

THE ABILITY OF BREADFRUIT STARCH NANOPARTICLE-STABILIZED PICKERING EMULSION FOR ENCAPSULATING CINNAMON ESSENTIAL OIL

BOVI WIRA HARSANTO¹, SUPRIYANTO¹, INDRIANA KARTINI² and YUDI PRANOTO^{1*}

¹Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

²Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Jl. Kaliurang KM. 5, Sekip Utara, Yogyakarta 55281, Indonesia

*E-mail: pranoto@ugm.ac.id

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ABSTRACT

Cinnamon essential oil (CO) is susceptible to decreased stability during storage, limiting its application in food products. Pickering emulsion stabilized by starch nanoparticles becomes a potential encapsulating method that can improve CO stability. This study aimed to investigate the ability of breadfruit starch nanoparticles-stabilized Pickering emulsion to encapsulate CO with various concentrations. Encapsulation process was carried out using the high-energy emulsification method with dispersing CO (0.05%; 0.1%; 0.5%; 1% w/w) in emulsion. The loading efficiency of CO and emulsion properties were evaluated. Retention of CO was also observed in 7 days-storage. Results showed that 0.5% and 1% CO were encapsulated effectively and stable in Pickering emulsion, with loading efficiency and CO retention ranging from 79.49-81.13% and 78.86-79.20%, respectively. The addition of 0.5% and 1% CO increased yellowness (+a*: 7.45-8.99) as well as decreased whiteness (+L*: 85.77-86.06) and viscosity (629.9-721.8 cP) of Pickering emulsion. However, differences in CO concentrations did not affect the emulsion index of Pickering emulsion. These findings concluded that breadfruit starch nanoparticles-stabilized Pickering emulsion could encapsulate up to 0.5% and 1% CO with the best properties among other treatments. Therefore, breadfruit starch nanoparticles-stabilized Pickering emulsion can be an alternative as encapsulation method, which can later expand the application of CO in food products.

Key words: Breadfruit starch nanoparticle, cinnamon essential oil, encapsulation, pickering emulsion

INTRODUCTION

The bark of the cinnamon species is one of the most popular spices around the world, especially its use in cooking. In practice, the cinnamon bark is widely processed into an essential oil. Cinnamon essential oil (CO) contains monoterpenes, sesquiterpenes, and phenylpropenes, contributing to the presence of a distinctive sweet and spicy flavor. Hence, the cinnamon essential oil can be used as a seasoning ingredient in meat, fish, sauces, baked goods, beverages, bakery, and pastry products (Ribeiro-Santos *et al.*, 2017).

Cinnamon essential oil is relatively hydrophobic, so it has limitations when mixing in hydrophilic or complex food systems (Chuesiang *et al.*, 2018; Chuesiang *et al.*, 2019). Moreover, flavor sources, like cinnamon essential oil, are volatile and chemically unstable during storage due to exposure to oxygen and light (Bhandari *et al.*, 1992). It can be said that the low stability and high hydrophobicity of cinnamon essential oil limit its application in food products.

Theoretically, different types of emulsions can facilitate the mixing of hydrophobic materials, such as nutrients, in complex food systems (Velikov & Pelan, 2008; McClements, 2010; Mao & Miao, 2015). On the other hand, Fasihi *et al.* (2019) explained that the encapsulation of essential oils in Pickering emulsion (solid particle-stabilized emulsion) has a protective effect against external factors. Previous studies have reported that oil-in-water emulsion stabilized by solid particles could protect curcumin against oxidation (Wang *et al.*, 2014).

Pickering emulsion is stable against coalescence and Ostwald ripening due to the high kinetic energy required to desorb solid particles at the oil-water interface (Marku *et al.*, 2012). Furthermore, effective Pickering stabilization can be achieved if a nano-scale particle is used (Dickinson, 2012). In the last decade, starch nanoparticles have attracted much attention as Pickering emulsion stabilizers because of biocompatibility and the high availability of starch sources in nature. Breadfruit (*Artocarpus altilis*) is a potential starch source that can be used as material nanoparticles. Our previous study found that breadfruit starch nanoparticles (mean size: 144.2 nm)

* To whom correspondence should be addressed.

could maintain the stability of Pickering emulsion for two weeks at room storage (Harsanto *et al.*, 2021). Starch nanoparticles were observed to be adsorbed at the oil-water interface to stabilize the Pickering emulsion (Tan *et al.*, 2014).

Recent studies had investigated the essential oil encapsulation based on Pickering emulsion stabilized by non-starch nanoparticles (Feng *et al.*, 2020; Jiang *et al.*, 2020). To date, there has been no report about breadfruit-based starch nanoparticles being used as emulsifiers in Pickering emulsion to encapsulate cinnamon essential oil. Therefore, this study aimed to evaluate the ability of Pickering emulsion stabilized by breadfruit starch nanoparticles to encapsulate various concentrations of cinnamon essential oil. In this study, the cinnamon essential oil was added in low to high concentrations to evaluate the encapsulation performance of the Pickering emulsion.

MATERIALS AND METHODS

Materials

Mature breadfruit (round-shaped and hard texture of flesh fruit) was obtained from local growers in Central Java, Indonesia. Breadfruit starch was obtained from wet-milled sediment of the Indonesian breadfruit. Then, breadfruit starch nanoparticle was produced using the nanoprecipitation method, as stated in Harsanto *et al.* (2021); n-hexane, was purchased from MERCK, Germany. MCT oil was procured from PT. Barco, Indonesia, and cinnamon essential oils (purity $\geq 99\%$) were obtained from Kleen and Kare, Indonesia.

Methods

Preparation of cinnamon essential oil (CO)-encapsulated Pickering emulsion

Preparation of emulsion was conducted using the high-energy method, according to Shah *et al.* (2016). Due to the high purity of cinnamon essential oil, the four concentration levels of cinnamon essential oil were varied, ranging from 0.05% to 1%. In the first step, cinnamon essential oil (0.05%; 0.1%; 0.5%; 1% (w/w)) was dissolved in MCT oil under continuous stirring at 500 rpm (magnetic stirrer, Thermo scientific, USA) for 5 min at ambient temperature (28 ± 2 °C) until the oil phase concentration becomes 40% (w/w). Under the same condition, breadfruit starch nanoparticles (3 g) were dispersed in distilled water (57 g) until the concentration of the aqueous phase became 60% (w/w). Later, the oil phase was added dropwise into the aqueous phase and then stirred at 500 rpm. Afterward, the mixture was homogenized using an ultra-turrax homogenizer (T50 Basic, IKA

WERKE, Germany) at 10000 rpm for 5 min at room temperature. In the final step, the Pickering emulsion was poured into a screw-cap plastic bottle and stored for seven days at room temperature for analysis. Pickering emulsion without cinnamon essential oil was also produced for comparison.

Loading efficiency and retention of cinnamon essential oil (CO)

After preparing the emulsion, cinnamon essential oil (CO) content was measured based on Li and Lu (2016). Initially, Pickering emulsion (1.5 mL) was mixed with deionized water (30 mL) and n-hexane (15 mL) and then stirred at 4000 rpm (ultra-turrax T50 Basic, IKA WERKE, Germany) for one min. Afterward, the mixture was heated in a water bath shaker (Julabo, Germany) at 45 °C and 50 rpm for 60 min. Then, the mixture was centrifuged at 4500 rpm for 30 min to obtain the top layer of the mixture. Later, the absorbance in the top layer of the mixture was measured at a wavelength of 285 nm (UV-VIS spectrophotometer, Genesys 10S, USA) and compared with the standard curve of CO in n-hexane to get the CO content of the emulsion. The loading efficiency of CO was determined based on the ratio between the CO content in emulsion and the added CO content, written in Equation (1) (Li & Lu, 2016). Retention of CO was calculated based on the remained CO content in emulsion after 7 days of storage and compared with the CO content in emulsion before storage, written in Equation (2) (Shah *et al.*, 2016).

$$\text{Loading efficiency of CO (\%)} = \frac{\text{CO content in emulsion}}{\text{CO added into emulsion}} \times 100$$

- Equation 1

$$\text{CO retention (\%)} = \frac{\text{CO content after 7 days storage of emulsion}}{\text{CO content before storage of emulsion}} \times 100$$

- Equation 2

Emulsion properties

Pickering emulsion was stored at room temperature for seven days and observed for parameters of droplet size ($d_{4,3}$), emulsion index, viscosity, whiteness, and yellowness. Observations were performed on day-0 and day-7. The emulsion appearance was also observed before storage using a pocket camera (Ixus I90, Canon, China) and an optical microscope (Olympus CX21LED, Japan).

The droplet size was analyzed using the method of Li *et al.* (2013). The 100 droplets observed using an optical microscope were measured for diameter using ImageRaster software (Informer Technologies, Inc.), and the $d_{4,3}$ value was calculated based on Equation (3) (Li *et al.*, 2013).

$$d_{4,3} (\mu\text{m}) = \frac{\sum \text{diameter}^4}{\sum \text{diameter}^3}$$

- Equation 3

Meanwhile, the emulsion index was analyzed using the ratio between the emulsified layer height and the total emulsion height and was then calculated based on Equation (4) (Saari *et al.*, 2017).

$$\text{Emulsion index} = \frac{\text{height of emulsified phase}}{\text{total height of emulsion}} \quad \text{- Equation 4}$$

Furthermore, emulsion viscosity was analyzed using a viscometer (Brookfield, USA). The S63 spindle was immersed in emulsion and operated for 30 sec at 30 rpm. Meanwhile, whiteness and yellowness were analyzed using chromameter (Konica Minolta, Japan) by measuring the +L* value and the +b* value, respectively which adopted the CIE L*a*b* color measurement system.

Statistical analysis

The experiments were carried out at least duplicate measurements. The differences between treatments were determined by one-way ANOVA through SPSS v.25 (IBM Inc., USA) and followed by Duncan's test at $\alpha = 5\%$. Significant differences were indicated by p -value < 0.05.

RESULTS AND DISCUSSION

Loading efficiency of cinnamon essential oil (CO)

In emulsion-based encapsulation systems, the term "loading efficiency" is often used to describe the entrapment ability of active ingredients in the emulsion (McClements *et al.*, 2009; Araiza-Calahorra *et al.*, 2018). It can be said that the high loading

efficiency of the active ingredient indicates successful encapsulation. Muhoza *et al.* (2019) stated that the effective ability to encapsulate active ingredients was shown by loading efficiency >80%. As seen in Figure 1, the loading efficiency of CO ranged from 70-85%. The addition of 0.1%, 0.5%, and 1% CO did not affect significantly ($p > 0.05$) loading efficiency but was higher than the addition of 0.05% CO ($p < 0.05$).

These results indicated that more essential oil would be trapped at a higher level of CO addition. Chuesiang *et al.* (2019) assumed that high initial cinnamaldehyde levels in lipid droplets would reduce the degradation of cinnamaldehyde fraction during storage. Figure 1 also shows that the high level of CO addition, up to 1%, could be trapped efficiently (80%) in the Pickering emulsion stabilized by breadfruit starch nanoparticles. Kaushik and Roos (2007) also found high encapsulation efficiency of limonene (84%) in emulsions through the high-pressure homogenizing method. The high loading efficiency of encapsulation could be attributed to MCT oil droplets in the Pickering emulsion system, which could play a role as a wall material for essential oil, as Muhoza *et al.* (2019) stated. In addition, the presence of MCT oil droplets was related to the availability of space to be occupied by the essential oil. This study used an oil phase with a high concentration (40%) so that there was a lot of space available for essential oils even at high concentrations. This resulted in the high loading efficiency of the Pickering emulsion at 0.5% and 1% CO (Figure 1).

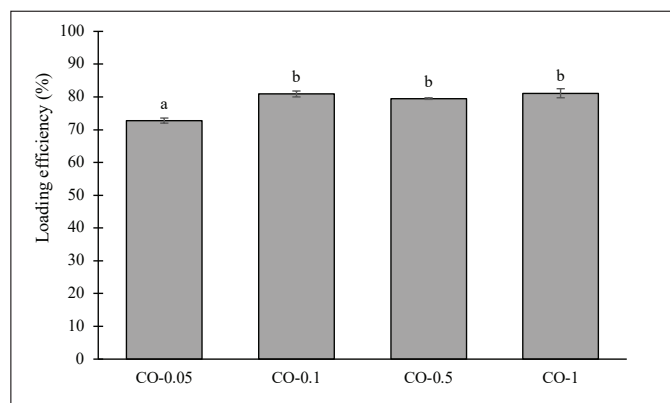


Fig. 1. Loading efficiency of CO encapsulated by Pickering emulsion with different added CO concentrations. CO-0.05; CO-0.1; CO-0.5; CO-1 represented the addition of 0.05%; 0.1%; 0.5%; 1% (w/w), respectively. The different letters outside the end of the bar diagram indicated significantly different treatments ($p < 0.05$).

Retention of cinnamon essential oil (CO)

The stability of active ingredients encapsulated in the emulsion can be evaluated from its retention during storage. This study observed the retention of CO in the Pickering emulsion after 7-days of storage. Figure 2 shows that the retention of CO was higher (78.86-79.20%) at the addition of 0.5% and 1% CO, compared to the addition of 0.05% CO ($p < 0.05$). Shah

et al. (2016) reported curcumin retention of 50% in Pickering emulsion after 5-days of storage.

The CO retention in the emulsion could be related to the diffusivity of essential oil from interior droplets into the continuous phase. The increase of retention indicated the lower diffusivity of the active ingredients in the matrix (Kaushik & Roos, 2007). In addition, the presence of covalent interactions between the

matrix and the active ingredients also affected the retention of active ingredients during storage (Lu *et al.*, 2014). Therefore, it was suggested that a higher added concentration of cinnamon essential oil might have lower diffusivity and more intensive covalent interaction with Pickering emulsion, compared to a lower added concentration of cinnamon essential oil. Another phenomenon was thought to be the presence

of nanoparticles that were strongly adsorbed and produce a solid layer on the droplet surface so that they could inhibit the migration of essential oils from the droplet interior to the outside (Jiang *et al.*, 2020). This causes high retention of a high concentration of cinnamon essential oil in the Pickering emulsion during storage.

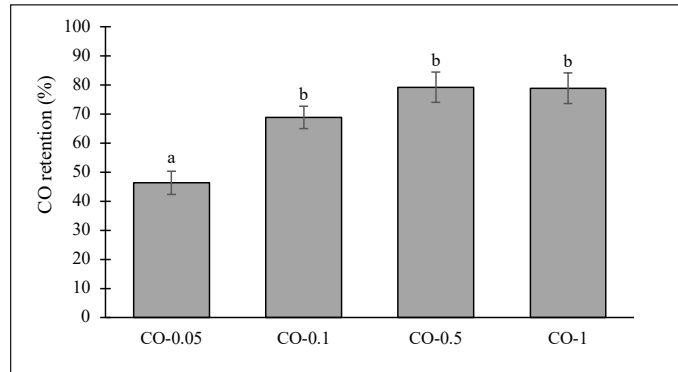


Fig. 2. Retention of CO encapsulated by Pickering emulsion with different added CO concentrations after 7-days of room storage. CO-0.05; CO-0.1; CO-0.5; CO-1 represented the addition of 0.05%; 0.1%; 0.5%; 1% (w/w), respectively. The different letters outside the end of the bar diagram indicated significantly different treatments ($p < 0.05$).

Emulsion properties

Appearance

This study performed a high-energy approach in the emulsification process, commonly used to break and mix the oil-water phase to produce small lipid droplets (Lee *et al.*, 2013; McClements & Gumus, 2016). The oil droplets seem to be the same size and surrounded by a layer of breadfruit starch nanoparticles (black layer) in all treatments (Figure 3). Therefore, the difference in CO concentration did not significantly affect the emulsion appearance. Shah *et al.* (2016) reported that curcumin encapsulation did not significantly affect emulsion droplet size.

One of the mechanisms of Pickering emulsion stabilization is the formation of a solid layer around the droplets containing essential oils (Dickinson, 2017). When two oil droplets are close to each other, the particles will envelop around the contact areas of droplets (Hunter *et al.*, 2008). Moreover, the driving force of the Pickering stabilization process was the reduction in Gibbs energy through adsorption of solid particles at the interface (Kralchevsky *et al.*, 2005; Sun, 2018). The addition of 3% breadfruit starch nanoparticles was assumed to form the structural barrier in the space among the droplets and stick to the oil-water interface to become protective against droplet coalescence (Dickinson, 2017).

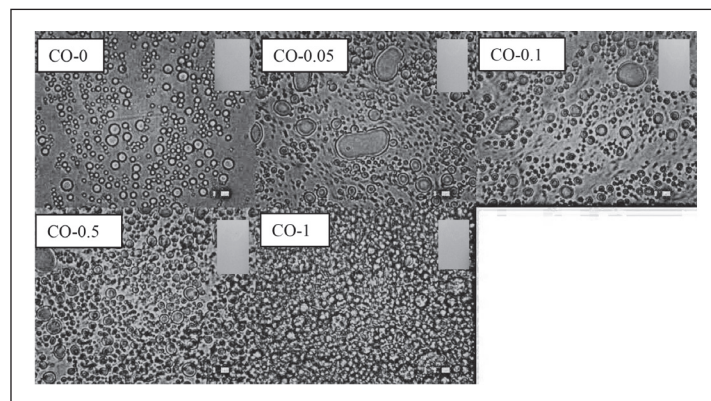


Fig. 3. Optical micrograph of the droplet in Pickering emulsion with/without CO addition before storage (magnification of 1000x). Scale bar (small white box at the bottom right of each image): 5 μm . CO-0 represented Pickering emulsion without CO addition; CO-0.05; CO-0.1; CO-0.5; CO-1 represented the addition of 0.05%; 0.1%; 0.5%; 1% (w/w), respectively. Inset: visual appearance of Pickering emulsion.

Droplet size, emulsion index, and viscosity

Table 1 shows that droplet size increased after 7 days of storage, compared to droplet size before storage of emulsion for all treatments. The increase in droplet size can be due to the coalescence phenomenon or Ostwald ripening during storage. Meanwhile, smaller droplet sizes were obtained at the addition of 0.5% and 1% CO, compared to other treatments (Table 1), which was likely related to the high retention of active ingredients. Small droplet sizes were better coated by particles so that they were better able to inhibit migration and maintain retention of an active ingredient during storage compared to large droplet sizes (Kaushik & Roos, 2007; Lu *et al.*, 2014).

According to Table 1, the emulsion index of 1 was formed in all treatments, either before or after

storage. Saari *et al.* (2017) stated that the emulsion index 1 indicated that the emulsion did not experience creaming or sedimentation. Theoretically, the particle aggregation could form the particle-droplet network at the interface resulting in creaming inhibition (Li *et al.*, 2013). The addition of 3% breadfruit starch nanoparticles and 40% MCT oil during emulsification was thought to play a role in forming stable Pickering emulsion. Ye *et al.* (2017) explained that high concentrations of starch nanoparticles could increase the interaction between starch nanoparticles and oil droplets, which led to good droplet coating and inhibition of phase separation. Several studies reported the role of high concentrations of starch nanoparticles and MCT oil in producing Pickering emulsion with an emulsion index of 1 (Ge *et al.*, 2017; Ye *et al.*, 2017).

Table 1. Pickering emulsion properties with/without addition before and after room storage. CO-0 represented Pickering emulsion without CO addition; CO-0.05; CO-0.1; CO-0.5; CO-1 represented the addition of 0.05%; 0.1%; 0.5%; 1% (w/w), respectively

	Droplet size ($d_{4,3}$) (μm) ^a		Emulsion index		Viscosity (cP) ^b	
	day 0	day 7	day 0	day 7	day 0	day 7
CO-0	20.98 ± 1.64	24.9 ± 1.06	1	1	763.8 ± 16.97 ^c	4769 ± 155.56 ^c
CO-0.05	23.62 ± 5.98	25.64 ± 5.65	1	1	789.8 ± 2.83 ^d	5059 ± 197.99 ^c
CO-0.1	17.81 ± 3.73	21.25 ± 4.49	1	1	813.8 ± 8.49 ^e	5849 ± 127.28 ^d
CO-0.5	11.66 ± 2.21	22.47 ± 5.27	1	1	721.8 ± 2.83 ^b	4249 ± 155.56 ^b
CO-1	14.28 ± 2.55	20.7 ± 3.91	1	1	629.9 ± 2.83 ^a	3209 ± 14.14 ^a

^aValue was expressed as mean size ± distribution

^bDifferent superscript letters indicated significantly different in the same column ($p < 0.05$)

The viscosity of Pickering emulsion ranged from 629.9 cP to 813.8 cP (before storage) and 3209 cP to 5849 cP (after 7-days of storage) (Table 1). The value of emulsion viscosity after storage was almost similar to the value of a high viscosity Pickering emulsion containing 50% MCT oil and stabilized with 0.25% corn starch nanoparticles (around 7000 cP), as reported by Ge *et al.* (2017). The increase in emulsion viscosity during storage was assumed to be due to the contact between starch nanoparticles that envelop adjacent droplets and cause particle zip, which was mentioned by Hunter *et al.* (2008). This results in the formation of particle bridges in the continuous phase thereby increasing the viscosity of the Pickering emulsion. This finding indicated that the breadfruit starch nanoparticle-stabilized Pickering emulsion has high viscosity. High viscosity positively impacts the stability of emulsions containing active ingredients, e.g., facilitating the inhibition of droplet damage in the short term, increasing encapsulation efficiency, and increasing resistance to external stress (Schmidt *et al.*, 2014; Muhoza *et al.*, 2019). The high viscosity of emulsion also can reduce the separation velocity (Mao & Miao, 2015) and might be associated with the emulsion index of 1 in all treatments for 7 days of storage (Table 1). Emulsion index 1 indicates the

absence of creaming of dispersed droplets. These results were following Stokes' law which relates high viscosity with low creaming speed (McClements & Gumus, 2016) so that there was no phase separation from the Pickering emulsion.

This study also found a significant decrease of emulsion viscosity when added 0.5% and 1% CO, compared to when added 0.05% and 0.1% CO ($p < 0.05$). This finding could be attributed to the decrease in droplet size before and after storage (Table 1) because the small droplet size would reduce the flow resistance of emulsion so that the viscosity was decreased. It was assumed that the addition of high CO concentrations during emulsification could increase the number of molecules in the oil phase, allowing intensive contact between the oil droplets and the homogenizer, leading to smaller droplets.

Whiteness and yellowness

Pickering emulsion contains droplets with $>1 \mu\text{m}$ in size (Table 1) thereby they scattered light. Hence, the Pickering emulsion looks opaque-white color (Rezaei *et al.*, 2019). McClements (2010) explained that emulsions containing micrometer-sized droplets will tend to look opaque because the droplet size is larger than the wavelength of visible light (400 nm),

which causes strong scattering of visible light. The addition of CO could reduce the whiteness level and increase the yellowness of Pickering emulsion, both on day-0 and day-7 of room storage (Figures 4A & 4B). The addition of 0.5% and 1% CO gave the lowest level of whiteness (+L*) and the highest level of yellowness (+b*) among other treatments ($p < 0.05$). This is thought to be related to the high loading efficiency of CO and CO retention of the Pickering emulsion in the addition of 0.5% and 1% CO.

These results indicated that the CO addition contributed to an increase of yellowish color

in the Pickering emulsion. The yellowish color might come from cinnamaldehyde (the major component of cinnamon essential oil). The addition of cinnamaldehyde had also been reported to give a yellowish color to the chitosan film, as reported by Chen *et al.* (2016). The natural yellowish color of cinnamaldehyde probably arises from reactions that occur during the biosynthesis of cinnamaldehyde, but this requires confirmation and further study. Previous studies had explained that cinnamaldehyde extracted from the cinnamon tree was a thick yellow liquid (Zhu *et al.*, 2017; Muhoza *et al.*, 2019).

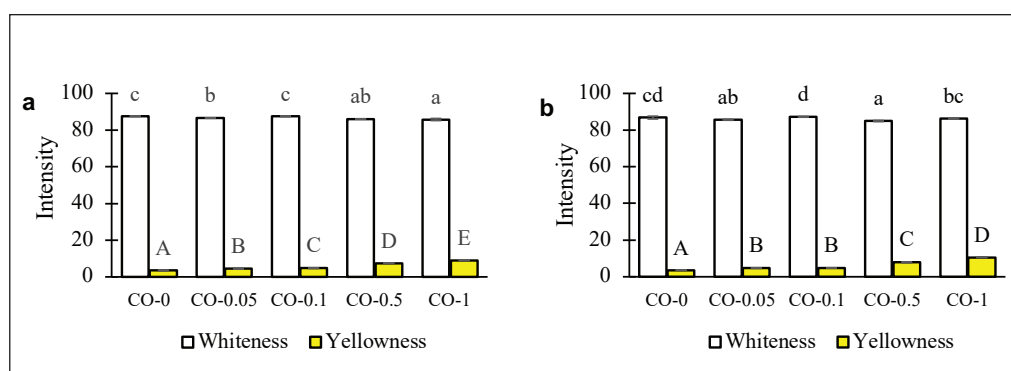


Fig. 4. Whiteness and yellowness level of Pickering emulsion with/without CO addition at a) day-0; b) day-7 of room storage. CO-0 represented Pickering emulsion without CO addition; CO-0.05; CO-0.1; CO-0.5; CO-1 represented the addition of 0.05%; 0.1%; 0.5%; 1% (w/w), respectively. The different letters outside the end of the bar diagram indicated significantly different treatments ($p < 0.05$).

CONCLUSION

Breadfruit starch nanoparticles could act as the stabilizer in the production of cinnamon essential oil-encapsulated Pickering emulsion. At the addition of 0.5% and 1% cinnamon essential oil, breadfruit starch nanoparticles-stabilized Pickering emulsion could encapsulate cinnamon oil with higher loading efficiency (79-80%) and higher retention (78.86-79.20%). It was stable for 7 days of room storage. In addition, the Pickering emulsion was viscous and yellowish color. Encapsulating materials based on Pickering emulsion is expected to be an alternative solution for expanding the cinnamon essential oil uses in food products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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