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Characterization of used cooking oil (UCO) and orange peels as the medium of insect repellent

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Abstract

Used cooking oil (UCO) is abundant waste oil in Malaysia, due to its daily use in frying food. The proper way to dispose UCO is yet to be found and creating awareness among Malaysians on disposing and recycling UCO required a long period of time. Therefore, alternative needs to be taken to solve this issue by transforming UCO into a value-added product such as insect repellent. Thus, the objective of this research is to investigate the presence of active functional chemical compounds in UCO and orange (*Citrus sinensis*) peels powder (limonene) that would help to transform UCO into a repellent. Based on GC-MS analysis, there was presence of fatty acids in UCO such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), myristic acid (C14:0) and capric acid (C10:0). FTIR analysis has proven that orange (*Citrus sinensis*) peels powder contained limonene compound due to presence of alkene (C=C) group. The results of UCO and orange peels analysis, as well as comparison made with previous studies show that UCO and orange peels powder are potential to be the medium of insect repellent.

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1.0 Introduction

Used cooking oil (UCO) is the oil that is being reused several times for cooking. Since Malaysian food is mostly prepared by frying, the consumption of cooking oil will never be null. UCO which is expected to undergo treatment to avoid detrimental effects to the human health and/or to the environment is being disposed of by consumers via sinks, bins, drains, toilet bowls, or directly into the immediate water bodies and lands (Kabir et al., 2014). It was found that UCO contains a few similar properties with an insect repellent. Insect repellent is defined as solutions which avoid insects from approaching a certain area which contains it. Both insect repellent and UCO contain fatty acids. Fatty acids can be considered as a potential important aerosol surfactant and it acts as an organic coating too. The main reported organic coatings are fatty acids of different carbon chain length which is up to the C_{32} in continental aerosols (Li et al., 2017).

Furthermore, another important raw material of this insect repellent is orange fruit peels. The chemical compound that is responsible to repel insects is limonene (Zewde and Jember, 2010). Orange fruits accumulate monoterpene limonene at high levels in the oil glands of their fruit peels (Rodriguez et. al., 2011). Apart from orange peels, a few essential oils and household remedies which were found to be good in repelling insects are cassia oil, pennyroyal, cedar, lavender, eucalyptus, peppermint, castor oil, menthol, nutmeg, crushed pepper, lemon juice and leeks (Paluch et al., 2010). Based on all the ingredients stated, only orange peels can be categorized as waste.

The downside of essential oils is the process to extract this oil from the origin plant is quite tedious. The processes which are usually used are hydro-distillation, supercritical fluid extraction, microwave-assisted hydro-distillation and ultrasound-assisted extraction (Raseem et al., 2016).

In this study, conventional process was preferred. Hence, the peels were turned into powder before being analyzed. Conventional process means a process that is commonly used without the need of any advanced equipment. Thus, this research was formulating both waste; UCO and orange peels, to be made into the final product; insect repellent. Then, Gas-Chromatography-Mass Spectrometry (GC-MS) analysis of UCO was done to determine the chemical compound that is responsible in making UCO a suitable medium as an insect repellent. Fourier Transform Infrared Spectroscopy (FTIR) was used to analyse the orange peels powder in order to determine limonene, which is known to be a chemical compound which can react as insect repellent.

2.0 Methodology

2.1 Materials

Three samples (200ml ± 50ml) were collected from households which used cooking oil to fry fish chips, potato chips and fresh fish. Each sample was labelled as Sample 1, Sample 2 and Sample 3 and stored in a 500ml dry drinking bottle. Four orange fruits *(Citrus sinensis*) were obtained from a hypermarket and were peeled. The size of the peels was random.

2.2 Pre-treatment of UCO

The samples of UCO were filtered as the first step using a kitchen sieve to remove any food particles in the oil.

2.3 GC-MS analysis

The GC-MS used was in Instrument Laboratory II at Faculty of Chemical Engineering, UiTM. Each sample was pipetted into blanks and labelled as Sample 1, Sample 2, and Sample 3. The GC-MS was equipped with flame ionization detector (FID). The temperature of both injector and detector was set to 250ºC. A ramp method was used as a part of the GC-MS analysis. The initial oven temperature was 70ºC and the sample was injected by auto-injector. The oven temperature was held at 70ºC for 2 minutes after the sample injection. The oven temperature was then ramped from 70ºC to 210ºC with the rate of 40 °C/min and from 210ºC to 230ºC at a rate of 7 °C/min. The oven temperature was held at 230ºC for 11 minutes to remove any remaining traces of the sample. After the sample run was over, the oven temperature was cooled back to 70ºC so that the next sample runs could be started (Abidin et al., 2013).

2.4 Drying of orange peels

The peels were scattered on a piece of aluminium foil with the inner part of the peels facing upwards since this part had the most moisture content. A universal oven was used with the temperature of 60 ± °C The sample weight was measured before and after each drying period. Each weighing process was done in triplicates and the average reading was taken. The sample was weighed from time to time to obtain the optimum percent of the moisture removed. The drying process stopped when the moisture content difference was found to be approximately ± 0.1 wt. %.

The formula used to calculate the percentage of moisture content being removed (M%) from the sample is as shown in Eq. (1):

$$M\ (\%) = \frac{\text{Original weight of sample-Dried weight of sample}}{\text{Original weight of sample}} \times 100\% \quad (1)$$

2.5 Grinding and sieving powder

The dried orange peels were then ground to a fine powder using a laboratory blender. The blender was set to a low speed for 3 minutes. The powder was then stored in an aluminium foil and kept in a clean plastic container at room temperature. Using a woven wire mesh sieve, the powdered sample was separated based on sizes of 125, 200 and ≥ 300 µm. This process was to determine the range size of the powder. The sieving process operated with the amplitude of 9 for 5 minutes.

2.6 Fourier-transform infrared spectroscopy (FTIR) analysis

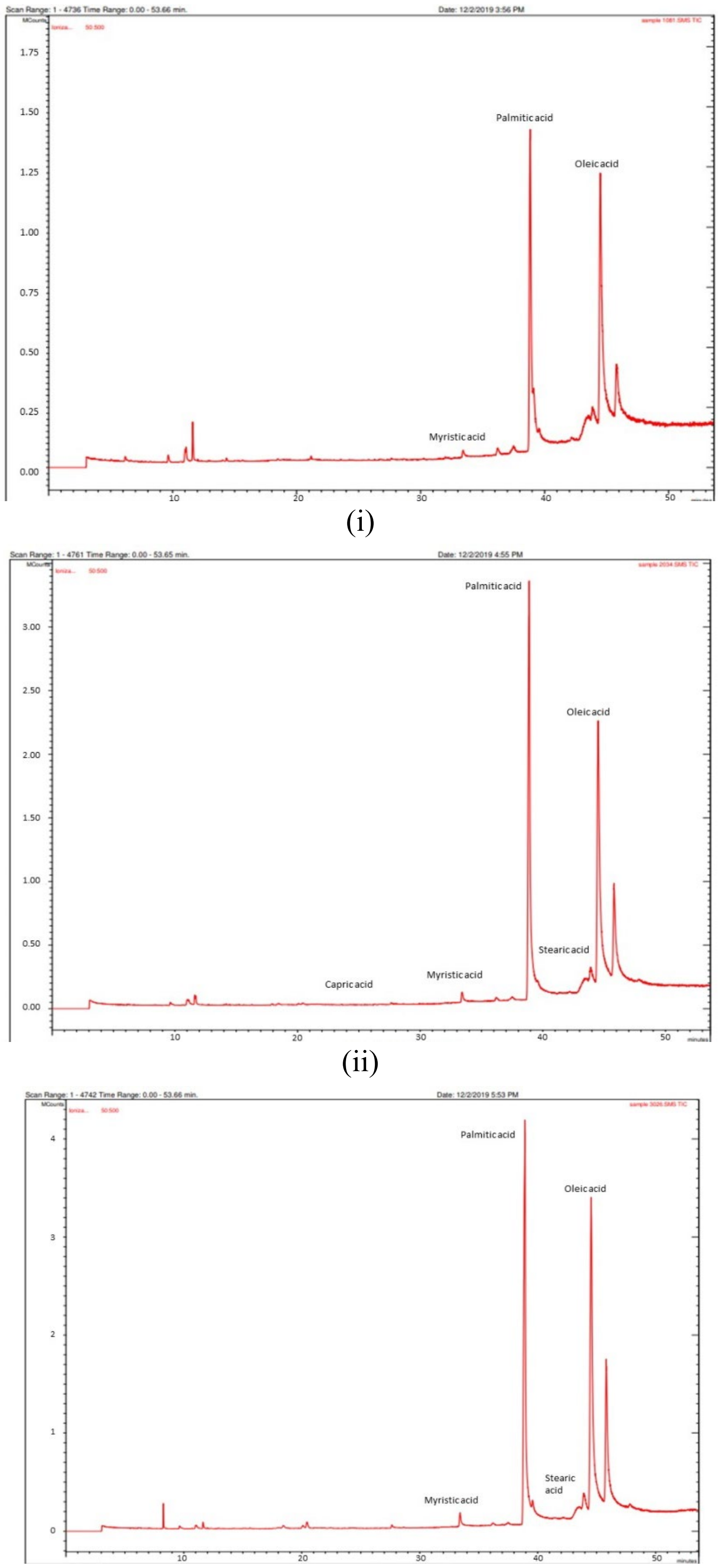
FTIR analysis is important to determine the presence of limonene in the orange peels powder. This analysis was done by using Perkin-Elmer spectrometer. It was equipped with diamante crystal for attenuated total reflection (ATR). The FTIR spectra were smoothed and their baselines were corrected using the "automatic smooth" and the "automatic baseline correct" functions of the built-in software of the spectrophotometer. Then, the intensities of the interesting peaks were measured (Badulescu et al., 2010).

3.0 Results and discussion

3.1 GC-MS analysis on UCO

The purpose of this analysis was to determine the presence of fatty acids found in the UCO samples. Fig. 1 shows the chromatography plots of each sample with their respective presence of fatty acids. Capric acid (C10:0) appeared at retention time of 27.6285 min for Sample 2 only. Palmitic acid (C16:0) was found to appear in Sample 1, Sample 2, and Sample 3 at retention time of 38.8371 min, 38.9904 min and 38.8997 min, respectively. Furthermore, stearic acid (C18:0) appeared at retention time of 38.8582 min in Sample 2 while at 43.9409 min in Sample 3. This is followed by oleic acid (C18:1) which appeared at the retention time of 44.5112 min, 44.4849 min and 44.6311 min for

Sample 1, Sample 2 and Sample 3, respectively. Myristic acid (C14:0) appeared in all three samples, wherein it appeared at the retention time of 33.4078 min for Sample 1, 33.4156 min for Sample 2, and 33.4211 min for Sample 3. Hence, it could be concluded that palmitic acid (C16:0), oleic acid (C18:1) and myristic acid (C14:0) were present in all the three UCO samples.

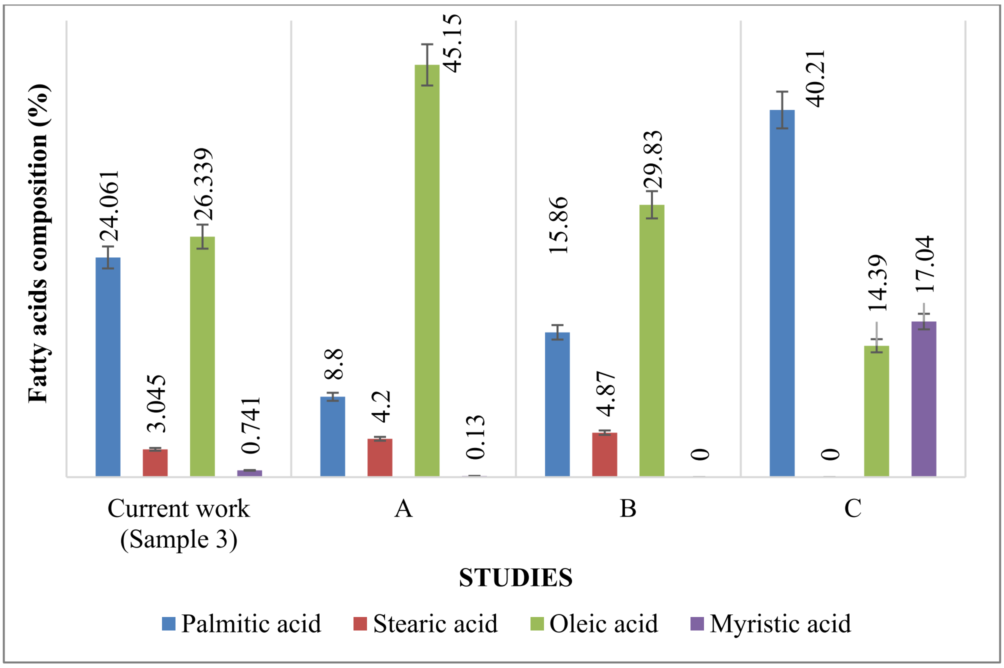


(iii)

Fig. 1: Chromatography plots of UCO analysis for (i) Sample 1, (ii) Sample 2 and (iii) Sample 3

Table 2: Composition of fatty acids in each sample with their respective areas.

Compound	Sample 1 Area (10^7)	Sample 1 Approximate composition (% v/v)	Sample 2 Area (10^7)	Sample 2 Approximate composition (% v/v)	Sample 3 Area (10^7)	Sample 3 Approximate composition (% v/v)
Capric acid	-	-	0.017	0.105	-	-
Palmitic acid	1.313	13.290	0.046	0.281	4.864	24.061
Stearic acid	-	-	3.877	23.806	0.616	3.045
Oleic acid	1.882	19.052	3.978	24.431	5.324	26.339
Myristic acid	0.036	0.361	0.097	0.593	0.150	0.741
Total		**32.703**		**49.216**		**54.186**



Current work (Sample 3)
(A)- Bautista, Vicente and Pacheco (2009)
(B)- Banani, Youssef, Bezzarga and Abderrabba (2015)
(C)- Awogbemi, Onuh and Inambao (2019)

Fig. 2 Comparison of fatty acids composition with other studies using data from Sample 3.

Table 2 summarizes the fatty acids composition in the three samples. It clearly shows that sample 1 consists of 32.703% (v/v) of total fatty acids while sample 2 contained 49.216% (v/v) of fatty acids from the total composition. On the other hand, sample 3 consists of 54.186% (v/v) of fatty acids. The remaining composition in each sample belonged to other components other than fatty acids. For sample 1, oleic acid content was made up of 19.052% v/v from the total composition followed by palmitic acid (13.29% v/v) and myristic acid (0.361% v/v). As for Sample 2, the decreasing order of fatty acids composition is oleic acid (24.431% v/v), stearic acid (23.806% v/v), myristic acid (0.593% v/v), palmitic acid (0.281% v/v) and capric acid (0.105% v/v). Lastly, Sample 3 contained four types of fatty acids which are oleic acid (26.339% v/v), palmitic acid (24.061% v/v), stearic acid (3.045% v/v), and myristic acid (0.741% v/v). From these compositions, it was concluded that although the UCO samples were used to fry different types of food with distinct nutritional values, fatty acids were still present with the minimum composition of 32.703% v/v.

As shown in Fig. 2, composition of fatty acids from three references was summarized and compared with the result of the analysis of Sample 3. Sample 3 has been chosen as an example because Palmitic acid (C16:0) and oleic acid (C18:1) were found present in this sample as well as in other three analysis results. The standard deviation for each fatty acid was calculated with the basis of each fatty acids mean value. It was found that the values were not in favor since it should be as low as possible. However, as shown in the figure, the standard deviation was found to be between 0.7538 and 11.7002 which was considered fairly high. This happened due to the difference of UCO taken as samples in each study. The past frying activity of UCO affected the final result of fatty acids present and hence, such a high standard deviation was obtained. Nevertheless, the objective of this analysis has been achieved which is to prove the presence of fatty acids in UCO which will be beneficial as the aerosol coating of the repellent (Li et al., 2017).

3.2 Drying, grinding and sieving of orange peels

To ensure that the peels were not burnt, it was observed from time to time and weighed every time it was brought out from the oven. Fig. 3 shows the condition of the orange peels before and after the drying process.

The drying process stopped after 78.5 hours of drying which was when the moisture content was observed to remain constant (± 0.1% w/w). The data obtained showed that the maximum percentage of moisture content that could be removed from the orange peels was 70.54%. To visualize the time duration needed for the orange peels to reach its maximum moisture content reduction, a graph of mass reduction versus time was plotted (Fig. 4).
Once the drying process was completed, the grinding process took place. Then, the powder was sieved to obtain different range in size. The result of this process showed that the size range of the powder was from 125 µm until >300 µm. The size range of the powder is important as it might affect the efficiency of the powder in repelling insects. However, the topic is not discussed in this current work but future research should be done.

(i) (ii)

Fig. 3: Drying orange peels in Universal Oven (i) before and (ii) after.

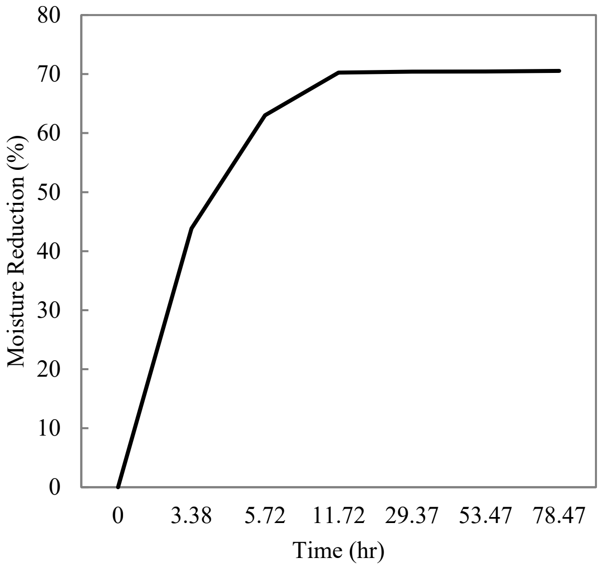


Fig. 4: Graph of moisture reduction of orange peels against time at 60 °C.

3.3 FTIR analysis on orange peels

The purpose of this analysis was to determine the functional group of limonene which is responsible for making this product a success. The IR spectra are shown in Fig. 5 and Table 3 shows the wavenumber of the functional group identified. A broad peak at 3000–3600 cm^{-1} indicated the functional group of hydroxyl which was caused by the stretching of free or −H bonded, −OH groups. It peaked at the wavelength of 3324.92 $cm^{-1}$and proved that the orange peels powder has strong OH-bond. This proved the presence of alcohol group in the peels. On the other hand, alkoxy functional group was found at the peak of 1000-1260 cm^{-1}, precisely at 1016.93 cm^{-1}. Alkoxy group which is bonded to –H will form an alcohol too.

The presence of hydroxyl and alkoxy groups proved that alcohol such as perillyl alcohol exists in citrus peels (Mansoor Al-Saadi et al., 2009). Perillyl alcohol in orange peels is responsible for the aroma of the citrus which also contributes to the insects' repellency.

Besides alkoxy and hydroxyl functional groups, another main peak occurred at 1600-1660 cm^{-1}which was due to the compounds containing C=C group known as alkene. Alkene was found present in orange peels powder because of terpenes. Terpenes are known to function as a solvent which dissolves the rest of the component in the peels (Hao Fan et al., 2015). In addition, the presence of this functional group is the most important part because presence of C=C bond indicated the existence of limonene compound. Limonene is made up of cyclic monoterpenes which are classes of terpenes with the chemical structure of $CH_2=C-CH=CH_2$. Hence, presence of alkene proved the existence of limonene in this orange (*Citrus sinensis*) peels powder. This analysis showed that this powder is a potential insect repellent since limonene was proven present (Dechi and Apeyuan, 2011).

3.4 Repellency test on insects (Comparative study)

Since UCO is the medium for the insect repellent, the effectiveness of the functional compound which helps to repel insects is being paid more attention in this comparative writing on the insects' repellency. The summarized repellency tests on insects based on seven past studies can be seen in Table 4.

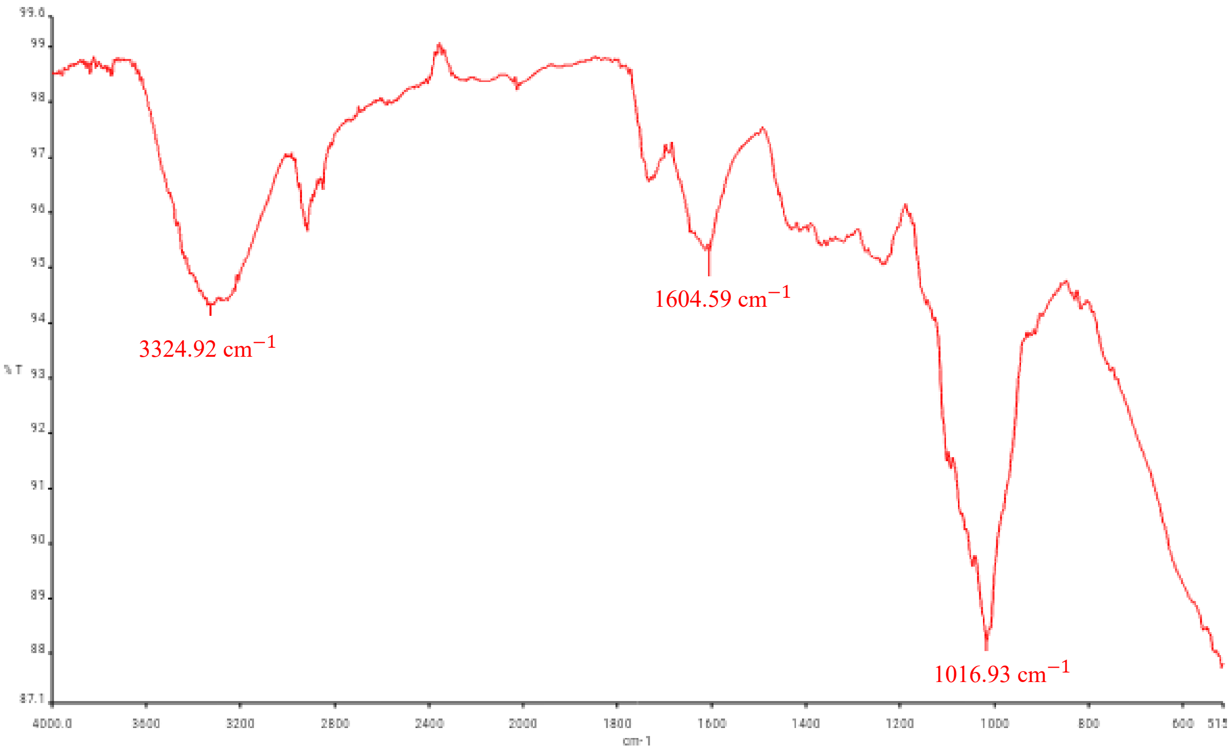


Fig. 5: FTIR spectrum of orange peels powder.

Table 3: Functional group obtained from FTIR spectrum.

Wavenumber (cm^{-1})	Bond (cm^{-1})	Functional group
3324.92	3200-3500	Hydroxyl (O-H)
1604.59	1600-1660	Alkene (-C=C-)
1016.93	1000-1260	Alkoxy (C-O)

Test 1 concluded that the chemical compound hetisine which was one of the substances found in alkaloids has the highest level of repellency (59.12%) against red flour beetles. Hetisine is a diterpene alkaloid which contains two terpene compounds (A. Ulubelen et al., 2001). Terpenes are also found in any citrus essential oil which confirms that *Citrus sinensis* will do well in repelling insects as proven by Test 1. Furthermore, test 2 and test 3 used *Citrus sinensis* powder as the repellent on *Callosobruchus maculatus* and *Zabrotes subfasciatus*, respectively. Both tests proved that the repellent would not only repel the insects but also may act as an insecticide although its efficiency would not be the same level as synthetic insecticides and citrus essential oil. The latter was said to contain high toxicity towards insects due to the presence of d-limonene (Zewde and Jember, 2010).

Both test 3 and test 4 chosen the red flour beetle as one of the insects to carry out the repellency test on. Ukeh and Umoetok (2011) reported that (R)-linalool is to be the major component of the essential oils which contributed to the repellent activity against *T. castaneum* and *R. dominica*. This strengthens the study of insecticidal properties which depending on the total monoterpenoid composition. It was found that the higher the monoterpenoid composition, the higher the level of repellency.

Comparison with N, N- Diethyl-meta-toluamide (DEET) was made in test 5 and it was found that essential oil of *Cymbopogon distans* (common name: lemongrass) which contains limonene, citronellol, citronellal, and trans-geraniol showed the similar repellency effect on insects as the common active ingredient of an insect repellent (Zhang et al, 2011).

Natural compounds citronella and citronellal was proven to repel mosquitoes. Kyu et al, (2005) stated that high dosage of citronella will lead to high repellent efficacy. Citronella is a terpenoid with citronellal as its constituents and terpenoid has been proven to be responsible in repellency (Ukeh and Umoetok, 2011). Lastly, test 7 used different types of oranges for the test which are *Citrus mobilis* and *Citrus medica*. Based on the findings by Harshani and Karunaratne (2019) both Citrus species exhibited good repelling effect and possess developmental suppressive properties. This is most likely due to presence of terpenes which are well-known for its insect repellent function and as insecticide.

Table 4: Summarized repellency tests on insects.

Test	**Repellent**	**Insects**	**Method**	**Results**
1	Diterpenoid and norditerpenoid alkaloids[a]	Red flour beetle (*Tribolium castaneum*)[a]	Four of the insects were exposed to the test area inside a glass ring[a]	Hetisine compound showed the highest levels of repellency (59.37%)[a]
2	*Citrus sinensis* peel powder[b]	Pulse beetle (*Callosobruchus maculatus*)[b]	The powder was mixed with un-infested black-eyed cowpea seeds in a container and five of the insects were introduced into it[b]	High ovicidal and larvicidal effect on the insects[b]
3	*Citrus sinensis* peel powder[c]	Leaf beetle (*Zabrotes subfasciatus*)[c]	Assessed in a choice and with no choice bioassay system in a 'Y' olfactormeter[c]	Orange powder was also effective in reducing the insects adult emergence though not as effective as orange peel oil and pirimiphos-methyl[c]
4	*Aframomum melegueta* and *Zingiber officinale* essential oils [d]	Red flour beetle (*Tribolium castaneum*) and lesser grain borer (*Rhyzopertha dominica* (F.))[d]	Recorded the time spent by each beetle in the different areas of the olfactometer and the number of visits into each area or odour zone[d]	Both pest species spent more time in the control olfactometer arms compared to the treated arms[d]
5	*Cymbopogon distans*[e] essential oil	Booklouse (*Liposcelis bostrychophila*) and red flour beetle (*Tribolium castaneum*)[e]	Twenty insects were released in the centre of each treated filter paper disk and a cover was placed over the Petri dish. Counts of the insects present on each strip were made after 2 and 4 h[e]	Compared with DEET, chemical compounds in the essential oil which are trans-geraniol and (+)-citronellol exhibited the same level of repellency against both insects[e]
6	Citronella and citronellal extract[f]	Mosquitoes (*Culex pipiens pallens*)[f]	Human-bait method was used. Bands, impregnated with citronella and citronellal were fastened in forearm. Data measured by the number mosquitoes' bites[f]	Both extracts have high repellent efficacy against mosquitoes not only in vitro but in field[f]
7	*Citrus nobilis* and *Citrus medica* peel powder[g]	Pulse beetle (*Callosobruchus maculatus*)[g]	Assessed by using a dual-choice olfactormeter made up of two plastic cups connected to a transparent plastic tube to its two ends. Peels were mixed with 60 un-infested cowpea seeds were placed in one cup. beetles were then introduced into the middle hole of the plastic tube. The number of insects that moved into treatment and control was counted[g]	Strong repellent effects of *C nobilis* and *C. medica*. *C. nobilis* showed an increasing trend in repellent activity with increasing exposure time[g]

[a]A. Ulubelen et al, (2001)
[b]A. Dechi and K. Apeyuan, (2011)
[c]Zewde and Jember, (2010)
[d]Ukeh and Umoetok, (2011)
[e]Zhang et al, (2011)
[f]Kyu et al, (2005)
[g]Harshani and Karunaratne, (2019)

For future research, the repellency test should be done by using an olfactormeter. The time of insects spent in the olfactormeter arm should be tested using a one-way analysis of variance (ANOVA) (Ukeh and Umoetok, 2011). The discussed results and comparative studies have a significant effect to the way of disposing UCO. UCO will not be disposed wrongly anymore if this repellent is a success besides consumers are able to get rid of insects at home by using this bio-insect repellent.

4.0 Conclusions

The potential of UCO as feedstock for an insect repellent by combining with orange peel has been examined. The observations have been carried out by analysing the composition and functional groups in UCO and orange peel. The addition of the powder is important as it contains an active chemical compound that repels insects while UCO will act as the medium. It is found that the UCO contains fatty acids; capric acid (C10:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and myristic acid (C14:0). Fatty acids are important as a coating chemical compound for the aerosol. As for the orange peels, the study revealed that orange (*Citrus sinensis*) peels powder consists of limonene compound which is responsible in repelling insects.

Based on past studies, each repellency test was using different types of repellent but the similarity of them with this present work's is that the properties or chemical compounds found in them with the compounds found in *Citrus sinensis* powder. For future research, it is recommended that an alcohol is introduced as a part of the insect repellent medium because alcohol will act as a solvent. It is also suggested that the combination of UCO with orange peels powder to be tested on insects.

As a conclusion, the characterization of both UCO and orange peel as the medium of an insect repellent was successfully carried out. There is a high possibility for the orange peel to work in repelling insects with UCO as the medium since both ingredients contain sufficient chemical compounds that would make the repellent works.

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