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# THE EFFECTIVENESS OF LEMONGRASS (*Cymbopogon citratus*) OIL AS AN ANTIMICROBIAL ADDITIVE IN KEROPOK LEKOR

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## ABSTRACT

*Keropok lekor* is a popular and highly savoured fish product in Malaysia. *Keropok lekor* has a short shelf life whereby it can be stored at room temperature for only one day. The aim of this study was to develop *keropok lekor* incorporated with lemongrass oil as an antimicrobial additive. In this study, *keropok lekor* were incorporated with lemongrass oil at different formulations: 0.8%, 1.2%, and 0% (control). The microbiological analysis (total plate count, yeast and mould, and coliform count) were conducted for samples stored at room temperature (25±2°C) for four days. The moisture content and sensory analysis were also evaluated. The results showed that the *keropok lekor* made using 1.2% lemongrass oil had significantly longer shelf life (4 days) when compared to control (1 day). In addition, significant reduction in total plate count, yeast and mould count, and moisture content were observed in this formulation ($p<0.05$). There were no coliforms detected in all samples. For sensory analysis, all samples recorded the same score for overall acceptability. Overall, *keropok lekor* made using 1.2% lemongrass oil was the best formulation. The results obtained support the use of lemongrass oil as a potential antimicrobial additive in *keropok lekor.*

**Key words:** *Keropok lekor,* lemongrass oil, antimicrobial additive, shelf life, sensory analysis

## INTRODUCTION

*Keropok lekor* or also known as fish cracker is one of the traditional snacks and a heritage of Malaysia. The main ingredients for making *keropok lekor* are tapioca flour, fish meat, and some seasoning. Good quality *keropok lekor* must have sufficient expansion on puffing for crispness, as well as low moisture content and low oil absorption (Nor-Khaizura *et al.,* 2010; Karrila, 2011). *Keropok lekor* is highly perishable due to microbial spoilage, thus it should be preserved appropriately to prolong its shelf life. Freshly made *keropok lekor* stored at room temperature is best consumed within one day. The signs of spoilage for this food include sliminess and the formation of spots on the surface, as a result of the bacterial growth (Nor-Khaizura *et al.,* 2009; Nor-Khaizura *et al.,* 2010). Recently, it was reported that microbial safety of food in terms of food contamination and spoilage by microorganisms are major concerns of consumers and food industries (Zulfa *et al.,* 2016).

Since a few decades ago, the antimicrobial properties of herbs and spices have been admitted and exploited for preservation of foods. The antimicrobial constituents of herbal medicines are usually found in the essential oil fraction, which commonly comprise of terpenoids and volatile simple compounds (Meyer *et al.,* 2002). Thus, treatment using plant-based medicine appears to be an alternative approach due to the adverse effects associated with the use of synthetic drugs (Mirghani *et al.,* 2012).

*Cymbopogon citratus*, commonly known as lemongrass, is a well-known species of this genus. Lemongrass oil is a volatile oil obtained from the lemongrass leaves, and has lemon-like odour. Lemongrass oil is mainly composed of citral which is a potential natural biocide used as a disinfectant. Previous studies on the antimicrobial activities of lemongrass oil reported its potential antibacterial, antifungal and many beneficial pharmacological

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effects (Tzortzakis, 2007; Vazirian *et al.,* 2012; Zulfa *et al.,* 2016). In addition to its antimicrobial activity, the safety of lemongrass oil makes it a good candidate as a natural antimicrobial additive in food products (Shah, 2011; Vazirian *et al.,* 2012).

In Malaysia, *keropok lekor* has been gaining popularity among health-conscious consumers as it is made traditionally by local producers (small scale industrialists). Therefore, this study was undertaken as part of efforts to evaluate the effects of lemongrass oil on the microbial content and sensory quality of *keropok lekor*.

## MATERIALS AND METHODS

### Preparation of *keropok lekor*

The materials needed to prepare *keropok lekor* are fish meat (Yellowstripe scad, *Selaroides leptolepis*), starch flour, salt, sugar, water and lemongrass oil (food grade). All the materials were purchased from local markets in Shah Alam (Selangor, Malaysia). *Keropok lekor* was prepared using lemongrass oil at different formulations. The samples include control (0% lemongrass oil), formulation 1 (0.8% lemongrass oil) and formulation 2 (1.2% lemongrass oil). The *keropok lekor* was prepared according to Wan-Md-Hatta (2015). *Keropok lekor* was boiled at 100°C until all the starch in the dough has gelatinized. The finished product was packed in a zip-lock plastic bag for further analysis.

### Microbiological analysis and shelf life study

The 3M Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis (3M Food Safety, 2010). *Keropok lekor* was stored at room temperature (25 ± 1°C) in zip-lock plastic bags for four days. Microbiological analysis of *keropok lekor* during storage was conducted daily by enumeration of total plate count, yeast and mould count and coliform count using Petrifilm plates (3M Center, MN, USA) according to Santhirasegaram *et al.* (2013). The results were expressed as log colony-forming units (CFU/g). The shelf life of *keropok lekor* was evaluated according to APHA (2001) and Nor-Khaizura *et al.* (2010).

### Moisture content

The moisture content of *keropok lekor* was analysed on dry matter basic using standard AOAC method (AOAC, 2002).

### Sensory analysis

The sensory evaluation of fried *keropok lekor* was carried out by distributing a simple questionnaire, as described by Santhirasegaram *et al.* (2015). The target evaluators were 100 semi-trained panellists from Management and Science University. The attributes that were evaluated are colour, aroma, taste, texture and overall acceptance of the *keropok lekor*. The attributes were based on hedonic scale from one (dislike extremely) to nine (like extremely).

### Statistical analysis

Data obtained were subjected to statistical analysis using SPSS 22.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard deviation (SD). The significant differences between mean values of *keropok lekor* samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (honestly significant difference) test at a significance level of $p<0.05$.

## RESULTS AND DISCUSSION

### Microbiological analysis

The data presented in Figure 1, 2 and 3 clearly shows the total plate count, yeast and mould, and coliform count of *keropok lekor* incorporated with lemongrass oil at different formulations (0%, 0.8% and 1.2%) during storage at room temperature (25 ± 1°C). Significant reduction in total plate count (5.20±0.1 log CFU/g) was observed in formulation 2 (1.2% lemongrass oil) when compared to control (10.91±0.2 log CFU/g). This proved that lemongrass oil showed potent antibacterial activity against contaminating bacteria (Jay, 2000). According to Adegbegi *et al.* (2012), lemongrass contains active components such as phenols, flavonoids, alkaloids, and terpenes. These compounds are widely being reported to possess hydrophobic characteristic that enable them to partition in the lipid component of bacterial membrane, thus causing membrane disruption. Additionally, these compounds affect the genetic material of microorganisms, therefore inhibiting the growth of microorganisms (Zulfa *et al.,* 2016).

Similarly, significant reduction in yeast and mould count (2.82±0.1 log CFU/g) was observed in formulation 2 (1.2% lemongrass oil) when compared to control (4.83±0.2 log CFU/g). The results showed that *keropok lekor* treated with higher concentration of lemongrass oil (1.2%) exhibited higher reduction of yeast and mould count followed by the lower concentration of lemongrass oil (0.8%). It is possible that the antifungal activity of the lemongrass oil is due to the antioxidants present such as phenolic compounds. Correspondingly, Shaaban *et al.* (2010) reported that phenols in lemongrass oil play an important role as antifungal agent in yogurt. In addition to its antimicrobial activity, lemongrass

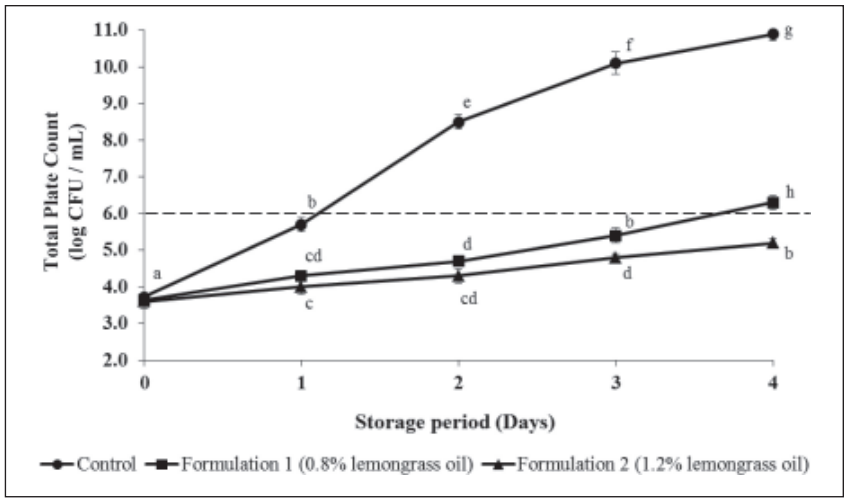


**Fig. 1.** Total plate count of *keropok lekor* samples during storage at 25°C.
Values followed by different letters are significantly different ($p < 0.05$); The dashed line (–) indicates the limit of microbial shelf-life; CFU, colony-forming unit.

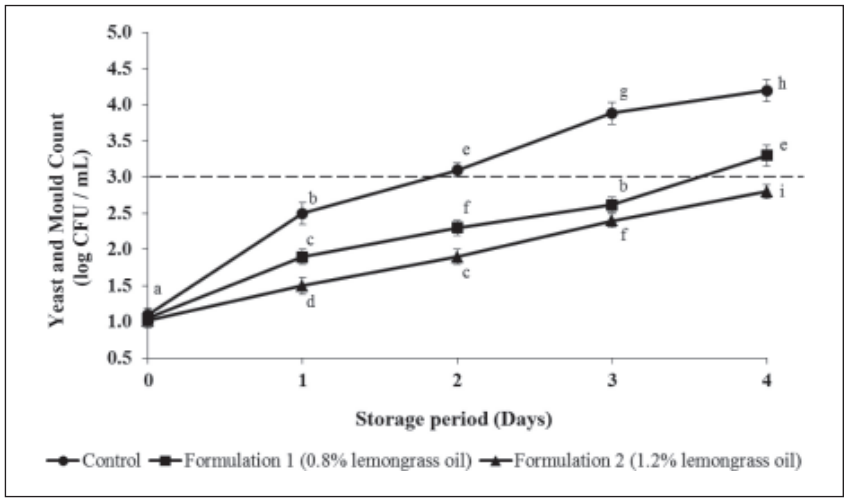


**Fig. 2.** Yeast and mould count of *keropok lekor* samples during storage at 25°C.
*Values followed by different letters are significantly different ($p < 0.05$); The dashed line (–) indicates the limit of microbial shelf-life; CFU, colony-forming unit.

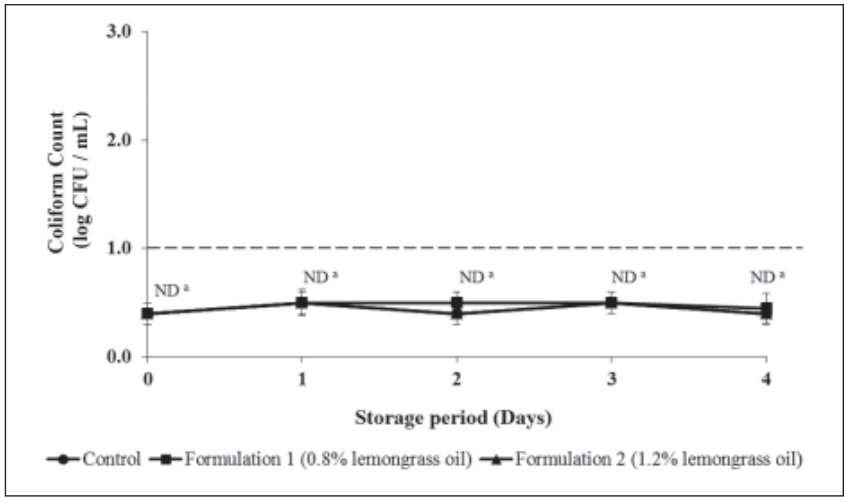


**Fig. 3.** Coliform count of *keropok lekor* samples during storage at 25°C.
*Values followed by different letters are significantly different ($p < 0.05$); The dashed line (–) indicates the limit of microbial shelf-life; CFU, colony-forming unit; ND, not detected.

essential oil has several medicinal properties and potential health benefits, which make it a good candidate as a natural antimicrobial preservative in food products (Vazirian *et al.,* 2012).

There were no coliforms detected (< 1 log CFU/g) in *keropok lekor* samples. The existence of coliform bacteria may not necessarily indicate a direct fecal contamination of the product, but more precisely as an indicator of poor hygiene and sanitary practices during processing and further handling. Therefore, the absence of coliforms in *keropok lekor* samples indicated good sanitary conditions during processing and no cross-contamination (Huss *et al.,* 2000; Nor-Khaizura *et al.,* 2010). The absence of coliform indicates that the samples were free from faecal contamination.

**Shelf life study**

Shelf life of *keropok lekor* samples were evaluated according APHA (2001) and Nor-Khaizura *et al.* (2010). The acceptable maximum microbial load was selected according to preliminary studies on *keropok lekor* to achieve quality retention and avoid microbial spoilage. Hence, the total plate count, yeast and mould count and coliform count limits of fish product (*keropok lekor*) is 6 log CFU/g, 3 log CFU/g and 1.0 log CFU/g, respectively. The rate of microbial growth observed in control was higher than *keropok lekor* incorporated with lemongrass oil during storage. The shelf life of *keropok lekor* prepared using formulation 2 (1.2% lemongrass oil) was the longest (4 days) when compared to formulation 1 (3 days) and control (1 day), in terms of microbial load limit. Thus, this indicated that the *keropok lekor* incorporated with 1.2% lemongrass oil did not undergo spoilage, and is safe to be consumed after 4 days of storage (Jay, 2000; Nor-Khaizura *et al.,* 2010).

**Moisture content**

The moisture content of *keropok lekor* incorporated with lemongrass oil at different formulations (0%, 0.8% and 1.2%) is shown in Table 1. The control exhibited the highest moisture content (63.7±0.5%) when compared to formulation 1 and formulation 2 which are 6.22 ± 0.02% and 6.20 ± 0.03%, respectively. Low moisture content is associated to low water activity in *keropok lekor*, thus ensuring the stability and extending the shelf life of *keropok lekor* during storage (Karrila *et al.,* 2011).

**Sensory evaluation**

The sensory evaluation of *keropok lekor* incorporated with lemongrass oil at different formulations (0%, 0.8% and 1.2%) is shown in Table 2. The sensory evaluation of the *keropok lekor* samples was carried out after the deep frying process. Sensory attributes are of great importance to measure consumer attitudes and their influence on food choice and acceptability (Santhirasegaram *et al.,* 2015). The overall result showed that there were no significant differences in the sensory attributes (colour, aroma, taste, texture and overall acceptance) after the incorporation of lemongrass oil. Eventhough, lemongrass oil has a strong lemon like odour (Vaibhav *et al.,* 2013), the panellists were unable to detect the difference in aroma within the

**Table 1.** Moisture content of *keropok lekor* samples

| Parameter | Control (0% lemongrass oil) | Formulation 1 (0.8% lemongrass oil) | Formulation 2 (1.2% lemongrass oil) |
|---|---|---|---|
| Moisture content (%) | 63.7 ± 0.5[a] | 61.1 ± 0.2[b] | 58.2 ± 0.4[c] |

*Values followed by different letters within the same row are significantly different (p< 0.05).

**Table 2.** Sensory analysis of *keropok lekor* samples

| Parameter | Control (0% lemongrass oil) | Formulation 1 (0.8% lemongrass oil) | Formulation 2 (1.2% lemongrass oil) |
|---|---|---|---|
| Colour | 6.57 ± 1.63 [a] | 6.57 ± 1.57 [a] | 6.40 ± 1.79 [a] |
| Texture | 6.07 ± 1.66 [a] | 6.37 ± 1.59 [a] | 6.23 ± 1.68 [a] |
| Aroma | 6.27 ± 1.57 [a] | 6.43 ± 1.67 [a] | 6.43 ± 1.61 [a] |
| Taste | 6.37 ± 1.45 [a] | 6.13 ± 1.28 [a] | 6.50 ± 1.91 [a] |
| Overall acceptability | 6.60 ± 1.57 [a] | 6.30 ± 1.37 [a] | 6.47 ± 1.66 [a] |

*Mean values followed by different letters within the same row are significantly different (p< 0.05); Score: 1: dislike extremely, 2: dislike very much, 3: dislike moderately, 4: dislike slightly, 5: either like or dislike, 6: like slightly, 7: like moderately, 8: like very much, 9: like extremely.

samples after the frying process. Thus, the levels of score indicated the importance of lemongrass oil in maintaining the sensory attributes of *keropok lekor* samples. Similar results were reported by Shaaban (2010) whereby lemongrass oil did not induce any significant changes in the sensory attributes of yoghurt.

## CONCLUSION

From the study, *keropok lekor* incorporated with 1.2% lemongrass oil showed the best results for significant reduction of total plate count, and yeast and mould count. This formulation also showed the lowest moisture content and absence of coliforms. The results showed that the *keropok lekor* made using 1.2% lemongrass oil had significantly longer shelf life (4 days) when compared to control (1 day). All samples recorded the same mean score of overall acceptability. By taking into consideration all the parameters studied, *keropok lekor* incorporated with 1.2% lemongrass oil was the best formulation. Thus, the results obtained recommended the usage of lemongrass oil as antimicrobial additive in food product can be utilized in the food industry to prevent the use of synthetic additive.

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