Research Article

DOI:10.22146/ifnp.70925 https://journal.ugm.ac.id/ifnp

ISSN 2597-9388

Indonesian Food and Nutrition Progress

Formulation and Characterization of Red Palm Oil Nanostructured Lipid Carriers Prepared by Microemulsion Method and Its Application in Yoghurt Drink

Dahlia Elianarni, Sri Raharjo, Supriyadi

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

*) Correspondence Email: dahliaelianarni6@gmail.com

ABSTRACT: Encapsulation of β-carotene compounds with nanostructured lipid carriers (NLC) is an effective way to prevent its dissolution and inactivation during application in beverage models and storage. This study aimed to investigate the characteristic of red palm oil nanostructured lipid carriers (RPO - NLC) made with the microemulsion method. The microemulsion procedures prepared with ratio Tween 80:Span 20 (80:20 ; 70:30), lipid : surfactant (1:3.75 ; 1:4), palm stearin : red palm oil (8:2 ; 7:3 ; 6:4). The best particle size was found at ratio 80:20, 1:4, 6:4 respectively with 24.73 ± 0.06 nm, zeta potential -7.43, turbidity 0.183% and polydispersity index at 0.06. This formulation indicated a loading capacity of 1.97%, encapsulation efficiency of 99.57%, FTIR, DSC, and stable emulsion using room temperature storage for four weeks. The best formulation was applied in drinking yoghurt with a ratio of nanostructured lipid carrier and yoghurt 1:18. The pH of yoghurt drink containing RPO-NLC increased from 4.13 to 4.37, while its encapsulation stability decreased from 78.45% to 49.29%. The results of the sensory evaluation showed no significant difference in colour and aroma, but there was a significant difference in the taste of drinking yoghurt compare with yoghurt – NLC.

Keywords: *β-carotene, encapsulation, lipid-based, storage stability*

INTRODUCTION

Red palm oil is oil obtained from palm mesocarp that has many nutrients such as saturated and unsaturated fatty acids, carotenoids, tocopherol, tocotrienol, sterol, phospholipid, squalene, and triterpenes (De Oliveira et al., 2015; Cazzonelli, 2011). Some of these phytonutrients are needed by the food and pharmaceutical industry (Mba et al., 2015). The natural pigment contained in red palm oil is carotenoid (Ruiz-zola et al., 2012). This carotenoid is soluble in oil. It has provitamin-A and antioxidant activity (Jomova et al., 2013). Because carotenoids in RPO are soluble in oil, it requires a suitable carrier system to be successfully dissolved into beverages or drink.

One of the lipid-based carriers is a nanostructured lipid carrier (NLC). NLC is better when compared to other carrier systems because it has high biocompatibility, long-term bioactive compound burst release, and imperfect crystal formation. Other than that, it has a high loading capacity, reduces the particle size, decreases the degree of gelation, and maintains the bioactive compound during storage (Khosa et al., 2018; Pardeike et al., 2019; Montenegro et al., 2016). Several methods that are commonly used for making nanostructured lipid carriers are hot homogenization, high-pressure homogenization, solvent evaporation, solvent emulsification-diffusion, microemulsion, sonication, and double emulsion (Lin et al., 2017; Shamsara et al., 2015).

In this study, the microemulsion method was selected because emulsion can form spontaneously with a ratio of surfactant and lipid of more than one (Yang et al., 2012). The combination of surfactant and co-surfactant was chosen for stabilizing the emulsion. Tween 80 as a surfactant has hydrophilic properties, and Span 20 as a co-surfactant have lipophilic properties (Suhendra et al., 2012). Moreover, the ratio between oil and solid lipid contributes to the characteristic of a microemulsion. The solid lipid used in this study is palm stearin. Palm stearin is the saturated fraction of about 86.65% that can encapsulate the bioactive compound (Liu et al., 2019; Wang et al., 2017).

The best NLC formula is applied to beverage products under acidic conditions. One of the beverage products that do not contain vitamin A and needs to be added with vitamin A is yoghurt. The addition of NLC into yoghurt drinks aims to fortify vitamin A in yoghurt drinks. The Recommended Dietary Allowance (RDAs) for vitamin A are 900 μg RAE for male and 700 μg RAE for female (Institute of Medicine, 2001).

MATERIALS AND METHODS

Material

Red palm oil was obtained from the Palm Oil Research Center (PPKS, Medan) and palm stearin was purchased from PT. Multimas Nabati Asahan. Aquabidest and Alcohol were purchased from CV. Progo Mulyo. Tween 80, Span 20, Standart beta carotene, Alcohol, n-hexane (Sigma Aldrich, Germany), and yoghurt commercial from the local market in Sleman, Special Region of Yogyakarta.

Preparation of Nanostructured lipid carrier

The microemulsion technique was selected to produce NLC dispersion. The ratio of palm stearin and red palm oil (6:4; 7:3; 8:2) was weighed and melted in the hot plate magnetic stirrer at 65 °C for 10 minutes. Then, the mixture surfactant of Tween 80 and Span 20 (80:20; 70:30) is heated to the same temperature and poured into the melted lipid phase little by little. Stir the mixture component at 65 °C for 10 minutes. 84 g of aquabidest heat at 65 °C and pour into the mixture little by little. Emulsification of this mixture at 65 °C for 20 minutes at 14000 rpm. The microemulsion was formed and continued with sonication for 5 minutes. The hot NLC was stored using room temperature overnight (Zhu et al., 2015).

Particle Size, Polydispersity Index, Zeta Potential

The average particle size, zeta potential, and polydispersity index NLC were determined using "Nanotrac Wave II-Model MN42x". 1 ml NLC was diluted with 9 ml aquabidest and stir it for 5 minutes at 500 rpm. The ready sample was injected into the sample cell (Pan et al., 2016).

Encapsulation Efficiency, Loading Capacity, Emulsion Stability

The efficiency encapsulation and loading capacity was measured with 0.5 ml NLC and 1 ml alcohol in a microtube. The system was centrifuged at 14000 rpm, 27 °C, for 30 minutes. The remaining supernatant was carefully removed using a micropipette, and its absorbance was registered as an unloaded beta carotene using a spectrophotometer UV-Vis at 450 nm. Each bioactive encapsulated (W total) mass was measured with the initial beta carotene minus unloaded beta carotene. The calculation of efficiency encapsulation and loading capacity use this equation, respectively.

EE(%) = (Wtotal – Wfree)100/Wtotal

LC (%) = (Wtotal–Wfree)100/ Wnp

W Total is the total beta carotene that is encapsulated in a NLC, W free is the total unloaded beta carotene in a NLC , and W np is the total lipid that adds to the NLC (D'Onofre Couto et al., (2021); Bashiri et al., (2020).

FTIR Spectroscopy Analysis

FTIR spectroscopic study of NLC was recorded using Shimadzu IR/FTIR spectrophotometer (435 U-40, Japan). The FTIR spectra were logged in a wavelength region of 4500 – 400 cm-1 at ambient temperature with a resolution of 4 cm-1. The NLC formula were separately mixed with potassium bromide (KBr) at ratio 1:100, then presses with a hydraulic press into discs for 5 min under continuous pressure of 5 tons (Nahr et al., 2018).

Thermal Analysis

Thermal analysis was performed on Differential scanning calorimetry with setting temperature from 30 – 300 °C. Put the complete information about the sample. The airflow is always controlled by the setting. Turn off the furnace until under 50 °C and clean the plate detector. Cool down the furnace until it reached room temperature (Das et al., (2017); Huang et al., (2017).

Thermal Analysis

Thermal analysis was performed on Differential scanning calorimetry with setting temperature from 30 – 300 °C. Put the complete information about the sample. The airflow is always controlled by the setting. Turn off the furnace until under 50 °C and clean the plate detector. Cool down the furnace until it reached room temperature (Das et al., (2017); Huang et al., (2017).

Turbidity

The turbidity of the NLC was measured with a spectrophotometer UV – Vis at 600 nm using room temperature. Aquabidest is set as a blank solution. The absorbance that we get calculated in this equation (Azevedo et al., 2021).

T(%) = 2.203 × sample absorbance (Fletcher dan Suhling, 1998).

Encapsulation Stability during Application in Yoghurt

The encapsulation stability of Yoghurt – NLC was determined with pour 0.5 ml Yoghurt – NLC and 1 ml ethanol 96% into a microtube. After that, the mixture was centrifuged at 14000 rpm, 27 °C, for 30 minutes. The supernatant was carefully removed, and measure the absorbance using a spectrophotometer UV-Vis at 450 nm. The absorbance of supernatant shows the amount of unloaded beta carotene. To calculate the stability encapsulation, this equation was used.

ES% = (WTR-WFR)100/WER1

WTR is the total beta carotene added into the NLC , WFR is the total unloaded beta carotene during application in the drinking model, and WER1 is the amount of beta carotene encapsulated in the NLC (Babazadeh et al., 2016).

pH measurement of yoghurt – NLC

The pH value of yoghurt – NLC samples was measured using a glass electrode pH Meter (Ohauss Starter 5000, China) which has been calibrated at 4.00, 7.00, and 10.01. The electrode was put into the sample, and measure the pH was with three repetitions. The measurement is taken once a week for a month in the refrigerator(Umeyor et al., 2021).

Sensory Evaluation

Twenty semi-trained panellists was choose to evaluated the sample. The method was based on a different test using paired comparison test with two sets of samples. Every sample sets is consists of two kinds of samples that is control and yoghurt – NLC. The attributes that were evaluated are color, flavour, and taste. The scale consists of two numbers, 1 for the attribute that has a difference and 0 for the attribute that doesn't have a difference (same). The sample was given to the panellist in coded plastic cups 20 ml (Lawles et al., 2010).

RESULT AND DISCUSSION

Characteristics of nanostructured lipid carrier formulation

Table 1 shows the particle size of the NLC before and after 30 days of storage. During storage, the particle size of droplet show increase in every formulation NLC. The smaller particle size found in the formula Tween 80:Span 20 = 80:20, Lipid:Surfactan = 1:4, Palm stearin:Red palm oil = 6:4. Until 30 days of storage, this formula has the smallest increasing size particle droplet. This indicates the higher concentration of surfactant, ratio of palm stearin and red palm oil affect the stability of droplet particle size. The type and concentration of surfactants used in NLC have an essential role in the size particle. Increasing the amount of surfactant in the emulsion system can decrease the particle size droplet. On the other hand, the ratio between lipid and oil impacts particle size diversity (Salvi & Pawar, 2019).

Table 1. The droplet particle size of RPO – NLC before and after 30 days of storage

Tween 80: Span 20	Lipid: Surfactant	Palm Stearin: Red Palm Oil	Particle Size (nm) Day 0	Day 30
80:20	1:3.75	6:4	36.60±0.98	63.37±1.77
		7:3	45.93±0.35	88.93±0.67
		8:2	62.63±1.85	74.56±1.72[a]
	1:4	6:4	24.73±0.22	51.17±0.25[b]
		7:3	44.47±0.55	86.83±1.65[b]
		8:2	26.41±0.26	51.20±0.60[b]
70:30	1:3.75	6:4	49.30±1.93	86.27±1.98[b]
		7:3	48.70±2.09	90.77±1.86[b]
		8:2	50.90±0.46	90.47±2.14[b]
	1:4	6:4	52.17±1.48	80.13±1.27[b]
		7:3	53.27±1.28	90.23±0.99[b]
		8:2	49.80±0.46	86.60±3.20[b]

Data expressed as mean ± SD, n = 3. Statistical analysis performed using analysis of variance (ANOVA) followed by T-test paired comparison. a statistically differs from the day 0 ($p < 0.05$). b statistically very differs from day 0 ($p<0.01$)

The appropriate combination between Tween 80 and Span 20 also contributes to the droplet particle size of the NLC. The ratio of 80:20 produces smaller particles compared to the ratio of 70:30. The different models of NLC need a different ratio of surfactant and co-surfactant. NLC from shea butter and Penthaclethra macrophylla use the ratio 1:1 of Tween 80:Span 20 with sodium dodecyl sulfate and plant care (Ajala et al., 2021). The proper combination of surfactant and co–surfactant can shrink the droplet particle size with adsorbing interface between the oil and water phase that makes a dense surfactant film layer (Shevalkar & Vavia, 2019).

During the storage of NLC, the droplet particle size in each formulation was increased. This cause aggregation droplet and agglomerate to the bigger NLC particle. Several factors influence the droplet particle size of NLC are lipid type, ratio of solid / liquid lipid, proportion, surfactant type, sonication, and emulsion cooling rate (Bashiri et al., 2020).

The best NLC was obtained in formula Tween 80:Span 20 = 80:20, lipid:surfactant = 1:4, and lipid : oil = 6:4 because it had the smallest size droplet on day 0 and storage for up to 30 days. The best formula was chosen to be applied to yoghurt drinks because it has the most stable emulsion so that it can maintain the β-carotene component in the RPO (Selvamuthukumar & Velmurugan, 2012). The best formula is first characterized by encapsulation efficiency, loading capacity, turbidity, functional group, and thermal profile before application to yoghurt drinks. Only the best formulas will be applied under acidic conditions because these formulas have the best droplet particle size stability to retain the bioactive components in them.

Characterization of best formula RPO-NLC

Efficiency encapsulation indicated the percentage of a β-carotene that was successfully encapsulated in this NLC. According to Table 2, the efficiency encapsulation of this formula is 99.6%. In a previous study, the efficiency of NLC from piceatannol is 94.91% (Krambeck et al., 2021). The encapsulation ability of each system is diverse according to the method and materials used. The main factor contributing to efficiency encapsulation is the surfactant concentration and solubility of β-carotene in the lipid phase (Cheow and Hadinoto, 2011).

Table 2. β-Carotene efficiency encapsulation and Loading capacity of NLC

Parameter	Percentage
Efficiency encapsulation	99.60% ± 0.15
Loading Capacity	1.97% ± 0.03

Loading capacity is the amount of β- carotene that can be plugged into the system when adding oil. The result of loading capacity analysis is 1.97%. In the other study, the loading capacity of NLC contain curcumin with the melt-emulsification-ultrasonication method is 5.0% (Sakellari et al., 2021). The variety of loading capacity is caused by the solubility of the bioactive compound in oil and total oil addition in the system (Houacine et al., 2020).

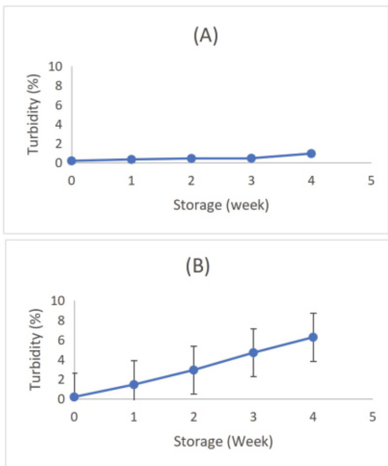

Figure 1. RPO-NLC stability during storage using room temperature (A) and 37 °C incubators (B).

Emulsion stability analysis was carried out by measuring turbidity every week for one month in two conditions, room temperature and incubator. Figure 1 shows that storage in the incubator has a relatively high increase in turbidity values compared with room temperature. NLC storage should be kept using room temperature because at 40 °C increasing particles happen from 206 to 286 nm (Raju et al., 2021). The best condition storage of NLC was found at 4 °C (Azevedo et al., 2021). The higher storage temperature caused the increase in kinetic energy in the NLC droplets. High kinetic energy can trigger coalescence between droplets so that there is an increase in droplet particle size due to agglomeration (Makoni et al., 2019).

FTIR spectroscopy analysis

FTIR spectroscopy was chosen to find out the functional group due to adding components and interaction between materials inside RPO-NLC. The result of spectra FTIR can be seen in Figure 2.

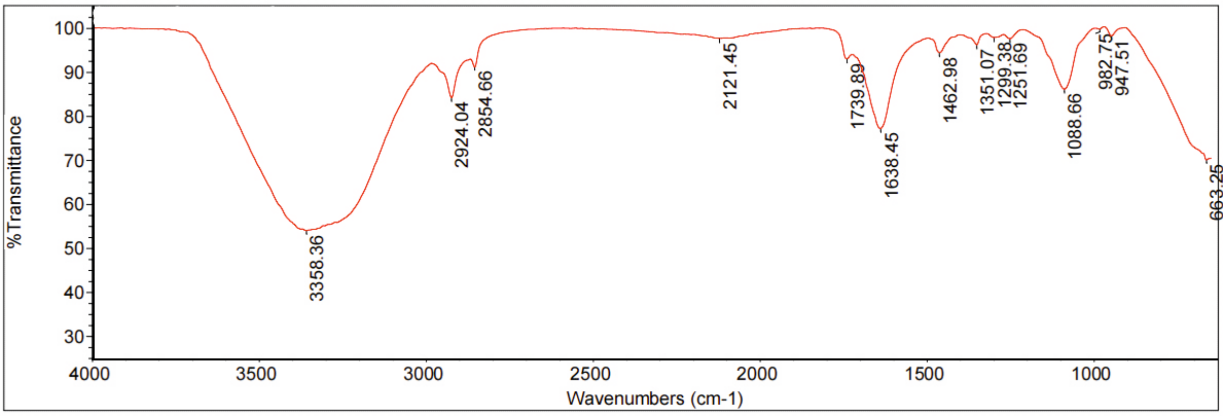

Figure 2. FTIR spectra of RPO-NLC

The first peak appears in 3358.36 cm-1 presence-free O-H group. Wavenumber between 3000-3700 cm-1 indicates the free OH groups (Karimi Khorrami et al., 2021). The peaks appearing in 2924.04 cm-1 dan 2854.66 cm-1 conduct the C-H group, especially CH2 and CH3. Streching vibration in range 2850-3000 cm-1 shows CH2 (2958.15, 2926.45) and CH3 (2868.23) (Eid et al., 2020). This functional group discovers the triacylglycerol component used as the lipid phase in NLC.

The peak observed at 2121.45 cm-1 is related to the existence of H-O-H groups that stretch from 2300 cm-1. H-O-H is the leading functional group that composes aquadest in the NLC. A similar result has been reported in the previous study that 2300 show the H-O-H group but shifted into 1845 cm-1, 2005 cm-1, 2047 cm-1, 2107 cm-1, and 2177 cm-1 due to the use of high temperature(Sohrabi et al., 2021).

Spectra found in wavenumber 1739.89 and 1462.98 are described as C=O (carbonyl ester) and C=C (aromatic ring, respectively. Similarly, the carbonyl ester group was found at wavenumber 1702.02 at stearic acid analysis (Khan et al., 2021). The absorption band at 1583 and 1461 are stretching peaks for aromatic rings (C=C)(Rajput & Butani, 2019).

Thermal Analysis

DSC thermograms reveal physicochemical characteristics during temperature changes. The thermogram obtained in the best formulation of NLC has been shown in Figure 3.

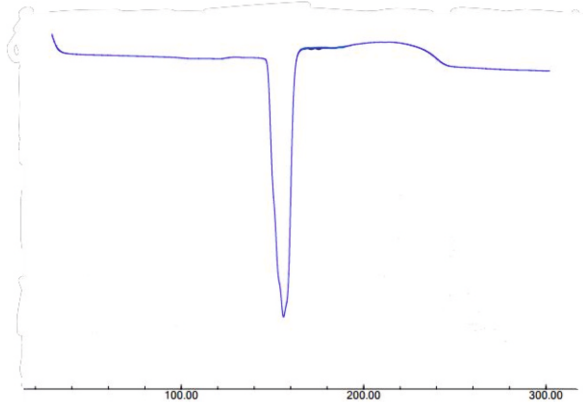

Figure 3. DSC curves of RPO - NLC

DSC thermograms of RPO – NLC generate two endothermic peaks at 83.97 °C and 146.03 °C. The first peak was detected because the utilization of palm stearin can encapsulate the beta carotene in a solid matrix. The peak is slightly low because of a bit of excess surfactant. It is related to a previous study that found a low peak at 82 °C in a bit addition of emulsifiers (Maretti et al., 2021). The sharpest peak at 146.03 °C describes the breakdown of encapsulated beta carotene in a NLC. Another study reported that the NLC used piperine and quercetin as bioactive compounds and had a sharp endothermic peak at 131 °C and 316 °C. When lyophilized drugs produce a sharp endothermic peak, the melting temperature of the drug can't be detected because of entrapment in the amorf matrix. In addition, there is no bioactive compound located on the NLC's surface (Chaudhari et al., 2021).

Application in drinking yoghurt model
Encapsulation Stability of β-carotene

After the emulsion was stored in a refrigerator, encapsulation stability of beta carotene was measured once a week for a month. The stability of beta carotene inside yoghurt is shown in figure 4.

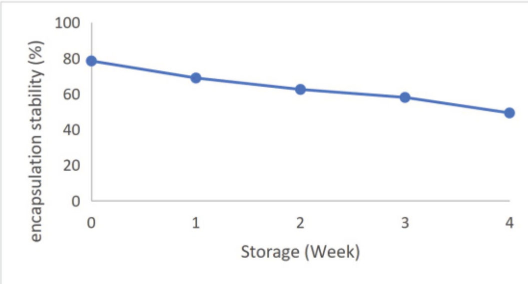

Figure 4. Stability encapsulation of β-carotene from NLC to yoghurt drink stored at 6 °C (refrigerator temperature).

The stability of β-carotene encapsulated with NLC applied in drinking yoghurt decreased during storage. Firstly the encapsulation stability is 78.45%, and along with the storage process, the stability decreased to 49.29%. Based on these results, it can see that storage time and pH conditions affect the stability of the encapsulation. Research conducted by (Huang et al., 2021) exhibits that the chemical stability of proanthocyanidin and β-carotene encapsulated with rice bran wax with W/O/W multiple emulsion decreased during storage. The impact of pH conditions is supported by (Liu et al., 2018) if β-carotene is applied in acidic conditions, the stability is reduced because of particle protonation, isomerization, and cis-trans degradation. The reduction of β-carotene stored for 24 days at 25 °C indicate a drastic decrease of about 70% from the initial amount.

pH measurement of yoghurt - NLC

The pH of yoghurt fortified RPO – NLC during four-week storage is shown in Figure 5. The result indicates that the pH is increasing but not significantly different for each week.

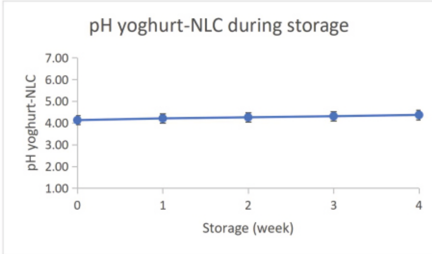

Figure 5. pH measurement of yoghurt drink containing RPO - NLC stored at 6 °C (refrigerator temperature).

The initial pH of the yoghurt – NLC was 4.13. The storage time indicates a rise in pH value in the following weeks at 4.21, 4.26, 4.31, and 4.37, respectively. In contrast (Qiu et al., 2021) show a reduction of pH value through the storage of yoghurt fortified with the edible rose effect of lactic acid production in the starter.

The slight rise in pH value of yoghurt – NLC is presumably caused by syneresis at the addition of a NLC. Syneresis can be caused by several things, such as the addition of stabilizers and the conversion of protein into amino acid by lactic acid bacteria. Conversion of protein is caused by lactose running out, so the lactic acid bacteria consume protein and change into amino acids. Supported by (Mohammadi-Gouraji et al., 2019) yoghurt enrich phycocyanin generates an increasing pH value in the 21 days of storage. Storage duration can increase syneresis due to damage of water-binding proteins.

Sensory Evaluation

The pair comparison test was chosen as a method of sensory evaluation. There are three parameters observed that are colour, aroma, and taste. The pair comparison test method was chosen to determine the difference between pure yoghurt and yoghurt – NLC. This pair comparison is used to determine the changes in sensory parameters in yoghurt during fortification, if there is no change after adding NLC then the fortification process can be said to be successful. If the sensory quality of the yoghurt added with NLC does not change, the fortification process does not affect the sensory quality of the yoghurt. Observations were carried out four times by 20 semi-trained panellists. The result of the sensory evaluation is shown in Table 3.

Table 3. Sensory evaluation : Comparison between control and Yoghurt NLC

Storage Time	Parameter		
	Colour	Aroma	Taste
Week 0	12^{a}	9^{a}	4^{b}
Week 1	15^{a}	8^{a}	3^{b}
Week 2	11^{a}	8^{a}	2^{b}
Week 3	11^{a}	7^{a}	4^{b}

The number indicates total of panellists who stated the same between the treatment and control samples.
a statistically not differs from the control sample. b statistically very differs from the control sample ($p<0.01$)

Based on Table 3 we know that the colour of yoghurt – NLC has no significant difference compared to pure yoghurt. The addition of RPO – NLC into the yoghurt was only 5.26%. These findings agree with data reported by (Zedan et al., 2021) addition 2%, 4%, and 6% tarragon essential oil into yoghurt produces no significant difference at colour evaluation for 15 days storage at 4 °C. The same result taken out by (Salama et al., 2021) yoghurt added with nanoemulsion essential oil about 25 mg and 50 mg generates no significant difference in colour.

Sensory evaluation during storage also resulted in a non-significant difference in aroma. According to research conducted by (de Campo et al., 2019), yoghurt fortified zeaxanthin with nanoparticle and nanoemulsion produces the same aroma compared with pure yoghurt. Furthermore, a study conducted by (Van Nieuwenhove et al., 2019) explained that yoghurt with pomegranate and jacandara oilseed has no significant difference in texture and aroma.

The different results were found in the colour parameters because they produce significantly different flavours from the first week to the end of storage. A similar result was described by (Baba et al., 2018) addition of walnut oil emulsion, flaxseed oil, and guar gum reduces acidity, texture, and creamy taste in fortified yoghurt. These changes are caused by the use of tween 80 and span 20 as emulsifiers. So, it contributes to produce an unwanted aftertaste. Suitable to results presented by (Zhong et al., 2018), yoghurt added with fish oil/γ-oryzanol low energy emulsification use tween 80 and span 20 as emulsifiers induce a significant difference compared to pure yoghurt.

CONCLUSION

The best formula of RPO – NLC found at ratio Tween 80: Span 20 = 80:20, Lipid: surfactant = 1:4, palm stearin : red palm oil = 6:4. The characteristic best formula of RPO – NLC has 99.60% encapsulation efficiency, 1.97% loading capacity, and stability using room temperature storage. Application of RPO - NLC into yoghurt has decreased encapsulation stability and increased pH value during storage. The sensory evaluation of yoghurt – NLC has no significant colour and aroma but a significant difference in taste.

ACKNOWLEDGEMENT

This work was funded by The Oil Palm Plantation Fund Management Agency, Republic of Indonesia, grant number PRJ-09/DPKS/2020.

REFERENCE

Ajala, T. O., Abraham, A., Keck, C. M., Odeku, O. A., Elufioye, T. O., & Olopade, J. O. 2021. Shea butter (Vitellaria paradoxa) and Pentaclethra macrophylla oil as lipids in the formulation of Nanostructured lipid carriers. *Scientific African*, *13*, 00965.

Azevedo, M. A., Cerqueira, M. A., Fuciños, P., Silva, B. F. B., Teixeira, J. A., & Pastrana, L. 2021. Rhamnolipids-based nanostructured lipid carriers: Effect of lipid phase on physicochemical properties and stability. *Food Chemistry*, *344*. 128670.

Baba, W. N., Jan, K., Punoo, H. A., Wani, T. A., Dar, M. M., & Masoodi, F. A. 2018. Techno-functional properties of yoghurts fortified with walnut and flaxseed oil emulsions in guar gum. *LWT - Food Science and Technology*, *92*, 242–249.

Babazadeh, A., Ghanbarzadeh, B., Hamishehkar, H. 2016. Novel nanostructured lipid carrier as a promising food-grade delivery system for ruitin. Journal of functional food. 26. 167 – 175.

Bashiri, S., Ghanbarzadeh, B., Ayaseh, A., Dehghannya, J., & Ehsani, A. 2020. Preparation and characterization of chitosan-coated nanostructured lipid carriers (CH-NLC) containing cinnamon essential oil for enriching milk and antioxidant activity. *Lwt*, *119*(July 2019), 108836.

Cazzonelli, C. I., 2011. Carotenoids in nature: insights from plants and beyond. Function Plant Biology. 38 (11). 833-847

Chaudhari, V. S., Murty, U. S., & Banerjee, S. 2021. Nanostructured lipid carriers as a strategy for encapsulation of active plant constituents: Formulation and in vitro physicochemical characterizations. *Chemistry and Physics of Lipids*, *235*, 105037.

Cheow, W.S., Hadinoto, K., 2011. Factors affecting drug encapsulation and stability of lipid–polymer hybrid nanoparticles. Colloids Surfaes B Biointerfaces 85, 214–220.

D'Onofre Couto, B., Novaes da Costa, R., Castro Laurindo, W., Moraes da Silva, H., Rocha da Silva, C., Sélia dos Reis Coimbra, J., Barbosa Mageste, A., de Cássia Dias, S., & José Boggione Santos, I. 2021. Characterization, techno-functional properties, and encapsulation efficiency of self-assembled β-lactoglobulin nanostructures. *Food Chemistry*, *356*. 129719.

Das, S., Ghosh, S., De, A. K., Bera, T. 2017. Oral delivery of ursolic acid loaded nanostructured lipid carrier coated with chitosan oligosaccharide: development, characterization, in vitro and in vivo assessment for the therapy of leishmaniasis. International journal of biological macromolecules. 102. 996 – 1008.

de Campo, C., Queiroz Assis, R., Marques da Silva, M., Haas Costa, T. M., Paese, K., Stanisçuaski Guterres, S., de Oliveira Rios, A., & Hickmann Flôres, S. 2019. Incorporation of zeaxanthin nanoparticles in yogurt: Influence on physicochemical properties, carotenoid stability and sensory analysis. Food Chemistry. 301. 125230.

De Oliveira, G. M., Ribeiro, A. P. B., Kieckbutsch, T. G. 2015. Hard fat improve technological properties of palm oil for application in fat-based products. LWT – Food Science and Technology. 63. 1155-1162

Eid, R. K., Ashour, D. S., Essa, E. A., El Maghraby, G. M., & Arafa, M. F. 2020. Chitosan coated nanostructured lipid carriers for enhanced in vivo efficacy of albendazole against Trichinella spiralis. *Carbohydrate Polymers*, *232*, 115826.

Fletcher, P. D. I., and Suhling, K. 1998. Interaction between weakly charged oil in water microemulsion droplets. Langmuir. 14. 4065 – 4069.

Houacine, C., Adams, D., & Singh, K. K. 2020. Impact of liquid lipid on development and stability of trimyristin nanostructured lipid carriers for oral delivery of resveratrol. *Journal of Molecular Liquids*, *316*, 113734.

Huang, W., Dou, H., Wu, H., Sun, Z., Wang, H., Huang, L. 2017. Preparation and characterization of nobiletin loaded nanostructured lipid carriers. Journal of nanomaterials. 215. 1 – 12.

Huang, Z., Guo, B., Deng, C., Tang, C., Liu, C., & Hu, X. 2021. Fabrication and characterization of the W/O/W multiple emulsion through oleogelation of oil. *Food Chemistry*, *358*, 129856.

Institute of Medicine. 2001. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.Washington, DC: National Academy Press.

Jomova, K., Valko, M. 2013. Health protective effects of carotenoids and their interactions with other biological antioxidants. Europe Journal Medicine Chemistry. 102 -110.

Karimi Khorrami, N., Radi, M., Amiri, S., & McClements, D. J. 2021. Fabrication and characterization of alginate-based films functionalized with nanostructured lipid carriers. *International Journal of Biological Macromolecules*, *182*, 373–384.

Khan, A. S., ud Din, F., Ali, Z., Bibi, M., Zahid, F., Zeb, A., Mujeeb-ur-Rehman, & Khan, G. M. 2021. Development, in vitro and in vivo evaluation of miltefosine loaded nanostructured lipid carriers for the treatment of Cutaneous Leishmaniasis. *International Journal of Pharmaceutics*, *593*, 120109.

Khosa, A., Reddi, S., Saha, R. N. 2018. Nanostructured lipid carrier for site-specific drug delivery. Journal of Biomedicine Pharmacoter. 103. 598 – 613.

Krambeck, K., Silva, V., Silva, R., Fernandes, C., Cagide, F., Borges, F., Santos, D., Otero-Espinar, F., Lobo, J. M. S., & Amaral, M. H. 2021. Design and characterization of Nanostructured lipid carriers (NLC) and Nanostructured lipid carrier-based hydrogels containing Passiflora edulis seeds oil. *International Journal of Pharmaceutics*, *600,* 120444.

Lawless, H. T., and Hildegarde, H. 2010. Sensory evaluation of food – principle and practices. Second edition. Springer.

Lin, C. H., Chen, C. H., Lin, Z. C., Fang, J. Y. 2017. Recent advances in oral delivery of drugs and bioactive natural product using solid lipid nanoparticles as the carriers. Review article. Journal of food and drug analysis. 25. 219 – 234.

Liu, C., Meng, Z., Chai, X., Liang, X., Piatko, M., Campbell, S., and Liu, Y. 2019. Comparative analysis of graded blends of palm kernel oil, palm kernel sterain, and palm stearin. Journal of Food Chemistry. 286. 636 – 643.

Liu, W., Wang, J., McClements, D. J., & Zou, L. 2018. Encapsulation of β-carotene-loaded oil droplets in caseinate/alginate microparticles: Enhancement of carotenoid stability and bioaccessibility. *Journal of Functional Foods*, *40*, 527–535.

Makoni, P. A., Kasongo, K. W., & Walker, R. B. 2019. Short-term stability testing of efavirenz-loaded solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) dispersions. *Pharmaceutics*, *11*(8).11080397.

Maretti, E., Leo, E., Rustichelli, C., Truzzi, E., Siligardi, C., & Iannuccelli, V. 2021. In vivo β-carotene skin permeation modulated by Nanostructured Lipid Carriers. *International Journal of Pharmaceutics*, *597*, 120322.

Mba, O.I., Dumont, M. J., Ngadi, M., 2015. Palm oil: processing, characterization and utilization in the food industry. A review. Food Bioscience. 10. 26-41.

Mohammadi-Gouraji, E., Soleimanian-Zad, S., & Ghiaci, M. 2019. Phycocyanin-enriched yogurt and its antibacterial and physicochemical properties during 21 days of storage. *Lwt*, *102*, 230–236.

Montenegro, L., Lai, F., Offerta, A., Sarpietro, M. G., Micicche, L., Maccioni, A. M., Valenti, D., Fadda, A. M. 2016. From nanoemulsion to nanostructured lipid carrier: A relevant development in dermal delivery of drugs and cosmetics. Journal of drug delivery science and technology. 32. 100 – 112.

Nahr, F. K., Ghanbarzadeh, B., Hamishehkar, H., Kafil, H. S. 2018. Food grade nanostructured lipid carrier for cardamom essential oil: preparation, characterization, and antimicrobial activity. Journal of functional food. 40. 1 – 8.

Pan, Y., Rohan, V. T., Nitin, N. 2016. Distribution of a model bioactive within solid lipid nanoparticle and nanostructured lipid carriers influences its loading efficiency and oxidative stability. International journal of pharmaceutics. 511. 322 – 330.

Pardeike, J., Weber, S., Haber, T., Wagner, J., Zarfl, H. P., Plank, H., Zimmer, A. 2011. Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. International journal of pharmaceutics. 419. 329 – 338.

Qiu, L., Zhang, M., Mujumdar, A. S., & Chang, L. 2021. Effect of edible rose (Rosa rugosa cv. Plena) flower extract addition on the physicochemical, rheological, functional and sensory properties of set-type yogurt. *Food Bioscience*, *43*.101249.

Rajput, A. P., & Butani, S. B. 2019. Resveratrol anchored nanostructured lipid carrier loaded in situ gel via nasal route: Formulation, optimization and in vivo characterization. *Journal of Drug Delivery Science and Technology*, *51*, 214–223.

Raju, M., Kunde, S. S., Auti, S. T., Kulkarni, Y. A., & Wairkar, S. 2021. Berberine loaded nanostructured lipid carrier for Alzheimer's disease: Design, statistical optimization and enhanced in vivo performance. *Life Sciences*, *285*, 119990.

Ruiz-Zola, M. A., Rodriguez-Concepcion, M. Carotenoid biosynthesis in Arabidopsis: a colorful pathway. The Arabidopsis Book/American Society of Plant Biologists. 10.

Sakellari, G. I., Zafeiri, I., Batchelor, H., & Spyropoulos, F. 2021. Formulation design, production and characterization of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for the encapsulation of a model hydrophobic active. *Food Hydrocolloids for Health*, *1*, 100024.

Salama, H. H., El-Sayed, H. S., Kholif, A. M. M., & Edris, A. E. 2021. Essential oils nanoemulsion for the flavoring of functional stirred yogurt: Manufacturing, physicochemical, microbiological, and sensorial investigation. *Journal of the Saudi Society of Agricultural Sciences*.10.001.

Salvi, V. R., & Pawar, P. 2019. Journal of Drug Delivery Science and Technology Nanostructured lipid carriers (NLC) system : A novel drug targeting carrier. *Journal of Drug Delivery Science and Technology*, *51*(990), 255–267.

Selvamuthukumar, S., & Velmurugan, R. (2012). Nanostructured Lipid Carriers: A potential drug carrier for cancer chemotherapy. *Lipids in Hea lth and Disease*, *11*(1), 1–8. https://doi.org/10.1186/1476-511X-11-159

Shamsara, O., Muhidinov, Z. K., Jafari, S. M., Bobokalonov, J., Jonmurodov, A., Taghvaei, M. 2015. Effect of ultrasonication, pH, and heating on stability of apricot gum lactoglobulin two layer nanoemulsion. International Journal of Biological Macromolecules. 81. 1019 – 1025.

Shevalkar, G., and Vavia, P. 2019. Solidified nanostructured lipid carrier (S-NLC) for enhancing the oral bioavailability of ezetimibe. Journal of drug delivery science and technology. 53. 101211.

Sohrabi, S., Keshavarz Moraveji, M., & Iranshahi, D. 2021. Morphological and structural insights into high aspect ratio lauric acid/TiO_2 nanowires: A low-temperature synthesis. *Ceramics International*, *47*(7), 9424 – 9436.

Suhendra, L., Raharjo, S, Pudji, H., Hidayat, C. 2012. Formulasi dan stabilitas mikroemulsi o/w sebagai pembawa fucoxanthin. Agritech. 32. 230-239.

mikroemulsi o/w sebagai pembawa fucoxanthin. Agritech. 32. 230-239.

Umeyor, C. E., Okoye, I., Uronnachi, E., Okeke, T., Kenechukwu, F., & Attama, A. 2021. Repositioning miconazole nitrate for malaria: Formulation of sustained release nanostructured lipid carriers, structure characterization and in vivo antimalarial evaluation. *Journal of Drug Delivery Science and Technology*, *61*, 102125.

Van Nieuwenhove, C. P., Moyano, A., Castro-Gómez, P., Fontecha, J., Sáez, G., Zárate, G., & Pizarro, P. L. 2019. Comparative study of pomegranate and jacaranda seeds as functional components for the conjugated linolenic acid enrichment of yogurt. *Lwt*, *111*, 401–407.

Wang, X., Hongyan, L., Fangqin, W., Guixue, X., Hongjun, L., Xiaojie, C., Ming, K., Ya, L., Chao, F., Xiguang, C., and Ying, W. 2017. Frontiers of material science. 11. 66 – 74.

Yang, Y., Marshall-Breton, C., Leser, M. E., Sher, A. A., McClements, D. J. 2012. Fabrication of ultrafine edible emulsions: comparison of high – energy and low – energy homogenization method. Food hydrocolloids. 29. 398 – 406.

Zedan, H., Hosseini, S. M., & Mohammadi, A. 2021. The effect of tarragon (Artemisia dracunculus) essential oil and high molecular weight Chitosan on sensory properties and shelf life of yogurt. *Lwt*, *147*, 111613.

Zhong, J., Yang, R., Cao, X., Liu, X., & Qin, X. 2018. Improved Physicochemical Properties of Yogurt Fortified with Fish Oil/γ-Oryzanol by Nanoemulsion Technology. *Molecules*, *23*(1), 56.

Zhu, J., Pan, Z., Lanla, L., Qi, S, feiwei, C. 2015. Preparation and characterization of novel nanocarriers containing krill oil for food application. Journal of functional food. 19. 902 - 912.