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Research Article

Assessing the Stability of Peppermint Oil Encapsulated in Hydrogel Beads

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ABSTRACT

Essential oil mainly contains volatile constituents making it vulnerable upon exposure to the external environment. The encapsulation method is known to protect the bioactive components of the essential oil from damage, in which alginate was used as the hydrogel in this study. This work investigates the physicochemical stability of the peppermint oil encapsulated within alginate beads (1.5% and 2.0%) during its five weeks of storage. Peppermint oil (PO) was added at four different weight ratios to alginate, which were 1:3, 1:2, 1:1, and 2:1. The encapsulation technique involves mixing alginate and oil using a homogeniser. Constituents profiling was done weekly using UV-Vis spectrophotometer and Gas chromatography-mass spectroscopy (GC-MS). Investigation revealed that 1.5% (*w/w*) alginate in a weight ratio of 1:1 has the highest encapsulation efficiency, which was 42.00%, while for 2.0% (*w/w*) alginate, the weight ratio of 1:2 gave a maximum encapsulation efficiency of 33.38%. Assessment of the beads' diameter with time showed little physical changes throughout storage time. The constituents profile of the oil indicates a decline in the chemical constituents between a pure sample and the encapsulated peppermint oil. This might be associated with the heat generated during mixing or exposure to the light during the preparation stage. Even so, the analysis of the encapsulated oil each week suggested no striking changes, indicating the stability of the peppermint oil encapsulated in the alginate beads.

Keywords: Alginate, Encapsulation, Essential oil, Hydrogels, Peppermint oil, Stability

Introduction

The global essential oil market is projected to be worth up to USD 16 billion by 2026 [1]. An increase in demand could also be observed in the domestic market. The application of essential oil covers the medical, pharmaceutical, cosmeceutical and food industries. Terpenes, alkaloids, and phenolics are among the common secondary metabolites in essential oils. These classes of secondary metabolites are the one that benefits humans in terms of food production and packaging, medical treatments, and cosmetics in which these metabolites could be used as sources of antioxidants [2, 3]. Secondary metabolites are known to be selectively produced under specified conditions affecting these organic solutions' total yield. Most secondary metabolites have low extraction yield, affecting this product's market price.

Despite the long list of essential oil benefits, the main impediment of essential oil is its vulnerability to oxidization upon exposure to light and high temperature [4]. The high volatility of essential oil tends to cause degradation of the bioactive compounds, thus reducing its effectivity after a few days of storage and losing its valuable benefits [5]. Overcoming these drawbacks, essential oils are mostly packed in opaque glass bottles, effectively reducing the temperature and light exposure. However, these materials are costly and provide limited protection. Other than tackling these issues through package design, attempts to encapsulate the oils minimized the exposure and losses. These entrapment methods preserve quality prolonging the shelf life altogether.

Encapsulation allows the entrapment of essential oil within the matrix of the material, providing a layer of protection for the bioactive compounds

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of the essential oil from being degraded by the harsh conditions of the external environment [6]. The encapsulated compounds are impermeable to the environment surrounding the capsules [4]. The encapsulation method improves the stability of the encapsulated essential oil, thus preventing loss due to evaporation or oxidation of the essential oil from occurring at a fast rate. Furthermore, the hydrophilic properties of the wall materials help improve the essential oil's hydrophobicity. While encapsulation enables the entrapment of the strong aroma of the essential oil, this method also assists in the controlled release of the essential oil, letting the consumers enjoy the essential oil longer [6, 7]. Alginate is widely used to encapsulate many particles due to its firm physical properties that give chemical and mechanical stability to the forming beads [4]. Alginate is a type of anionic polysaccharide which is naturally synthesized by brown algae (also called seaweed), a type of algae categorized under the class of Phaeophyceae [8]. Alginate mainly consists of 1,4-linked β-D-mannuronate (M) residues, 1,4-linked α-L-guluronate (G) residues, or alternate sequences of mannuronate and guluronate units as in Figure 2.4 [9, 10]. Alginate is cross-linked with divalent and trivalent ions such as Ca^{2+}, Mg^{2+}, Sr^{2+}, and Al^{3+} [11, 12]. However, only guluronic alginate successfully creates alginate hydrogel because of the interaction between the G residues and calcium ions Ca^{2+} [8]. The interaction between Ca^{2+} and G residues would form gels that are known as "egg-box" dimers, illustrated in [10]. This chemically set method involving no heat makes alginate superior as the hydrogel of choice in many fields as most biological samples are known to be sensitive and vulnerable to any thermal fluctuation during product development processes.

Many studies found that different alginate concentration affects the gel strength. This anionic polymer strength is affected by parameters such as pH, ionic strength and the chemical properties of the encapsulated material. However, different ratios of essential oil being loaded in the alginate could affect gel strength and the encapsulation efficiency of the gel system. As the main purpose of the encapsulation method is to protect the essential oil, many studies have yet to assess the stability of the encapsulated essential oil. If proven effective, hydrogel such as alginate may be commercialized on its ability to maintain the stability of the essential oil for a longer time. Hence, this study aimed to investigate encapsulation efficiency together with the stability of the peppermint oil throughout storage time.

Material and Methods

Formation of alginate beads with encapsulated peppermint oil (PO)

For the extrusion of the alginate beads, 96-wells caviar dropper kit was used. The caviar syringe was clamped at a distance from the surface of the 1.5% (*w/w*) calcium chloride solution and pushed to extrude the alginate droplets in a container of 1% calcium chloride at a constant speed and force using hands. The formulation ratios are 0:1, 1:3, 1:2, 1:1 and 2:1. The droplets were left in the calcium chloride solution for 10 minutes and sieved using a plastic sieve. The wet beads were left dried for 5 minutes before being put in a labelled plastic petri dish and kept chilled in the refrigerator at 4°C.

Determination of the size of alginate beads

The diameter of the alginate beads was measured using a digital vernier calliper. For each sample, the diameter was taken from the longest diameter of the formed spherical beads as some of the alginate beads. Data collection was done in triplicate for each week for five weeks.

Determination of the amount of encapsulated PO using ultraviolet-visible spectrophotometer (UV-Vis spectrophotometer)

Beads for each weight ratios of PO: alginate was weighed. 10 mL of sodium citrate was the added to the beads. Mixture was vortexed for 15 to 20 minutes until the alginate beads completely disintegrated. The vortexed solution (1 ml) was pipetted into the cuvette. The blank used was sodium citrate, which was the solution used to digest the beads. The absorbance of the solution was measured using UV-VIS spectrophotometer at the wavelength of 270 nm. Encapsulated were quantified using the standard curve.

Determination of encapsulation efficiency

The encapsulation efficiency (EE) of the PO encapsulated in the alginate beads for each weight ratios of PO: alginate was calculated using equation 1.

$$(\text{EE}\%) = \frac{\text{Mass of encapsulated PO (g)}}{\text{Initial mass of PO (g)}} \times 100\% \quad \ldots 1$$

Gas chromatography-mass spectroscopy (GC-MS) analysis

For GC-MS analysis, the GC (model Clarus^{TM} 680 of the Perkin Elmer brand) coupled to MS (Clarus^{TM} SQ 8T MS) was used to identify the compounds in the extracted PO. The Elite-5MS capillary column of the Perkin Elmer brand (30 m length × 0.25 mm × 0.25 µm film thickness) coated with fused-silica was used in the GC to separate the components of PO. Helium gas was used as the carrier gas.

During the analysis, 1 µl of the sample was injected into the GC. The operating conditions of the GC-MS were as in Table 3.4. The retention time and chemical composition mass spectra of the detected elements were compared with the standard PO and the National Institute of Standards and Technology) library. The quantitative analysis of the compounds was done using equation 2 based on the peak area of the compound. The percentage of the peak area represented the amount of the compound present in the peppermint oil.

$$\text{Area (\%)} = \frac{\text{Peak area of the compound}}{\text{Total area of the compounds}} \times 100\% \quad \ldots 2$$

Statistical analysis

Data were analyzed using SPSS software version 26. The results were recorded as mean ± standard deviation. An independent sample t-test was used to get the mean diameter of the empty beads after extrusion. Two-way ANOVA analysis was conducted to check for the significant difference. The post-hoc test was done using the Tukey and least significant difference (LSD) at the significant level of $p < 0.05$.

Results and Discussion

Characterization of the alginate PO-loaded beads

Alginate is a versatile hydrogel whose physical properties (viscoelasticity) could be tailored specifically for applications. Viscoelasticity could be toggle by manipulating parameters such as pH, ratio of compositions mainly guluronic and mannuronic acid, molecular polymer concentration [8]. An increase in hydrogel concentration will directly affect the water holding capacity, which could consequently influence the bead size and diameter. Table 1, indicates a significant difference between the alginate concentration and the diameter of the PO-loaded beads. Analysis revealed the mean diameter of the 1.5% (*w/w*) beads was 3.36 ± 0.23 mm while for 2.0% (*w/w*) beads, the mean diameter was 3.70 ± 0.20 mm, which the latter produced larger beads than the former. In agreement to [10], the size of the beads increased as the alginate concentration increased from 0.5% (*w/v*) to 1.5% (*w/v*) and the beads formed were more spherical. However, in Table 2, findings revealed no significant differences were seen in the diameter observed within the PO ratios in both alginate concentrations. This research work also preceded in observing the size changes through storage week (5 weeks). As shown in Table 3, beads containing PO appears cloudy as compared to empty beads resultant from the presence of hydrophobic elements which might cause some internal entanglement during the cross-linking or polymerization process [13]. Recorded diameter throughout storage time shows no significant physical changes. Minor physical changes considered a desirable trait which signifies stability and longer storage shelf life.

Table 1. The quantitative results determining the relationship between the alginate concentration and/or weight ratios with the diameter of the alginate.

Alginate concentration (*w/w*)	Weight ratios of the PO: alginate	Diameter (mm)*
1.5%	1:3	3.15 ± 0.15^{acd}
	1:2	3.40 ± 0.21^{acd}
	1:1	3.30 ± 0.15^{acd}
	2:1	3.56 ± 0.29^{acd}
2.0%	1:3	3.71 ± 0.02^{bcd}
	1:2	3.66 ± 0.09^{bcd}
	1:1	3.88 ± 0.39^{bcd}
	2:1	3.67 ± 0.08^{bcd}

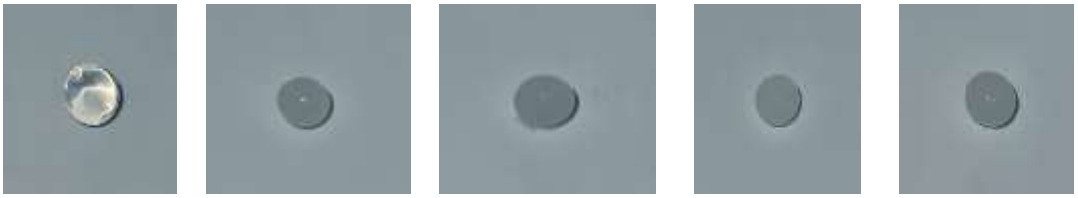
Notes: Statistical analysis using two-way ANOVA, Tukey statistical test (95% confidence interval and $p < 0.05$ were expressed using alphabetical superscript.

Table 2. The quantitative results determining the relationship between the alginate concentration and/or weight ratios and/or weeks with the diameter of the PO-loaded beads.

Alginate concentration (*w/w*)	Weight ratio	Diameter (mm)*				
		Week 1	Week 2	Week 3	Week 4	Week 5
1.5%	1:3	3.15 ± 0.15^{acfg}	3.01 ± 0.12^{acf}	3.09 ± 0.15^{acfg}	3.31 ± 0.08^{acg}	3.46 ± 0.18^{acg}
	1:2	3.40 ± 0.21^{acdfg}	3.34 ± 0.08^{acdf}	3.26 ± 0.29^{acdfg}	3.37 ± 0.19^{acdg}	3.41 ± 0.21^{acdg}
	1:1	3.30 ± 0.15^{adefg}	3.19 ± 0.17^{adef}	3.23 ± 0.02^{adefg}	3.41 ± 0.10^{adeg}	3.44 ± 0.25^{adeg}
	2:1	3.56 ± 0.29^{aefg}	3.58 ± 0.06^{aef}	3.62 ± 0.07^{aefg}	3.46 ± 0.19^{aeg}	3.51 ± 0.17^{aeg}
2.0%	1:3	3.71 ± 0.02^{bcfg}	3.51 ± 0.12^{bcf}	3.71 ± 0.14^{bcfg}	3.82 ± 0.08^{bcg}	3.87 ± 0.20^{bcg}
	1:2	3.66 ± 0.09^{bcdfg}	3.54 ± 0.14^{bcdf}	3.55 ± 0.16^{bcdfg}	3.69 ± 0.15^{bcdg}	3.65 ± 0.16^{bcdg}
	1:1	3.88 ± 0.39^{bdefg}	3.83 ± 0.07^{bdef}	3.95 ± 0.25^{bdefg}	3.71 ± 0.16^{bdeg}	3.95 ± 0.07^{bdeg}
	2:1	3.67 ± 0.08^{befg}	3.47 ± 0.09^{bef}	3.72 ± 0.12^{befg}	3.83 ± 0.20^{beg}	3.59 ± 0.13^{beg}

Notes: Statistical analysis using two-way ANOVA, Tukey statistical test (95% confidence interval and $p < 0.05$ were expressed using alphabetical superscript.

Table 3. Empty and PO-loaded alginate beads at different weight ratios and alginate concentrations.

Alginate (*w/w*)	Weight ratio of PO to alginate				
	0:1 (empty)	1:3	1:2	1:1	2:1
1.5%					
2.0%					

Encapsulation efficiency (EE) of the PO loaded beads

Table 4 depicted that 1.5% (*w/w*) alginate has significantly higher EE as compared 2% (*w/w*). Total EE for all PO ratio at 1.5% (*w/w*) ranges from 27.62% to 42.00% as compared to 2.0% (*w/w*) that could only encapsulate maximally 33.85%. The mean EE for 1.5% (*w/w*) alginate was 35.62 ± 5.55 %, higher than the EE for 2.0% (*w/w*) alginate of 20.05 ± 9.64 %). Soliman et al. (2013) observed that from 0.25 to 8.0% (*v/w*) alginate, the loading capacity and encapsulation efficiency reached their maximum encapsulation at 2.0% (*w/v*) for thyme, cinnamon, and clove oil, which may differ depending on the types of EOs used. Increment in the concentration may limit the ability to encapsulate more oils. This is due to the crosslinking reaction that occurs between the G residues of the alginate and the Ca^{2+} ions, resulting in the formation of denser network that gives smaller pore size to encapsulate the EO [4, 14]. Based on the result, it could be said that 1.5% (*w/w*) alginate gave the maximum EE, that further increase on the concentration of the alginate yielded no increase or may reduce the EE. Moreover, the ratio of mannuronic and glucuronic acid of the alginate, which was not stated on the packaging of the alginate used for this experiment, in

Table 4. The quantitative results determining the relationship between the alginate concentration and/or weight ratios with the encapsulation efficiency (EE%).

Alginate concentration (*w/w*)	Weight ratio of PO: alginate	Encapsulation efficiency, EE (%)
1.5%	1:3	36.74 ± 0.93^{ac}
	1:2	36.15 ± 0.67^{ad}
	1:1	**42.00 ± 0.30^{ad}**
	2:1	27.62 ± 3.05^{ac}
2.0%	1:3	11.35 ± 6.03^{bc}
	1:2	**33.38 ± 4.66^{bd}**
	1:1	21.82 ± 2.22^{bd}
	2:1	13.63 ± 0.79^{bc}

Notes*:* Statistical analysis using two-way ANOVA, Tukey statistical test (95% confidence interval and $p < 0.05$ were expressed using alphabetical superscript.

Table 5. The quantitative results determining the relationship between the alginate concentration and/or weight ratios and/or weeks with the amount of encapsulated PO.

Alginate concentration (*w/w*)	Weight ratio	Amount of encapsulated PO (g)*				
		Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**
1.5%	1:3	0.131 ± 0.003^{acgh}	0.046 ± 0.006^{acg}	0.156 ± 0.032^{aci}	0.061 ± 0.012^{ach}	0.123 ± 0.010^{aci}
	1:2	0.193 ± 0.004^{adgh}	0.156 ± 0.005^{adg}	0.162 ± 0.029^{adi}	0.102 ± 0.025^{adh}	0.214 ± 0.022^{adi}
	1:1	**0.447 ± 0.003^{aegh}**	**0.400 ± 0.009^{aeg}**	**0.530 ± 0.002^{aei}**	**0.660 ± 0.058^{aeh}**	**0.744 ± 0.091^{aei}**
	2:1	0.589 ± 0.065^{afgh}	0.693 ± 0.016^{afg}	0.944 ± 0.098^{afi}	0.692 ± 0.060^{afh}	0.774 ± 0.063^{afi}
2.0%	1:3	0.054 ± 0.029^{bcgh}	0.064 ± 0.008^{bcg}	0.102 ± 0.014^{bci}	0.076 ± 0.004^{bch}	0.136 ± 0.022^{bci}
	1:2	**0.238 ± 0.033^{bdgh}**	**0.151 ± 0.005^{bdg}**	**0.247 ± 0.055^{bdi}**	**0.168 ± 0.001^{bdh}**	**0.339 ± 0.072^{bdi}**
	1:1	0.312 ± 0.033^{begh}	0.178 ± 0.007^{beg}	0.228 ± 0.043^{bei}	0.162 ± 0.008^{beh}	0.282 ± 0.062^{bei}
	2:1	0.390 ± 0.023^{bfgh}	0.451 ± 0.024^{bfg}	1.020 ± 0.174^{bfi}	0.717 ± 0.024^{bfh}	0.806 ± 0.123^{bfi}

Notes*:* Statistical analysis using two-way ANOVA, Tukey statistical test (95% confidence interval and $p < 0.05$ were expressed using alphabetical superscript.

fact could be another factor that affect the cross-linking of the M and G residues in the $CaCl_2$ solution, in which a higher ratio of M/G residues might cause the cross-linking to less occur due to the fact that only G residues are able to crosslink with Ca^{2+} ions [15], leaving the alginate beads permeable to leakage of the essential oil [4].

The relationship between weight ratios and EE, based on analysis, showed a significant difference ($p < 0.05$) in which EE of the weight ratios of 1:2 (34.77 ± 3.34 %) and 1:1 (31.89 ± 11.13 %) was significantly higher than 1:3 (24.05 ± 14.43 %) and 2:1 (20.63 ± 7.92 %). Higher EE was obtained as the volume of EO added increased due to the loss of water in the alginate, leaving more space for the encapsulation of the EO [14]. Plus, a higher amount of PO loaded in the alginate solution means more PO could be encapsulated upon crosslinking the alginate with Ca^{2+}. Even so, Chan [7] demonstrated that a drastic decrease in EE could be measured once the volume of oil added exceeded the capability of the alginate to encapsulate the oil efficiently. The addition of 50% of the oil in the alginate solution showed the highest EE of 90%, but during the addition of 60% of the oil,

the EE decreased significantly to 30%. The finding correlates with the result obtained in the experiment in which from the weight ratios of 1:3 to 1:2, it showed an increasing trend, but after the addition of a 1:1 weight ratio of PO: alginate, the EE started to decrease gradually and became drastically lower after the addition of PO twice the weight of the alginate. This indicated that after alginate reached its cut-off for encapsulation of PO, the polymer would lose its capability to retain a large amount of the input PO in its matrix, resulting in a large amount of the PO to remain in the gelation solution instead of being encapsulated. The emulsion stability before ionic gelation also plays role to determine the EE, which was proven by Chan [7] that addition of oil more than half of the weight of the alginate solution resulting in half of the initial PO to separate from the oil-alginate emulsion, producing immiscible layers that are harder to be encapsulated. Hence, lower EE would be measured. Encapsulation efficiency is usually improved by the addition of surfactant which may later improve this preliminary attempt to encapsulate the EO.

In comparison between the alginate concentration, weight ratios, and EE, for 1.5% (*w/w*) alginate, the maximum EE was achieved from the weight ratio of PO: alginate at 1:1 while for 2.0% (*w/w*) alginate, the maximum EE was achieved from the weight ratio of 1:2. Analysis showed that both alginate concentration and ratios differed significantly with the encapsulation efficiency ($p < 0.005$). Similarly, [7] reported the same significant relationship between these three variables. As the volume of oil loaded in the solution and the alginate concentration increased, the size of the beads started to vary but the shape of the beads was highly affected, which the beads started to deform at higher alginate concentration of 3.5% (*w/w*) with the highest oil loading of 50% from the weight of the alginate, forming tear-shaped beads. [15] emphasized that the mechanical and chemical properties of aspherical beads may be reduced compared to spherical beads. This suggested that the gel strength of the aspherical beads would decrease, leading to the leakage of the EO from the alginate matrix, thus lower EE was obtained upon increasing the alginate concentration and oil loading volume. Hence, this supported the finding from the experiment that between the two different alginate concentrations of 1.5% (*w/w*) and 2.0% (*w/w*) alginate, the weight ratio of 1:1 and 1:2 have the highest EE due to the formation of the most spherical beads compared to other weight ratio. Plus, [16] showed an inverse relationship between the size of the beads and the encapsulation efficiency which it was similar with the observation from this experiment that the bigger the size of the beads, the higher the encapsulation efficiency.

Most of the data in Table 5 showed different and inconsistent amount of encapsulated PO being released after the digestion of the alginate beads. ANOVA analysis illustrated a significant relationship between the amount of encapsulated PO with the alginate concentration, weight ratio, and week. However, based on the Pearson correlation analysis, the amount of PO remained encapsulated in the alginate beads during 5 weeks of storage was highly correlated with the weight ratio of the PO to alginate.

Previously mentioned that as the emulsion was not stable, it caused high polydispersity of the PO in the alginate solution. The higher the weight ratio, the higher the higher the tendency for the emulsion to become less stable and forming creaming phenomenon. As the PO was not uniformly dispersed, there was a tendency for some of the beads to encapsulate more of the PO during the extrusion of the top layer of the emulsion. Furthermore, excessive EO may present on the surface of the alginate beads, leading to the agglomeration of the loaded beads as a result of the attraction between the surface of the alginate and the EO [17]. Due to these reasons, the oil droplets tended to merge into larger droplets again if the droplets come into contact together, known as coalescence process [5, 16]. The coalescence of the oil droplets may give different amount of PO present on the surface of the oil, resulting in some of the beads to give different amount of PO at different weeks.

Essential oil (PO) composition characterisation for PO loaded alginate beads

Based on the GC-MS analysis of the original PO listed in Table 6, it was found that (±)-Menthol and l-Menthone were the most abundant metabolites in the PO, representing 65.4% of the total relative peak area. [18] found menthol and menthone were the most abundant compounds in steam-distilled PO present in peppermint and chocolate mint while [19] summarized neoiso-Menthol and iso-

Table 6. The initial percentage compounds composition different alginate concentration and PO weight ratio.

Compound (%)	Percentage composition (%)								
	Original PO	1.5% (*w/w*) alginate concentration				2.0% (*w/w*) alginate concentration			
	1:0	1:3	1:2	1:1	2:1	1:3	1:2	1:1	2:1
Eucalyptol	8.7	-	1.6	3.5	-	-	-	-	4.4
l-Menthone	27.4	9.4	23.8	26.2	12.1	20.8	-	5.1	29.5
Isomenthyl acetate	11.0	-	-	-	9.1	-	3.8	-	6.8
Caryophyllene	5.7	3.9	3.3	3.3	3.7	3.3	-	3.6	2.6
Menthyl acetate	-	10.3	8.8	8.5	-	9.1	-	8.9	-

Menthone as the two major compounds present in distilled PO. Meanwhile, isomenthyl acetate present only in a small amount, constituting about 0.2% of the relative peak area, much lower than the percentage of menthyl acetate. However, the analysis of the original PO reported no detection of menthyl acetate, instead, isomenthyl acetate was the third highest compound after (±)-Menthol and l-Menthone. Other than that, β-Pinene, D-Limonene, (-)-β-Bourbonene, and caryophyllene from the GC-MS analysis in the experiment were also present in the PO, but of different isomers such as α-Pinene, limonene, β-Bourbonene, and caryophyllene oxide.

Nevertheless, (±)-Menthol, the highest compound in the original PO was not detected in the liberated PO. One of the reasons that may explain the percentage composition difference was due to the different preparation methods prior to the PO analysis between the original and separated PO. The original PO was diluted with sole dichloromethane to separate the PO from the alginate beads. Sodium citrate was first used to digest the alginate beads, releasing the encapsulated PO. Water (10.2), which was added during the preparation of sodium citrate solution, has a higher polarity index than dichloromethane (3.1) [20]. There was a possibility that the (±)-Menthol constituent, which is polar due to the presence of hydroxyl group, was highly attracted to the sodium citrate than the dichloromethane after extraction, due to the strong mint odour in the sodium citrate layer after the separation of the supernatants.

Like other essential oils, PO consists primarily of volatile organic compounds (VOC) such as terpenes, aromatic hydrocarbons, aldehydes, and many more [21]. Reduction in certain volatile compounds such as caryophyllene [22], eucalyptol, l-menthone, and D-limonene [23] could be due to the vulnerability to factors that lead to oxidation, such as exposure to light for a long time during the preparation of PO-alginate emulsion and heat generated during the long duration of homogenization. The percentage composition of -pinene and menthone in [24] gradually decreased from 50 to 70 °C. Even though some of the PO constituents decreased or were lost throughout the emulsion preparation and beads formation, the alginate was still able to retain the l-menthone in both different alginate concentrations and most of the weight ratios except for 2.0% (*w/w*) in the 1:2 weight ratio, indicating alginate as a stable wall material for successful encapsulation of essential oils.

According to Tables 7 and 8, the analysis of the released PO from the alginate beads, which initially had the highest EE, demonstrated the ability of the microencapsulation method to protect the volatile compositions of the PO from being volatilized by the harsh environment, with the majority of the PO constituents remaining present in the encapsulated PO. 2.0% (*w/w*) alginate with a weight ratio of 1:2 PO to alginate showed better retention of all four abundant PO components except for eucalyptol during storage.

In agreement to [5], FTIR spectrum analysis shows the PO was present in all the encapsulates, with menthol as the dominant compound detected in the PO. This indicated the stability of the encapsulation method to retain the components of the PO. [4] observed that after 8 days of storage, the encapsulated clove and thyme oil able to sustain about 50% of their fungal activity by inhibiting the growth of the fungi. [16] observed a slight decrease of eugenol in the encapsulated clove oil after storage of almost a month, due to the heat generated during the homogenization of the PO in the viscous alginate solution, causing loss of the heat-sensitive constituents of the clove oil. Other than coalescence, the absence of (±)-Menthol at certain weeks was explained by the fact that polar compound is able to be solubilized in the continuous phase of the emulsion, either the sodium alginate solution or the sodium citrate used to digest the alginate beads. But still, encapsulation method

Table 7. The percentage composition of the compounds in the extracted PO at weight ratio 1:2 for 5 weeks.

Compound	Percentage composition (%)									
	Week 1		Week 2		Week 3		Week 4		Week 5	
	1.5	2.0	1.5	2.0	1.5	2.0	1.5	2.0	1.5	2.0
(±)-Menthol	-	-	63.2	63.0	65.5	-	-	-	10.6	61.0
l-Menthone	23.8	23.6	-	25.4	20.9	24.1	18.7	23.0	8.1	21.8
Isomenthyl acetate	-	3.78	7.06	6.2	7.3	6.5	7.5	7.2	3.6	10.7
Eucalyptol	1.6	-	1.7	2.7	-	2.2	-	1.80	-	-
Caryophyllene	3.3	-	3.16	2.7	3.5	3.0	4.0	3.3	1.6	5.1

Table 8. The percentage composition of the compounds in the extracted PO at weight ratio 1:1 for 5 weeks.

Compound	Percentage composition (%)									
	Week 1		Week 2		Week 3		Week 4		Week 5	
	1.5	2.0	1.5	2.0	1.5	2.0	1.5	2.0	1.5	2.0
(±)-Menthol	-	-	62.6	-	-	70.1	-	-	-	-
l-Menthone	26.2	5.1	24.0	22.1	23.6	18.9	20.6	15.0	13.4	17.5
Isomenthyl acetate	-	-	6.9	6.9	7.5	7.4	7.9	8.0	4.2	10.3
Eucalyptol	3.5	-	2.1	1.6	-	-	-	-	-	-
Caryophyllene	3.3	3.6	3.1	3.1	3.4	3.6	4.0	4.8	1.5	5.4

enabled the protection of the EO constituents better than the free EO that may lose 90% of its activity after two days [4].

Conclusion

Findings revealed entrapment of PO in beads were a success however the encapsulation efficiency is considered moderate. The entrapment of PO is dependent on the PO alginate ration and the concentration of the alginate polymer. Physical assessment in size and EE are considered stable through the storage time as there were no significant changes were observed in the size and remnants of the PO were still detected. Traces of the constituents were also seen to reduce gradually throughout storage time which indicates slow release and some form of protection provided by the alginate layers.

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