International Food Research Journal 25(4): 1349-1356 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

# β-Carotene bioavailability of palm oil emulsion drink in rats *(Rattus norvegicus)* blood plasma and liver

[1*]Harlen, W. C.,[1,2]Muchtadi, T.R. and [1]Palupi, N. S.

[1]*Department of Food Science, Faculty of Agricultural Technology, Bogor Agricultural University, Dramaga, Bogor, Indonesia16002*
[2]*Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University, Dramaga, Bogor, Indonesia16680*

**Article history**

*Received: 16 March 2017*
*Received in revised form: 11 May 2017*
*Accepted: 13 May 2017*

**Keywords**

*β-Carotene*
*Bioavailability*
*Palm oil emulsion drink*
*Retinol accumulation factor (RAF)*

**Abstract**

Palm oil emulsion drink (POED) is a kind of oil-in-water emulsion considered to be a good source of vitamin A as β-carotene but its bioavailability was not well known. The aim of this research were (1) to review the chemical composition and β-carotene content of POED and(2) to evaluate bioavailability of β-carotene based on *in vivo* study. The chemical composition were determined using proximate analysis, while bioavailability evaluation was carried out by measuring Retinol Accumulation Factor (RAF). Thirty-six male Sprague-dawley rats received a standard diet as an adaptation period and a low-vitamin A-diet as depletion period. In the repletion period, rats were divided into four groups: control (C) group, Positive Control (PC) group, Negative Control (NC) group and Palm Oil Emulsion Drink (POED) group. C group received standard diet, while NC group received depletion diet. The other two groups received depletion diet enriched with pure synthetic β-carotene (for PC group) and POED (for POED group). The POED-enriched-diet and pure-synthetic-β-carotene-diet contained 180 µg β-carotene. At the end of repletion period, all rats were sacrificed then its plasma and liver were colected to provide retinol data. β-carotene content of POED was 211.08 µg/g. After repletion, plasma retinol in POEDgroupincreased up to 1.39 µg/ml in average. Meanwhile, its total liver retinol increased up to 385.76 µg in average. RAF proposed for the test group was 1/9.97, which indicated that 9.97 µg vitamin A from this product was needed to accumulate 1 µg retinol in the liver. Relative bioavailability of POED was 70.91%. It appeared that β-carotene in POED can be well-absorbed by the vitamin A-depleted-rats.



## Introduction

Palm oil was extracted from the fleshy palm oil mesocarp which contains vitamin A as β-carotene. β-carotene is the major vitamin A with the highest activity found in plants pigment. Like any other carotenoid, β-carotene as pro-vitamin A are prone to isomeration and oxidation (Gleize *et al*., 2012). Being highly unsaturated, the major cause of β-carotene destruction during processing and storage are light and high temperature exposure (Xu *et al.,* 2013). Therefore, carotenoid activity are getting lower after high temperature processing as in cooking oil processing (Roohinejad, 2015). Vitamin A metabolism within enterocytes needs fat as a carrier. Retinol and β-carotene absorption depends on the function and absorption of dietary fat (Ball, 2006; D'Ambrosio *et al.*, 2011).

Considering the complexity of β-carotene characterization and metabolism, many products were developed to maintain β-carotene activity in palm oil, which is stated as one good source of pro-vitamin A. Several researches have developed products based on oil-in-water emulsion system, such as nanoemulsion and microemulsion, as a delivery system for β-carotene. Those kind of delivery system have been widely used in functional food or pharmaceuticals (Yi *et al*., 2014). Emulsion system was made to protect bioactive compound that susceptible to oxidation such as carotenoid groups in palm oil, prevent degradation, and also improve its bioavailability (Shin *et al*., 2015).

Many studies have illustrated that β-carotene bioavailability were determined by several factors, such as food matrix, food processing, and nutrition interaction. Carotenoid like β-carotene are easily damage if treated with bleaching process in cooking oil production which lead to bioavailability reduction. Otherwise, fat was needed to optimize β-carotene absorption due to its lipophilic characterization. Minimum fat consumption suggested to reach optimum β-carotene absorption are 2-5 g/day (Ball, 2006). β-carotene in food oils has better bioavailability if compared to food with lower fat

*Corresponding author.
Email: *harlen@gmail.com*

content (Yi *et al.*, 2014).

Emulsion system is a kind of liquids dispersion or suspension, with one of the liquids dipersed as small spherical droplets in the others. The droplets diameter size are ranging from 0.1-50 µm. Palm oil emulsion drink (POED) is an oil-in-water emulsion. Its basic formula were palm olein, water, and emulsifier. Other ingredients were sweetener, flavoring agent, and food preservatives (Sabariman, 2007). Droplet formed in emulsion system was a great carrier for lipophilic bioactive compound. It also support β-carotene digestion and absoption. Lipid droplet may also acted as a nonpolar solvent which involved in β-carotene release from food matrix (McClements *et al.*, 2015).

According to Surfiana (2002), β-carotene content of POED were 211.85-310.87 µg/g. Emulsion particle size plays an important role for β-carotene bioavailability. Smaller particle size would increase β-carotene absorption in GIT (Salvia-Trajillo *et al*., 2013). Mubarok (2011) reported that POED droplet diameter which formulated based on Surfiana (2002) was ranging from 0.7-18 µm. Based on in vitrostudy, β-carotene bioavailability may increase 5-10% when emulsion droplet diameter was decreased from 0.7-18 µm (Yi *et al.*, 2014).

Theoritically, POED may be a good source of vitamin A. However, its metabolism and biological activities inside the body need to be examined deeply because β-carotene bioavailability are depends on many physiological factors (Donhowe *et al*., 2014). Therefore, The aim of this study were to determined biological value of POED by using *in vivo* bioavailability study and also to evaluate nutritional value of POED based on its β-carotene content and chemical composition.

## Materials and Methods

*Materials*

Main materials to make POED was Crude Palm Oil (CPO) purchased from PT Salim Ivomas Pratama Jakarta. Other materials were potassium sorbate, butyl hydroxy toluene (BHT), Tween-80 emulsifier, flavoring agent, and High Fructose Syrup (HFS), all purchase from Setiaguna Chemical Store, Bogor. Chemicals used for analysis were $H_2SO_4$, NaOH, NaCl, acetic acid ($CH_3COOH$) glacial, KOH, $K_2SO_4$, HgO, $H_3BO_3$, HCl, $AgNO_3$, methanol, $Na_2SO_4$ anhydrous, heksan, isopropanol, and acetonitrile, all purchased from Merck, Germany.

Thirty-six male Rattus norvegicus rats *(Sprague-dawley)* were purchased from Faculty of Veterinary, Bogor Agricultural University at the age of 3 months. Ingredient for formulating rats feed were casein technique, carboxymethylcelulose (CMC), corn oil, cornstarch, vitamin mix, mineral mix, palm oil emulsion drink (POED), and aquades. The standards for chromatographic analysis were crystal β-carotene (Sigma 7235-40-7, >95%) and all trans-Retinol (Sigma R-7632, >96%). Nitrogen liquid and gas was bought from local dealers.

Equipments we used for purifying CPO and to prepare POED were Deodorizer (SM 100-Be), Neutralizer (SM 100-Ne), and Homogenizer (Armfield silverstone). Other equipment were analytical scale (Sartorius model BSA 224) and Centrifuge (Eppendorf). The HPLC-UV Vis recorder to analyze plasma and liver retinol was from Shimadzu and column C18 was from Zorbac Eclipse (150 mm in lenght and 4.6 mm in diameter). To analyze β-carotene concentration in POED, reversed-phase HPLC-UV Vis from Agilent and the C18 colomn from Zorbac Eclipse were used.

*Palm oil emulsion drink formulation*

Formulation of POED used for this experiment was a modificiation from Surfiana (2002). CPO was degumized, deacidified, deodorized, and fractionized untill liquid fraction (olein) and solid fraction (stearin) was formed. Only olein used for this experiment. Olein mixed with BHT (200 µg/kg) and EDTA (200 µg/kg) then homogenized in 8000 rpm for 1 minute. Water was mixed with Potassium Sorbate (0.2%) and emulisifer Tween-80 (1%) then homogenized for 1 minute. Both mixtures were homogenized together for 3 minutes then fructose syrup (15%) and flavoring agent (1.5%) was added. The mixture then homogenized once again for 4 minute and pasteurized in 70°C for 15 minutes. Olein and water amount ratio used for this formulation was 7:3 (w/w).

*Preparation of laboratory diet and composition*

The standard diet (S) composition was referred to AOAC (2012) and so were vitamin and mineral mix. Water was given in ad libitum. There were four type of diet composition used for this experiment (Table 1). The depletion diet (R1) was basically S diet made without vitamin A addition. Diet for positive control (PC) group were enriched with synthetic β-carotene as vit A substitute (R2), while diet for palm oil emulsion drink (POED) group were enriched with POED (R3). Diet for those group was calculated to meet the requirement of 180 µg β-carotene per feed per rats, as suggested by Zakaria-Rungkat (2000). Fat content on POED-repleted-diet was adjusted based on proximate analysis of POED.

Table 1.Ingredient Composition of Repletion Diets

| Ingredient | Unit | Type of Diet | | | |
|---|---|---|---|---|---|
| | | S | R1 | R2 | POED |
| Casein | g | 116.5 | 116.5 | 116.5 | 116.5 |
| Corn Oil | g | 79.8 | 79.8 | 79.8 | 49.95 |
| Mineral mix | g | 45.9 | 45.9 | 45.9 | 45.9 |
| CarboxymethylCelulose (CMC) | g | 10 | 10 | 10 | 10 |
| Water | g | 45.2 | 45.2 | 45.2 | 45.2 |
| Cornstarch | g | 692.6 | 692.6 | 692.6 | 692.6 |
| Vitamin mix | % | 1 | - | - | - |
| Vitamin mix (without vitamin A) | % | - | 1 | 1 | - |
| Synthetic β-carotene | mg | - | - | 9 | - |
| POED | ml | - | - | - | 47.38 |

*Ingredient listed above was used to make 1 kg diet (50 portions)

*Laboratory animal care and handling*

Ethical clearance was issued by the Institution of Research and Community Service (LPPM), Bogor Agricultural University by November 15th 2015 (Ethical approval number: 03-2015). All rats were put on 10 days of adaptation, received standard diet (S) and then divided into two groups. Control group (C, n=6) received the standard diet, while the others received depleted diet ($R^2$ diet) for 60 days of depletion period. The depletion period was followed by repletion period for 14 days. In the repletion period, all depleted-rats was divided into three groups: the positive control (PC) group (n=6) that received $R^2$ diet, the palm oil emulsion drink (POED) group (n=6) that received R3 diet, and the negative control (NC) group (n=6) that received R1 diet. The control (C) group that received standard diet at depletion period still received similar diet in repletion period. Rats were put in individual cage. Feeds were changed at the same time every day and all the left-over was measured to obtain rats' intake per day. All rats were weight every three days and the weight gain was recorded to observe their growth.

*Rats termination and sample handling*

At the end of the adaptation period, six rats were sacrificed in order to obtain the retinol plasma and liver concentration in a normal vitamin A status. At the end of depletion period, six rats from the depleted group was also sacrificed in order to get the retinol plasma and liver concentration after being suffered from vitamin-A-depleted diet. Termination was done using the cervical dislocation method (National Research Council, 2011). Rats would receiveisoflurane as an inhalant anaesthetic before given cervical dislocation. The same termination method was done to the remaining rats at the end of repletion period. When the rats were unconscious, the chest was cut open and blood was withdrawn from the heart using syringe and placed into vacutainer filled with EDTA anticoagulant to prevent from blood coagulation. Separation of blood plasma from the serum was done using centrifuge (Eppendorf) at 3000 rpm for 15 minutes. The blood plasma were collected and put into microtubes then kept at -20°C. The livers were taken out, weighted, put in a plastic pouch and freezed with liquid nitrogen to achived flash freezing. The livers then wrapped with alumunium foil, put into plastic box, and kept at -20°C.

*Proximate analysis*

Protein, fat, moisture, ash and carbohydrate content from POED were determined with the procedure recommended by AOAC (2012).

*β-Carotene analysis of palm oil emulsion drink*

Sample preparation method was a modification of AOAC (2012). One gram sample was placed inside the test tube then homogenized with 10 ml KOH 5% in methanol. Gas nitrogen was blown into the tube for 30 seconds then the tube was immediately closed to prevent β-carotene oxidation. Saponification was done at 65°C for 30 minutes inside a waterbath. The extract were cooled and then 5 ml deionized water and 10 ml hexane were added. After centrifugation, the top layer were withdrawn. The extraction process was done three times and combine with the first extract, evaporated under nitrogen, and dissolve in 1 ml HPLC mobile phase (acetonitrile:isopropanol= 65:35). Twenty microliters of extract was injected into the HPLC column with 1 ml/min flow rate and wavelenght 450 nm. The β-carotene concentration was calculated using :

$$\beta - \text{carotene Standard (mg)} \times \frac{\text{sample volume (ml)}}{100\ \text{ml}} \times \text{Dilution factor}$$

*Retinol analysis for plasma and liver*

Samplepreparation method for plasma retinol analysis was evaluated according to Domitrovic *et al.* (2008). Into 0.5 ml blood plasma, the same amount of ethanol was added. Then 4 ml hexane was added and the solution was homogenized. After centrifuged, the top layer were withdrawn. The extraction process was done three times and combined with the first extract, evaporated under nitrogen, and dissolved with 1 ml HPLC mobile phase (methanol:acetronitrile = 1:1).

Liver retinol sample preparation was referred to Furusho *et al.* (2000). Livers (0.5 g) were crushed, put into a test tube, and 4 ml of 30% KOH and 0.5% ascorbic acid on ethanol was added. Saponification was done at 60 °C for 30 minutes inside a waterbath. The extract were cooled and then 4 ml hexane was

added. After centrifugation, the top layer were withdrawn. The extraction process was done three times and combined with the first extract, evaporated under nitrogen, and dissolved with 1 ml HPLC mobile phase (methanol:acetronitrile = 1:1). Twenty microliters of extract was injected into the HPLC column with 1 ml/min flow rate and wavelenght 325 nm. The method to calculate retinol concentration was similar to that of β-carotene.

*Retinol accumulation factor (RAF)*

β-carotene bioavailability was obtained by Retinol Accumulation Factor or RAF (Zakaria-Rungkat *et al.*, 2000; Carillo-Lopez *et al.*, 2010). RAF wasthe reciprocal value of β-carotene total intake in µg/liver during repletion perioddivided by the liver retinol accumulation (LRA) in µg.

Liver retinol accumulation (LRA) was calculated by substracting total liver retinol after repletion period from total liver retinol after depletion period. Total liver retinol was measured by multiplying liver retinol obtained by analysis (µg/g) with liver weight (g). β-carotene bioavailability evaluation of POED was evaluated by RAF relative to synthetic β-carotene. RAF relative was obtained by dividing the RAF of PC group bythe RAF of POED group.

*Statistical analysis*

These statistical analysis was carried out for weight gain, plasma retinol, liver retinol, total liver accumulation, and RAF.Results were expressed as the mean value ± deviation standard of the mean. Analysis of Variance (ANOVA) was used to determine differences among diet treatment and continued by Duncan Multiple Range Test (α=0.05). Independent T-test was used to compared RAF of POED to RAF of synthetic β-carotene.

## Results and Discussion

*Palm oil emulsion drink chemical composition*

As the result of HPLC analysis, palm olein contained 311.70 µg/g β-carotene. After fully processed, POED contained 211.08 µg/g β-carotene. It showed that POED formulation was able to maintain 67.72% β-carotene from palm olein that used as a main ingredient. As expected, the result corresponded with previous experiments showed that POED contains 211-310.87 µg/g β-carotene (Surfiana, 2002). It was higher than POED formulated by Meridian (2000) and Wulandari (2000). The Chemical composition of POED were shown in Table 2.

POED fat content was 68.98%. It may has potential benefit as a good source of vitamin A.

Table 2.Chemical composition of palm oil emulsion drink

| Parameter | Value |
|---|---|
| Protein (g/100 g) | 0.065±0.005 |
| Fat (g/100 g) | 68.98±0.18 |
| Ash (g/100 g) | 0.015±0.0003 |
| Moisture (g/100 g) | 22.812±0.110 |
| Carbohydrate (g/100 g) | 8.125±0.300 |
| β-carotene (µg/g) | 211.08±5.88 |

High fat content may boostvitamin A bioavailability (Ball, 2006). Higher fat content could increase micelles solubilization in small intestine and support β-carotene absorption. Bioactive compound should remain stable within delivery system. Emulsion-based delivery systems are especially suitable for protecting and delivering oil-soluble vitamins (McClements *et al.*, 2015).

*Animal growth*

On the first day of adaptation period, the average weight of the rats was 145.78 g. In the end of adaptation period, the average weight gain was 18.45 g or increasing up to 12.66%. After received adaptation period, rats was divided into two group. The C groups received standard diet which is the same diet that was given on adaptation period, while the depleted groups received depleted diet. At the end of depletion period of 60 days, the group intended to received standard diet gained less weight (71.92 g) and the group intended to received depleted diet gained more weight (86.63 g). The depleted groups gained less weight in the middle of depletion period, while the C group have gained weight persistenly untill the end of depletion period. It may happened because of metabolism alteration due to loss of liver vitamin A storage (Fernandez-Garcia *et al*., 2012).

In the end of repletion period, C group gained the most weight if compared to another groups, while NC group which received depleted diet gained the least weight. NC group experienced inconsistent weight gain after the third day of repletion period. Vitamin A deficiency that occured in NC group may played an important role in rats growth. Weight gain could be an indirect indicators to vitamin A deficiency status (Nguyen *et al.*,2015). Meanwhile, there was no significant difference in weight gain between PC group and palm oil emulsion group, as represented in Table 3 (α=0.05). Previous experiments by McClementset al. (2015) showed that emulsion delivery system might increase weight gain of vitamin A-depleted-rats.

Table3.Average of rats diet consumption and weight gain during repletion period

| Groups of Rats | Total Consumption (g) | Weight gain (g) |
|---|---|---|
| C | 262.43 ± 20.26$^{a}$ | 23.00 ± 9.45$^{a}$ |
| PC | 215.16 ± 12.05$^{bc}$ | 15.29 ± 3.59$^{ab}$ |
| NC | 225.83 ± 20.13$^{c}$ | 9.17 ± 1.61$^{b}$ |
| POED | 238.85 ± 20.38$^{b}$ | 15.86 ± 6.09$^{ab}$ |

*Plasma and liver retinol*

Rats have been used to evaluate the efficiency of β-carotene conversion to retinol by monitoring changes in plasma and liver vitamin A stores. Retinol plasma levels at the end of adaptation period was 0.38 µg/ml, while liver retinol levels was 85.11 µg/g. At the end of depletion period, the retinol plasma level was reduced to 0.09 µg/ml, while retinol liver was reduce to 14.36 µg/g. It showed that retinol plasma and liver was depleted up to 76.31% and 83.13%, respectively if compared to retinol levels at the end of adaptation period.

Concomitant to retinol plasma reduction, liver retinol was also decrease. That would lead to hyporetinolemia, even though plasma retinol was controlled by the homeostatic (Zakaria-Rungkat *et al*., 2000). However, in depletion period the homeostatic regulation was inhibited by the lack-of-vitamin A-diet leads to low level of retinol liver. The homeostatic regulation was also affected by the Retinol Binding Protein (RBP) in blood plasma and liver (Souganidis *et al.*, 2013). RBP was synthesized and secreted by liver (Ball 2006). RBP was involved in retinol transportation from liver tissue to blood circulation. Vitamin A deficiency inhibited RBP secretion in liver parenkim cells which leads to retinol plasma reduction (Gieng *et al*., 2005).

β-carotene absorption and conversion into retinol was increased in vitamin A deficient rats (Faulks and Southon, 2005). It caused plasma and liver retinol after repletion period increased significantly. Retinol plasma level of PC group increased up to 92.97%, while POED group retinol plasma increased higher (93.58%). Liver retinol level was also increased up to 72.42% and 67.26% for PC group and POED group, respectively. There was no significant difference between POED group's retinol liver level and PC's retinol liver level (Figure 1B). This result indicated that POED has a contribution to increase liver retinol level nearly as high as syntetic β-carotene.

*β-Carotene bioavailability of palm oil emulsion drink*

β-carotene bioavailability study of palm oil emusion drink was carried out using Retinol Accumulation Factor (RAF). RAF value for positive control group was 1/7.07, meanwhile RAF value for POED group was 1/9.97 (Table 4). It could be stated that 7.07 µg synthetic β-carotene intake and 9.97 µg β-carotene intake from POED results in 1 µg liver retinol, respectively. Relative Bioavilability of POED was 70.91%.

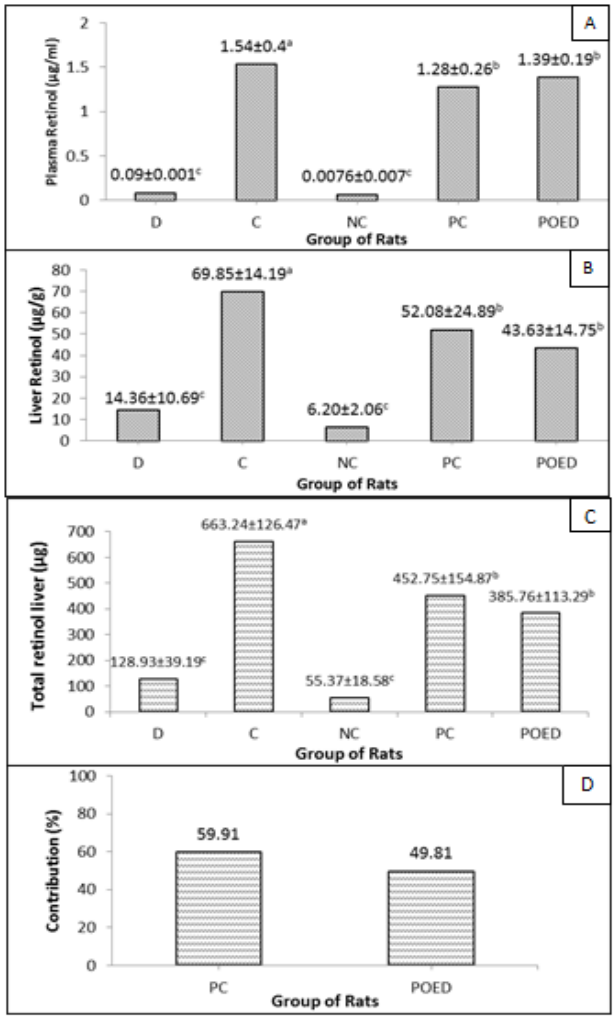
Table4.Retinol Accumulation Factor(RAF) for positive control group and palm oil emulsion drink group

| Groups of Rats | Liver Retinol Accumulation (µg) | β-carotene Total Intake (µg) | RAF* |
|---|---|---|---|
| PC | 323.82 ± 136.31 | 1870.77 ± 177.22 | 1/7.07 ± 4.23$^{a}$ |
| POED | 256.83 ± 112.06 | 2179.17 ± 166.02 | 1/9.97 ± 4.28$^{a}$ |

*RAF was the reciprocal value of β-carotene total intake in µg/liver during repletion perioddivided by the liver retinol accumulation (LRA) in µg.

Previous experiment by Meridian (2000) stated that the RAF of POED was 1/9.09 and the relative bioavailability was 33.33%. The RAF of POEDfrom this research was higher than the previous experiment. It may cause by different formulation we used. The β-carotene content of palm olein as a main ingredient was also higher than palm olein used by Meridian (2000) that appeared to have β-carotene degradation due to unsuitable storage and handling. POED's RAF value was higher than boiled vegetables and tubers such as carrot, brassica, sweet potato, water convolvulus, and cassava leaves measured by Zakaria-Rungkat (2000). Some of the vegetables had lower RAF value than POED, which could indicated better absorption. However, their relative bioavailability was lower than the POED. The high content of fibre in vegetables may interfere with β-carotene bioavailability, as reported in some publications (Ornelaz-Paz *et al*., 2010; Verrijsen *et al.*, 2014). Fibre may reduce β-carotene bioavailability through inhibition of lipase and reduce fat absoption (Salvia-Trajillo *et al*., 2013; Bonet *et al.*, 2015). Absorption of vitamin A and carotenoids depends on the proper digestion and absorption of dietary fat. In the intestine, the free carotenoids congregate in fatty globules and form micelles (Ball, 2006). If there is not enough fat to form a micelles, vitamin A and carotenoids absorption will decrease (Graebner *et al.*, 2004).

There was no significant difference between total liver retinol of POED and total liver retinol of PC group (Figure 1C). At the end of repletion period, the total liver retinol level of POED group was increased to 58.16% of the total liver retinol of C group. Meanwhile, the total liver retinol level of PC group was increased to 68.16% of the total liver retinol of C




D=Depletion, C=Control, NC=Negative Control, PC=Positive Control, POED=Palm Oil Emulsion Drink. There was no significant difference between groups which data labels followed by the same alphabet (α=0.05)

Figure 1. Rats plasma retinol level (A), liver retinol level (B), total retinol liver accumulation (C), and the contribution of synthetic β-carotene and palm oil emulsion drink in recovering liver retinol accumulation (D)

group. It could be suggested that POED was able to restore total retinol liver of vitamin-A-depleted rats level nearly as high as synthetic β-carotene.

The contribution for POED and synthetic β-carotene in recovering liver retinol accumulation was measured as a ratio of total liver retinol level of POED or PC group and total liver retinol level of C group. The contribution of POED in recovering liver retinol accumulation was 49.81%, while the synthetic β-carotene contribution to recover liver retinol accumulation was 59.91% (Figure 1D). It means that the POED could regain liver retinol accumulation up to 83.14% if compared to synthetic β-carotene.

Liver retinol concentration would increase significantly when β-carotene was given in oil-in-water emulsion system. Oil-in-water emulsion could effectively improving liver retinol concentration in vitaminA-depleted rats if compared to normal rats (Siqueira *et al.*, 2007). Unlike β-carotene in fruit and vegetables, β-carotene in POED did not bound in chloroplast as a pigment so that it could release easily from food matrix (Meridian 2000). That would simplify β-carotene transportation in GIT and absorption within erytrocyte where β-carotene was heavily absorb and converted into retinol (Faulks and Sounthon, 2005; Xu *et al.,* 2013). Those kind of situation would improve β-carotene bioavailability. Emulsion system was able to prevent β-carotene loss from oxidation. Moreover, addition of antioxidant such as EDTA in POED would inhibit β-carotene degradation and maintain emulsion stability (Xu *et al.*, 2013).

The chemical structure of lipid molecule determined the rate of lipid digestion within the gastrointestinal tract. It have important consequences to identify solubilization capacity of the mixed micelle phase formed within the small intestine, which is related to β-carotene bioavailability. Generally, carotenoid bioavailability could be elevated by using Long Chain Triglycerides (LCT) as an emulsion based lipid. The main lipid composition of palm oil were kinds of Long Chain Fatty Acids (LCFA) which turn into LCT when metabolized in intestine. LCFA derived from LCT would form longer non-polar domain than carotenoid's molecular dimension so that it could accomodate carotenoid better than Medium Chain Tryglycerides (MCT) (McClements *et al*., 2015).

## Conclusion

The formulation of palm oil emulsion was able to maintain β-carotene composition of palm olein up to 211.08 µg/g. β-carotene in POED could be effectively absorbed and be used by vitamin-A-depleted rats receiving vitamin-A-repletion period. The RAF value of POED was 1/9.97, which indicated that 9.97µg β-carotene intake from POED results in 1 µg liver retinol.Relative bioavailability of POED was 70.91%. The contribution of POED to recover liver retinol accumulation was 49.81%. It seems from this bioavailability study that POED was a good source of pro-vitamin A.

## Acknowledgements

The authors thank to Indonesia Endowment Fund for Education (LPDP) and the Indonesian Ministry of Education Directorate General of Higher Education (DIKTI) for financing this research through Hibah Kompetensi 2 on behalf of Prof. Dr. Tien Muchtadi,

MS.

## References


Association of Official Analytical Chemist (AOAC). 2012.Official methods of analysis.New York(USA): Gaithersburg, Md.

Ball, G.F. 2006.Vitamins in foods: analysis, bioavailability, and stability. Boca Raton:Taylor and Francis Group.

Bonet M.L., Canas, J.A., Ribot, J.and Palou, A. 2015. Carotenoids and their conversion products in the control of adipocyte function, adiposity, and obesity. Archives of Biochemistry and Biophysics 572: 112-125.

Carillo-Lopez, A., Yahia, E. and Ramirez-Padilla, G. 2010.Bioconversion of carotenoids in five fruits and vegetables to vitamin a measured by retinol accumulation in rat livers.American Journal of Agricultural and Biological Sciences 5(2): 215-221.

D'Ambrosio, D.N., Clugston, R.D. andBlaner,W.S. 2011. Vitamin a metabolism: an update.Nutrient 3: 63-103.

Domitrovic, R., Tota, M. and Milin, C. 2008. Differential effect of high dietary iron on α-tocopherol and retinol levels in the liver and serum of mice fed olive oil-and corn oil-enriched diets. Nutrition Research 28(4): 263-269.

Donhowe, E.G., Flores, F.P., Kerr, W.L., Wicker, L. and Kong, F. 2014.Characterization and in vitro bioavailability of β-carotene: effects of microencapsulation method and food matrix. LWT-Food Science and Technology 57: 42-48.

Faulks, R.M. and Sounthon, S. 2005. Challenges to understanding and measuring carotenoid bioavailability. Journal of Biochemistry and Biophysics 1740: 95-100.

Fernandez-Garcia, E., Carvajal-Lerida, I., Jaren-Galan, M., Garrido-Fernandez, J., Perez-Galvez, A. and Hornero-Mendez, D. 2012.Carotenoids bioavailability from foods: from plant pigments to efficient biological activities. Food Research International 46: 438-450.

Furusho, T., Kataoka, E., Yasuhara, T., Wada, M. and Masushige, M. 2000. Retinol equivalence of carotenoids can be evaluated by hepatic vitamin a content. International Journal of Vitamin and Nutrients Research 70(2): 43-47.

Gieng, S.H., Raila, J. and Rosales, F.J. 2005.Accumulation of retinol in the liver after prolonged hyporetinolemia in the vitamin a-sufficient rat. Journal of Lipid Research 46: 641-649.

Gleize, B., Steib, M., Andre, M. andReboul, E. 2012. Simple and fast hplc method for simultaneous determination of retinol, tocopherols, coenzyme q10, and carotenoids in complex samples.Food Chemistry 134: 2560-2564.

Graebner, I.T., Siquera, E.M., Arruda, S.F. and de Souza, E.M. 2004. Carotenoids from native brazillian dark-green vegetable are bioavailable: a study in rats. Nutrition Research 24: 671-679.

Meridian, Y.A. 2000.Beta-carotenebioavailability study of palm oil carotene emulsion drink in rats *(rattus norvegicus)* blood plasma and liver.Bogor, Indonesia: Bogor Agricultural University, BScThesis

McClements, D.J., Saliva-Trajillo, L., Zhang, R., Zhang, Z., Zou, L., Yao, M. and Xiao, H. 2015. Boosting the bioavailability of hydrophobic nutrients, vitamins, and neutraceutical in natural products using excipient emulsion. Food Research International 88A: 140-152.

Mubarok, A.Z. 2011. Scaling up emulsification process of emulsion drink from red palm oil and their rheological characterization. Bogor, Indonesia: Bogor Agricultural University, MSc Thesis.

Nguyen, L.M., Scherr, R.E., Linnell, J.D., Ermakov, I.V., Gellermann, W., Jahns, L., Keen, C.L., Miyamoto, S., Steinberg, F.M., Young, H.M. and Zidenberg-Cherr, S. 2015. Evaluating the relationship between plasma and skin carotenoids and reported dietary intake in elementary school children to assess fruit and vegetable intake. Archives of Biochemistry and Biophysics 572: 73-80.

National Research Council (NRC). 2011. Guide for the care and use of laboratory animals. 8th edition. Washington DC: The National Academies Press

Ornelaz-Paz, J., Yahia, E.M. and Gardea-Behar, A.A. 2010. Bioconversion efficiency of β-carotene from mango fruit and carrots in vitamin a. American Journal of Agriculture & Biological Sciences 5(3): 301-308.

Roohinejad, S., Oey, I., Wen, J., Lee, S.J., Everett, D.W. and Burritt,D.J . 2015. Formulation of oil-in-water β-carotene microemulsions: effect of oil type and fatty acid chain length. Food Chemistry 174: 270-278.

Sabariman, M. 2007. Rheology and physical properties of palm oil emulsion drink rich in β-carotenefrom red palm oil emulsified by several emulsifiers. Bogor: Indonesia: Bogor Agricultural University, MSc Thesis

Salvia-Trajillo, L., Qian, C., Martin-Belosso, O. and McClements, D.J. 2013.Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. Food Chemistry 141 (2): 1472-1480.

Shin, G.H., Kim, J.T. and Park, H.J. 2015. Recent developments in nanoformulations of lipophilic functional foods. Trends in Food Science and Technology 15:172-177.

Siqueira, E.M., Arruda, S.F., Vargas, R.M. and Souza, E.M. 2007. β-carotene from cassava *(manihot esculenta crantz)* leaves improves vitamin a status in rats. Comparative Biochemistry and Physiology C 146: 235-240.

Souganidis, E., Laillou, A., Leyvraz, M. andMoench-Pfanner, R. 2013.A comparison of retinylpalmitate and red palm oil β-carotene as strategies to address vitamin a deficiency.Nutrients 5: 3257-3271.

Surfiana. 2002. Formulation of emulsion drink rich in β-carotene from red palm oil. Bogor, Indonesia: Bogor Agricultural University, BSc Thesis.

Verrijssen, T.A., Vanierschot, M., Ongena, S.I., Cardinaels, R., Van den Bulck, E., Van Loey, A.M., Hendrickx, M.E. and Van Buggenhout, S. 2014. Role of

mechanical forces in the stomach phase on the in vitro bioaccessibility of β-carotene.International Journal of Food Research 55: 271-280.

Wulandari,O.V. 2000.Utilization of red palm oil to produce β-carotene emulsion as vitamin a supplement. Bogor, Indonesia: Bogor Agricultural University, BSc Thesis.

Xu, D., Wang, X., Jiang, J., Yuan, F., Decker, E.A. andGao, Y. 2013. Influence of ph, edta, α-tocopherol, and wpi oxidation on the degradation of β-carotene in wpistabilized oil-in-water emulsion. Journal of Food Science and Technology 54: 236-241.

Yi, J., Li, Y., Zhong, F. and Yokoyama, W. 2014.The physicochemical stability and *in vitro* bioaccessibility of beta-carotene in oil-in-water sodium caseinate emulsions. Food Hydrocolloid 35, 19-27.

Zakaria-Rungkat, F., Djaelani, M., Rumondang, E. andNurrochmah. 2000. Carotenoid bioavailability of vegetables and carbohydrate-containing foods measured by retinol accumulation in rat livers.Journal of Food Composition and Analysis 13: 297-310.