implications. These insights lay the groundwork for future research and contribute to establishing safety guidelines that maintain the cultural relevance of this traditional food while safeguarding public health.

Acknowledgement

The authors would like to thank the staff at the Environmental and Occupational Health Laboratory Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for the support for the research. In addition, the authors would like to thank all respondents who volunteered to participate in this study and their cooperation given throughout the data collection process.

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