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Microencapsulation of red palm oil and its stability during accelerated storage

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ABSTRACT

Introduction: Sensitivity of red palm oil (RPO) towards oxidation is known to result in degradation of nutritional value and organoleptic properties. This study aimed to determine the stability of microencapsulated RPO during accelerated storage at 65˚C for 24 days. **Methods:** Microencapsulated was undertaken by co-extrusion technology using sodium alginate with high methoxyl pectin, and calcium chloride solution enhanced with chitosan as cross-linking agent in the presence of Tween® 80 as surfactant. The encapsulated beads were freeze dried and the physical properties, antioxidant activities and total carotenoid content of dried powder were determined. Microencapsulated red palm oil (MRPO) was then subjected to accelerated storage at 65°C for 24 days. **Results:** Antioxidant activity of both RPO and MRPO measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was significantly decreased, with higher percentage loss in MRPO during accelerated storage. RPO and MRPO also experienced decreases in percentage inhibition with higher percentage of loss as measured by Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS) radical scavenging activity. Both RPO and MRPO showed decreasing trends in total carotenoid content, with higher content in MRPO than RPO at end of storage period. Antioxidant activities of RPO and MRPO correlated well with the carotenoid content, with best correlation coefficient in RPO between the ABTS assay and total carotenoid content measured by high performance liquid chromatography (r=0.952). Very strong association between DPPH and ABTS values (r=0.871) for the MRPO, and between DPPH and total carotenoid content (r=0.856) were noted. **Conclusion:** The study showed that microencapsulation effectively protected the carotenoid content in MRPO, but not its other natural antioxidants.

Keywords: Carotenoids, microencapsulation, co-extrusion, antioxidants, accelerated storage

INTRODUCTION

Crude palm oil extracted from the mesocarp of ripened fruit of oil palm tree, *Elaeis guineensis,* is a complex mixture consisting more than 99% glycerides. This oil is also known as red palm oil (RPO). It differs from other plant and animal oils in that it contains 50% of saturated fatty acids, 40% unsaturated fatty acids and 10% polyunsaturated fatty acids. Palm oil and its products play prominent roles in several manufacturing industries and are beneficial to human and animal diets. They are widely consumed and

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exported in many forms, including refined, bleached and deodorized (RBD).

Red palm oil contains carotenoids, of which 80-90% are α- and β- carotene, as well as vitamin E (tocopherols and tocotrienols) (Sadiq, Rasool & Sharif, 2006; Andreu-Sevilla *et al.*, 2009). These compounds are essential as antioxidants and may contribute to the oxidative stability of the oil. The oil also contains appreciable amounts of components, such as sterols, phospholipids, glycolipids, ubiquinones and squalene, which are nutritious and provide benefits to human health (Alyas, Abdullah & Idris, 2006; Atawodi *et al.*, 2011; Bakry *et al.*, 2015). However, RPO as a cooking oil is not favoured by consumers due to its red colour, Thus, RPO has been modified resulting in the oil having a light golden color without the carotenes (NorAini *et al.*, 1998; Okonkwo, 2012).

Microencapsulation technology has been applied in the food industry for various benefits (Estevinho *et al.*, 2013). Encapsulation is a process to entrap a substance within another substance resulting in the production of small particles with diameters ranging from few micrometers to few millimeters. The inner substance is known as the core material, while the outer substance is called the wall material or encapsulant (Zuidam & Nedović, 2010). Microencapsulation allows the encasement of micron-sized particles of either solid, droplet of liquids or gasses in inert shell, which isolate and protect them from the external environment. Therefore, microencapsulation is able to protect the sensitive ingredients and organoleptic properties including colour, taste and odour from external effects (Jyothi *et al.*, 2010). In addition, microencapsulation techniques are regarded as an alternative method to control the release of substances over prolonged periods under specific conditions (Estevinho *et al.*, 2013).

The selection of appropriate encapsulation technology, core material, wall material and capsule properties are critical in promoting its successful commercial application (Lim & Nyam, 2016). There are numerous techniques of microencapsulation of food compounds available, including spray drying, spray chilling, freeze-drying, coacervation and co-extrusion (Sagis, 2015; Chew & Nyam, 2016). The choice of microencapsulation techniques depends on the physical and chemical properties of the materials to be encapsulated (Jyothi *et al.*, 2012). Each technique has its own advantages and disadvantages.

MATERIALS AND METHODS

Red palm oil (Carotino, Johor) was purchased by convenience from a supermarket in Kuala Lumpur. Wall materials made from sodium alginate were acquired from R & M Marketing (Essex, UK), while high methoxyl pectin (HMP) was purchased from a local food ingredient supplier. All chemicals and reagent used in this study were of analytical grade (Merck, Darmstadt, Germany and Sigma-Aldrich, Germany).

Microencapsulation of RPO using co-extrusion technology

Sodium alginate solution (1.50% w/w) and HMP solution (1.50% w/w) were prepared and gently homogenised at 12000 rpm for 2 min and 7200 rpm for 1 minute (min), using digital Ultra-Turrax® homogenizer (T25, IKA, Germany). The HMP-alginate solution was mixed according to the method of Chew *et al.* (2015) at a volume ratio of 2:1 and stored overnight at 4°C.

The hardening solution made from calcium chloride solution (3.00% w/v) enhanced with chitosan coating (0.10% w/v) was freshly prepared. Tween® 80 (0.10% w/v) was added and mixed together until fully dissolved. The solution was adjusted to pH 5.0-5.5. Subsequently, any insoluble material was filtered out and the solution was topped up to 1 L. The chitosan solution

was incubated in the water bath set at 50°C until further usage (Lab Companion, Korea).

The microencapsulation of RPO by co-extrusion technology was carried out using Encapsulator B-390 (Buchi, Switzerland) with the concentric nozzle (150 µm for inner nozzle and 300 µm for outer nozzle). The air pressure was set at 600 mbar to give a core-shell fluid stream, which was sprayed out through the nozzle. The frequency was set at 300 Hz under amplitude of three with an additional electrostatic field of 1.5 kV between the nozzle and hardening solution to minimise the potential dissolution of core-shell droplets when approaching hardening solution surface.

Microcapsules obtained were hardened when dropped into the calcium chloride solution enhanced with chitosan coating and Tween® 80. The microcapsules were incubated for 10 min in the hardening solution with gentle stirring to avoid clumping of microcapsules. The microcapsules were next collected with nylon sieve, rinsed and drained until no further moisture was found. The microcapsules were spread thinly and evenly to increase the drying rate.

After forming, the microcapsules were dried using freeze dryer (SciQuip, United Kingdom) for 23 hours. All the dried microcapsules were collected in aluminum foil-wrapped Schott bottle, following which the bottle was flushed with 99.9% nitrogen to remove oxygen, and stored in a freezer (E388, Fisher and Paykel, Australia) at -20°C for later use.

Physical analysis

The morphology of fresh and dried microcapsules was observed using optical microscope under magnification of x4 (Nikon Instruments Inc., United State) and their size measured with the aid of a stage micrometer slide (Ladd Research, United State). The moisture content of the microcapsules was determined using oven, while the water activity of the microcapsules was analysed using water activity analyzer (Aqua Lab, United State) (Chew & Nyam 2016). The moisture content was calculated using the equation shown below:

$$\text{Misture content (\%)} = \frac{(W1 - W2)}{W1} \times 100\%$$

where W1 is the weight of the samples before drying (g) and W2 is the weight of the samples after drying in g.

Microencapsulation efficiency (MEE)

The MEE of MRPO after drying was determined according to Thamaket & Raviyan (2015) and MPOB P2.6: 2004 analytical method. The microencapsulation efficiency was determined using the following equation.

$$\text{MEE} = \frac{\text{Total carotenoids in microcapsules} - \text{Surface carotenoids}}{\text{Total carotenoids input}} \times 100\%$$

Total carotenoids in the microcapsules were obtained by dissolving 10 mg of microcapsules in 25 mL of sodium citrate solution with gentle agitation. Hexane (25 mL) was added and the mixture was shaken vigorously to facilitate the transfer of oil into the hexane layer. The obtained hexane layer was evaporated using a Multivapor P-6 (BÜCHI Labortechnik AG, Switzerland) at 40°C, 125 mbar for 30 min with speed six. The residual solvent was next removed by flushing with 99.90% nitrogen.

Total carotenoids input content was determined by dissolving 0.04 g of oil in 10 mL volumetric flask with hexane. It was then gently shaken until it was fully dissolved and measured at 446 nm using ultraviolet-visible (UV-Vis) spectrophotometer (Secomam, France) with hexane as the blank.

The surface carotenoids content was determined by adding 25 mL of hexane to 50 mg of microcapsules, followed by

agitating it using orbital shaking plate (Vision Scientific, Pakistan) for 15 seconds at 100 rpm. The supernatant collected was measured spectrophotometrically at 446 nm.

Total carotenoids in microcapsules, surface carotenoids and total carotenoids input content were then calculated using the formula as shown below:

$$\text{Total carotenoids content} = V \times \frac{383}{100W} \times (As - Ab)$$

where V is the volume used for analysis (mL), 383 is the extinction coefficient for carotenoids, W is weight of sample (gram, g), As is the absorbance of sample at 446 nm, and Ab is the absorbance of blank at 446 nm.

Storage stability

The microcapsules were subjected to an accelerated storage condition at 65°C for 24 days, whereby 1 day of storage reflected 1 month of storage at room temperature (Ng *et al.*, 2014). The antioxidant activity tests carried out were DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activities. The total carotenoid content in the RPO and MRPO were examined using high performance liquid chromatography (HPLC) (Agilent Technologies, United State) and UV-Vis spectrophotometer (Secomam, France) every 6 days. RPO was used as a control for comparison.

Assessment of antioxidant activities upon accelerated storage

DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity was determined according to the method of Cheong, Tan & Nyam (2016), Chew *et al.* (2015) and Chew *et al.* (2016). The antioxidant activity of the oil samples was expressed as mg Trolox equivalents (mg Teq/100 g oil) with calibration equation of y=61.633x–0.2148 (R^2=0.9889). The percentage of inhibition (%) was calculated based on the equation below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activity

The ABTS radical scavenging activity was performed according to Cheong, Tan & Nyam (2016) and Chew *et al.* (2015; 2016). The antioxidant activity of the oil samples was expressed as mg Trolox equivalents (mg Teq/100 g oil) with calibration equation of y=210.67x – 1.0596 (R^2=0.9861). The percentage of inhibition (%) was calculated based on the equation below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Assessment of total carotenoid content upon accelerated storage

High performance liquid chromatography (HPLC)

The total carotenoid contents in MRPO and RPO were determined according to the modified method of Jain *et al.* (2016). An aliquot (20 µL) of sample was injected and analysed by HPLC (Agilent Technologies 1200 series, United State). The carotenoid content was expressed as mg/kg. HPLC analysis was carried out isocratically at 35°C using C18 column (250x4 mm i.d., particle size 5 µm) (Merck, Germany). The mobile phase used was methanol/acetonitrile/ethyl acetate (80:10:10 v/v). The peaks were quantified at 446 nm.

UV-Vis spectrophotometer
The total carotenes content in MRPO and RPO was evaluated based on MPOB P2.6:2004 analytical test. The absorbance was measured at 446 nm using UV-Vis spectrophotometer.

Statistical analysis
The results obtained were subjected to statistical analysis utilising MINITAB 17 (Minitab Inc, Pennsylvania, United State). One-way analysis of variance (ANOVA) was performed and significant differences (p<0.05) were determined using Tukey Honestly Significant Differences (HSD) multiple comparison test. Independent t-test was conducted to compare the differences of antioxidant activities and total carotenoid contents between RPO and MRPO on the same day of storage. Pearson correlation was determined to quantify the relationship between the two data samples. The results were expressed as mean±standard deviation for both physical analysis and storage stability tests.

RESULTS AND DISCUSSION

Physical analysis
The RPO was successfully encapsulated within the wall materials forming a bulbous shape. The microcapsules had a consistent size with regular and smooth surface. After subjecting the microcapsules to freeze drying, the microcapsules shrunk and appeared to be slightly irregular with darker yellow colour. The surface of dried microcapsules was fibrous and not smooth, compared to freshly produced microcapsules (Figure 1). The average particle size of fresh MRPO (626.67±75.28 μm) was significantly higher compared to the dried MRPO (545.00±110.91 μm). This was expected as fresh MRPO contained water, which was removed during freeze drying, resulting in the smaller size of dried MRPO.

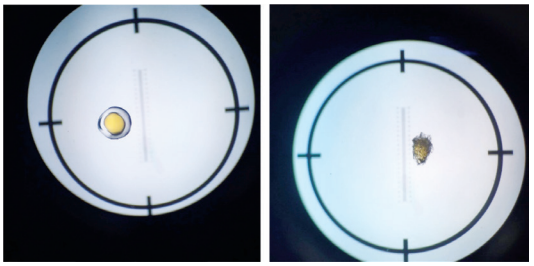

Figure 1. Microencapsulated red palm oil before drying (left) and after drying (right) under light microscope viewed with 4x magnification

The MRPO was found to have low moisture content (1.98±0.11%). According to Onwulata (2005), microencapsulation of fats is able to reduce the adhesiveness and improve the handling properties during storage, transport and blending with non-fat ingredients. Through water activity analysis, it was found that the MRPO is categorised as a low water activity product (0.36±0.02) as the water activity was less than 0.70 (Gurtler, Doyle & Kornacki, 2014). Due to its low water activity, dried MRPO had a lower risk of microbial spoilage and therefore able to prolong its shelf life by preventing the growth of microorganisms.

Microencapsulation efficiency (MEE)
Microencapsulation efficiency plays a significant role in this study because MEE refers to the proportion of oil that is surrounded by the shell wall matrix and less exposed to the surrounding environment and thus, having an effect on the oxidative stability of the product (Chew & Nyam, 2016). The MEE of MRPO was found to be 76.95±5.42%, which is higher than reported by Chew & Nyam (2016). Therefore, the wall materials selection and its proportions can be considered to be optimum in this study.

Analysis of DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of RPO and MRPO during accelerated storage

The percentage inhibition of DPPH value of both RPO and MRPO showed a decreasing trend from day 0 to day 24 with an initial value of 40.23±6.03% and 27.30±5.34 %, respectively (Figure 2). MRPO experienced a higher decrease compared to RPO starting from day 0. This could be due to oxidation as the oil had longer exposure to the oxygen and water during the microencapsulation. Results obtained indicated that the percentage of inhibition was significantly higher for RPO at day 0. The total decrease of DPPH values in RPO and MRPO were 77.50% and 81.21%, respectively. Therefore, the results indicated that microencapsulation could not protect and stabilise the natural antioxidants in RPO during storage.

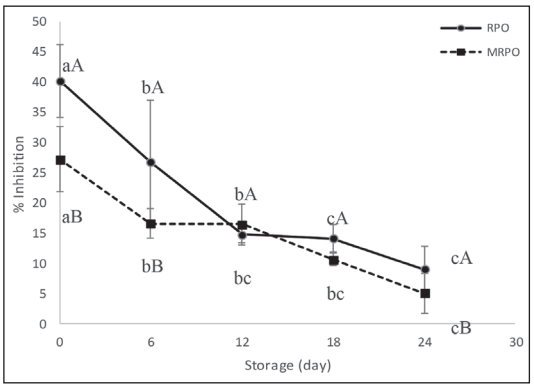

Figure 2. Total percentage of inhibition of DPPH in RPO and MRPO upon accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abc} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Analysis of Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activity of RPO and MRPO during accelerated storage

Percentage inhibition values of ABTS for both RPO and MRPO declined from day 0 to day 24 (Figure 3). The decreasing trend in ABTS might be due to the encapsulation process having an impact on the oil oxidation. The longer the exposure time of oil to the environment, the higher the likelihood of degradation of the antioxidants in the oil. This brought about a lowered capability in free radicals scavenging. At day 0, RPO samples showed significantly higher ABTS value compared to the other days, while the MRPO samples showed no significant difference with the ABTS value on day 6. The differences in % inhibition between both RPO and MRPO became smaller from day 12 of storage due to the degradation of antioxidants in RPO. In brief, the total percentage decrease in inhibition values for ABTS in RPO and MRPO were 88.07% and 73.54%, respectively.

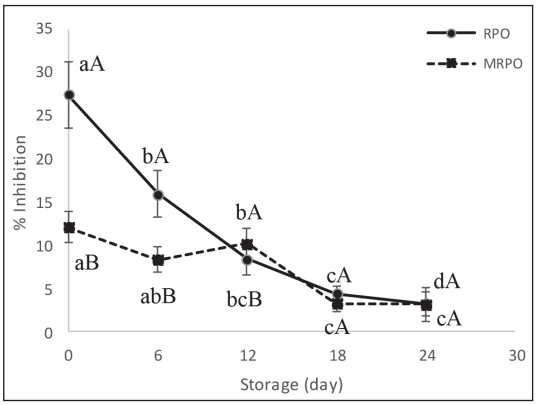

Figure 3. Total percentage of inhibition of ABTS in RPO and MRPO upon accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abcd} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Total carotenoid contents of RPO and MRPO during accelerated storage

Decreasing trends of the total carotenoid contents in RPO and MRPO were observed during accelerated storage. The initial total carotenoid content of RPO was higher than in MRPO and decreased continuously till a low value of about 10 mg/kg on day 24 (Figure 4). However, for MRPO, after an initial decline, the carotenoid content was observed to increase from 65.92±7.38 to 88.91±2.63 mg/kg between day 6 and day 12. The carotenoids showed considerable fluctuations depending on environmental conditions. After day 12, the carotenoid content in MPRO continued to decrease but the level at the end of storage (day 24) was higher than that of RPO.

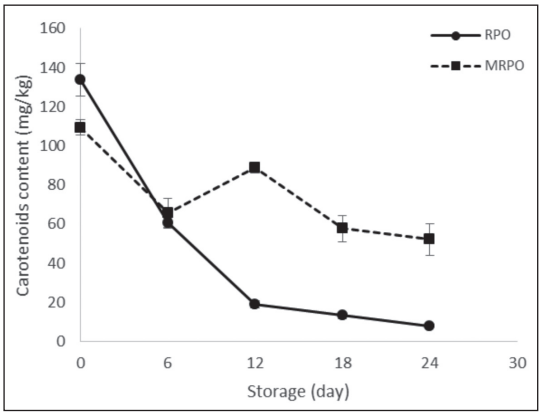

Figure 4. Total carotenoid content by HPLC in RPO and MRPO during accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abcd} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Similarly, the initial total carotenoid content of RPO was higher than MRPO. However, over the storage period, there was less decline in carotenoid content for MRPO and on the last day of storage (day 24), the carotenoids content in MRPO was significantly higher than that in RPO (Figure 5). It could be concluded that through microencapsulation, the carotenoids content in the oil in MRPO was comparatively better retained than RPO during storage.

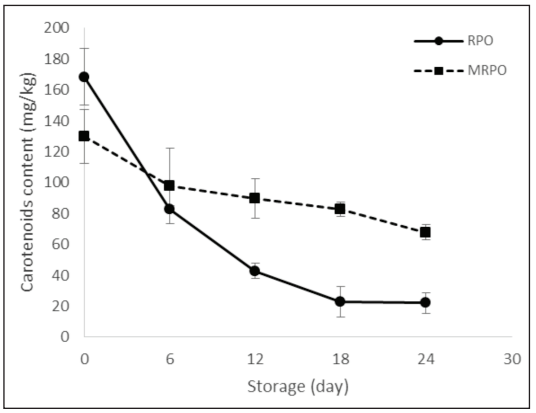

Figure 5. Total carotenoids content by UV-Vis spectrophotometer in RPO and MRPO during accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abc} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Correlation assessment among RPO traits

There was a significantly positive and strong association between the antioxidant activities, measured by DPPH and ABTS, and the total carotenoid content in RPO. The best correlation coefficient was found between the ABTS assay and total carotenoid content measured by HPLC (r=0.952). The total carotenoid content as measured by HPLC and UV-Vis spectrophotometer showed strong and significant positive relationship with one another. According to Martysiak-Żurowska & Wenta (2012), DPPH method has more limitations as characterised by a lower sensitivity and slower reaction with most antioxidants compared to ABTS assay.

Correlation assessment among MRPO traits

Pearson's correlation coefficients (r) among DPPH, ABTS and total carotenoids in MRPO showed significant positive relationships. Very strong association between DPPH and ABTS values (r=0.871), as well as between DPPH and total carotenoid content determined using HPLC (r=0.856) were noted from the results. This was in agreement with the study by Dudonné *et al.* (2009), where DPPH and ABTS assays showed a strong positive correlation. The lowest correlation was observed between ABTS value and total carotenoids measured spectrophotometrically (r=0.704). As the results demonstrated positive linear relationships, it can be interpreted that the antioxidant assays, (DPPH and ABTS) employed in this study were suitable and can be used to measure the antioxidant activity of both RPO and MRPO (Razmkhah *et al.*, 2013).

CONCLUSION

Red palm oil, which was micro-encapsulated by co-extrusion technology and dried using freeze drying method showed relatively low moisture content and water activity. This indicates that microencapsulated RPO (MRPO) has good stability and low risk of microbial contamination. The microencapsulation efficiency of MRPO was considered relatively high, hence, the combination of the wall materials utilised is considered suitable as coating agent. It is suggested that more advanced tests, such as Fourier transform infrared spectroscopy (FTIR) and immobilized horseradish peroxide assay be conducted along with DPPH and ABTS assays to quantify the actual amounts of scavenging activity.

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Authors' contributions

KLN, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; FA, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript.

Conflict of interest

The authors declared no conflict of interest

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