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Influence of a Red Palm Oil Emulsion on the Level of Retinol in the Plasma of Primary School Children in the Coastal Area of Makassar City

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Abstract: Beta-carotene, one component of palm oil, is a precursor of vitamin A and functions as pro-vitamin A; therefore, palm oil has the potential to be used as a food source to prevent the occurrence of vitamin A deficiency (VAD). This study aimed to determine the effect of a red palm oil emulsion on the plasma retinal levels of primary school children in a coastal area of Makassar City. Quasi-experimental research with a pre-test post-test design was conducted in the Cambaya Elementary School Sub District of Ujung Tanah Makassar City and involved 36 sixth-grade students who were divided into three treatment groups; each group consisted of 12 students. The first group was given 5 ml/day of red palm oil emulsion for one month. The second and the third group were given 10 and 15 ml/day, respectively, for one month. The plasma retinal levels were measured before and after one month of administration of the red palm oil emulsion. The research began with a hedonic test to determine the level of preference and consumer acceptance of the emulsion product and it continued with the intervention stage. The results showed that the most preferred emulsion product was an emulsion of red palm oil using a melon flavor. Giving the emulsion at a dose of 10 and 15 ml/day leads to a significant effect on the plasma retinal levels, which is characterized by a significant difference between the mean plasma retinal levels before and after the intervention (p<0.05); administration of the emulsion at a dose of 5 ml/day did not provide a significant effect, although it appears there was an increase in plasma retinal levels after the intervention. Finally, it can be concluded that the red palm oil emulsion did have an effect on the plasma retinal levels of primary school children.

Key words: Palm oil, red palm oil, carotene, vitamin A deficiency, retinal, plasma retinal

INTRODUCTION

The development of palm plantations in Indonesia shows that the rate of expansion is increasing every year. In 1990, palm plantations covered 1,126677 ha in 1995 this number increased to 2,024,986 ha, in 2000 to 4,158,077 ha in 2005 to 5,453,817 ha and in 2010 it increased to 8,430,027 ha. In line with the increase of plantations, national palm oil production also increased from year to year. CPO production in 1990 amounted to 2412.612 tons, in 1995 it increased to 4.47967 million tons, in 2000 to 6.35 million tons, in 2005 to 11,861,615 tons, in 2010 to 21800,000 tons and in 2014 production increased to 31,000,000 tons (The Directorate General of Plantation, 2014).

Palm oil, as a source of oil, also contains a number of active components that are critical to the improvement of health, including the carotenoids (a, ~ y-carotene and lycopene), tocopherols, tocotrienols, phytosterols and essential fatty acids (Winarno, 1999; De Witt and Chong, 1998; Muhilal, 1998). β-carotene, as one component of palm oil, is a precursor of vitamin A and serves as pro-vitamin A (Sikorski, 1997). β-carotene has several biological activities that are beneficial to the body, including helping to cope with blindness due to xerophthalmia, to prevent cancer and to prevent premature aging (May, 1994; Umegaki and Ikegami, 1994; Poppel and Goldbohm, 1995) because the antioxidants can further reduce the chances of degenerative diseases (Iwashaki and Murakoshi, 1992; Basu *et al.*, 2001). Judging from the amount of pro-vitamin A activity, the carotenoid content of palm oil has 10 times greater activity than a tomato (Tan, 1987).

Vitamin A deficiency (VAD), in addition to increasing the risk of blindness, also has a role in the high mortality rate in Indonesia and has the potential to lower labor productivity. Studies conducted in Indonesian children (Somer *et al.*, 1984; Wolf and Keusch, 1999) found that children with moderate xerophthalmia have an increased risk of respiratory infections and diarrhea compared to children who do not have eye abnormalities. The lack of vitamin A, which is characterized by the fact that xerophthalmia cases have not disappeared, is proven by the Nusa Tenggara Timur, who still reported cases of xerophthalmia in 2000.

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There is a possibility that other provinces that have not managed to cover >80% of vitamin A capsules that are distributed to the children, will rediscover xerophthalmia cases. If so, then another solution is needed to deal with VAD, especially in children. The prevalence of subclinical VAD is still high, which suggests that this nationwide problem requires us to keep our attention on programs to alleviate malnutrition in the future. It is estimated that each year there are three to ten million children, most of whom live in developing countries, that experience xerophthalmia and between 250000 to 500,000 become blind. The number of cases of xerophthalmia found in ten provinces in Indonesia showed as many as 20 cases of infant patients; 75% of those with xerophthalmia were 3 years old and most had not received vitamin A capsules within the last 6 months (Herman *et al.*, 2007).

The abundance of palm oil production allows for its use in overcoming the problem of vitamin A deficiency by providing natural pigments and being a healthy food that is very useful in improving public health. Research using crude palm oil as a whole for the prevention of vitamin A deficiency (VAD) was carried out in the 1960s in Central Java and in 1967 at the Research Institute of Nutrition Bogor. The results of these two studies revealed that the administration of one teaspoon of crude palm oil every day can prevent VAD and one tablespoon of palm oil every day can cure xerophthalmia. There are some disadvantages of the previous studies, namely that crude palm oil tastes bad so children do not want to eat it every day, which requires administering it by force. Thus, it is necessary to diversify the crude palm oil products to give them a better taste and to increase the utilization of palm oil as a food rich in beta carotene red palm oil is especially useful in food consumption and nutrient diversity.

MATERIALS AND METHODS

This study was quasi-experimental with a pre-test post-test design. In practice, this research was conducted in three stages. The first stage was the production of a red palm oil emulsion rich in carotene; the second stage was a hedonic test to determine the level of preference and consumer acceptance of the product emulsion; the third was the intervention stage to determine the efficacy of the red palm oil emulsion carotene-rich product, which was determined by its influence on the plasma retinol levels of primary school children in the coastal area of Makassar City. The design of the study for the efficacy test is as follows:

$$O1 \xrightarrow{X} O2$$

01: Plasma retinol levels before administration of RPO emulsion
02: Plasma retinol levels after administration of RPO emulsion
X: Administration of RPO emulsion

Time and place of the research: This study was conducted from June 2014 to November 2014 at the Integrated Laboratory School of Public Health University of Hasanuddin and PTPN XIV Luwu for palm oil sampling and a state primary school located in the coastal area of Makassar; in this case, the selected primary school was Cambaya Elementary School Sub District of Ujung Tanah Makassar City because this school was cooperative, both in research and community service.

Materials and equipment

Ingredients: The raw materials used in this study were: crude palm oil (CPO) from PTPN XIVMills Luwu District in South Sulawesi Province, fructose and additional material flavor, such as vanilla melon and citrus. Materials for the analysis of the levels of beta-carotene included a beta carotene standard Sigma C-9750), hexane, acetone, acetic acid and nitrogen. The materials for the analysis of plasma retinol levels included standard retinol (Sigma), n-heptane, absolute ethanol, SOS, butyl hydroxy toluene (BHT), methanol, acetonitrile and dichloromethane. The equipment for the analysis of the carotene levels included a stoppered test tube, flask, beaker, flask, water bath, aluminum foil, vortex, centrifuge and a spectrophotometer and High Pressure Liquid Chromatography (HPLC) system.

Population and sampie: The population in this study included the crude palm oil (CPO) produced by PTPN X Luwu District of South Sulawesi and the students of Cambaya Elementary School, Sub District of Ujung Tanah Makassar City.

Sample research: Samples were drawn from the study population and are described as follows:

a. Unit of observation:

i: Emulsion with the addition of red palm oil as an experimental unit
ii: Panelists or persons who provide an assessment of the quality of emulsion
iii: Elementary school students as study subjects

b. Unit of analysis: All the variables inherent in the observation unit, including the nutrient content of the palm oil emulsion, the organoleptic quality of the emulsion and the plasma retinol levels of the primary school children before and after the intervention with the RPO emulsion.

c. Sampies size: All sixth grade students in the Cambaya Public Elementary School District of Ujung Tanah Makassar City who were willing to participate in the research; willingness to participate was determined by the signing of a letter of approval or informed consent. The number of students who agreed to participate was 36 students; but at the moment, there are 3 students whose post test cannot be found, so the number of samples is 33.

Implementation of the research

Analysis of total carotenoid content (Parker, 1992): Analysis of the total carotenoid content in the RPO was conducted by the following procedure: First, we pipetted 10 ml of red palm oil, put it in a test tube that has a lid, then saponified it by adding 2 ml of KOH-methanol solution of 5% (w/v). The tube was then closed and wrapped with aluminum foil to prevent oxidation due to light. It was then heated on a water bath at 60°C for 1 hour, which aimed to enhance the reaction and it was shaken every 10 min. After one hour, the results of saponification cooled and were extracted with 1 ml of distilled water and 3 ml of hexane. Then, the tubes were shaken using a vortex for 30 sec and the upper layer (organic layer) was separated into a clean test tube. The bottom layer was extracted once again with 3 ml of hexane, shaken using a vortex for 30 sec and the upper layer was separated again and combined with the top layer from before. The combined top layer was further neutralized to remove the remaining soap and residual KOH that was not saponified with the addition of 1 ml of 5% acetic acid. Then, the tube was shaken for 30 seconds and we again separated the top layer. The next layer above the filtrate was blown dry using nitrogen gas. To the obtained dry extract, we added 3 ml of an acetonitrile-methanol mixture (1:1) and the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 460 nm. For measurements using the HPLC, the dried extract was dissolved in a mixture of acetonitrile: dichloromethane: methanol (60:35:5%).

Production of a red palm oil emulsion rich in carotene: An emulsion of red palm oil was made using the formula of Surfiana (2002) and Rita (9) with the following composition: Oil: Water Ratio (7:3), Emulsifier (Tween 80) 1%, fructose 10%, flavor 1%, BHT 200 ppm and potassium sorbate 0.1%. The emulsion was made in the following manner. All of the material was put in the homogenizer and stirred with a speed of 250 rpm until it was homogenous. The emulsion products were then put into a bottle and pasteurized at 70°C for 10 min.

Product acceptance (hedonic test): This phase was conducted to determine the subjects' preferred color, flavor and taste. The emulsion products were tested on 10 trained panelists, 30 semi-trained panelists and 30 untrained panelists. The assessment was conducted by giving a form assessment to the panelists with the procedures and criteria as follows: (1) Love = 5, (2) Like = 4, (3) Kindly Love = 3, (4) Dislike= 2 and (5) Do not like = 1.

Assessment procedures

1: Panelists were given an explanation about the purpose and how to fill out the form
2: Samples to be assessed were placed in a particular code
3: Organoleptic assessment was conducted on color, flavor and aroma
4: To neutralize the taste and aroma of each product, the panelists were given water

Assessment criteria: Each attribute was assessed by the hedonic test according to the level of preference panel. The scale is transformed into a numerical scale as follows: Strongly like = 5, Like = 4, Rather like = 3, Dislike = 2, Strongly dislike = 1. The assessment criteria were further simplified into two categories, namely the criteria of like, which was taken from the strongly like and like (5 and 4) responses and the criteria of do not like, which was taken from the rather like, dislike and strongly dislike (3, 2 and 1) responses. This process intended to facilitate the assessment of the final result. The selected products here were subsequently used for the efficacy product test.

Efficacy test of palm oil emulsion product: This test was conducted to determine the effect of a red palm oil emulsion product on the plasma retinal levels of mentary school students. Students who participated were divided into three groups. The first group was administered as much as one tablespoon (5 ml) of the red palm oil emulsion once a day, the second group was administered 10 ml of the emulsion and the third group was administered 15 ml of the emulsion. Each group took the emulsion for one month and the plasma retinal levels were measured before and after the intervention. Vitamin A (retinal) levels were assessed in the serum.

Preparation of standard retinol: A total of 0.00123 g of standard retinal was diluted with absolute ethanol to 100 ml (0.00123 g/100 ml = 1230 μg/dl as the stock standard). Then, from the standard stock we made the following dilutions to give concentrations of 5, 10, 20, 40 and 60 ug/dl, which were made in absolute ethanol. Absorbance was read at a 325 nm wavelength. We then diluted each with 200 μL of solvent and injected 50 μL into the HPLC (the standards used for analysis ranged between 36-40 μg/dl).

Preparation of serum quality control (QC): Healthy respondents gave blood, as many as 10 samples and each blood draw was as much as 10 ml. We then separated the serum (the serum of 10 respondentswas put in an Erlenmeyer flask, shaken and vortexed until homogeneous) and then it was distributed into the serumvials (eachvial contained250 µL of serum). The serum was then stored in the freezer. The serum QC samples were analyzed,as many as 20 samples. Once the levels of the vitaminswere known,we calculated the standard deviation (SD) and created graphs.

Analysis of retinal levels (AOAC, 1984): 100 µL of serum sample I/QC serum was added to 100 µL 10 mmol SOS then shaken for 45 sec; then, we added 200 µL absolute ethanol. It was then whipped by using a vortex for 1 min. Then, we added 1000 µL heptane containing0.5 g BHT/L.It was then vortexedor 2 min and centrifuged for 10 min at 1000 to 1500 rpm. 700 µL of supernatant was taken and transferred into a tube and dried under a stream of N_2 gas. Once dry, we added 200 µL of mobile phase/solvent.50 µL of each sample was injected into the HPLC.

Data analysis: The datawere analyzedboth descriptively and analytically. The data on the characteristics of the participants (research subjects) were processed descriptively and presented in the form of a frequency distribution table. The test results of the children's favorite substituted palm oil emulsion were analyzed usingANOVAor the Kruskal-Wallistest and if the results of the analysis showed a difference, then the data analysis was followed by the least significant difference test (LSD)to determinethe most preferred product.

To determinethe effect of the RPO emulsion on plasma retinol levels, we then compared plasma retinol levels before and after the intervention using a paired t-test or Wilcoxon test at the 95% confidence level (a= 0.05).

RESULTS

This research was conducted at the Laboratory of the Integrated School of Public Health, University of Hasanuddin, from June to November 2014 using a sample of crude palm oil (CPO) from Palm Oil Mill PT. Perkebunan NusantaraXIV in Luwu South Sulawesi for efficacy testing was conducted at the State Primary School Cambaya New Patingaloang Village, District Ujung Tanah Makassar City, which is one of the locations in the coastal area of Makassar.

Production of red palm oil emulsion rich in carotene: The production of the red palm oil emulsion was made using a formula from Surfiana (2002) and Rita (2009). This basic formula was developed or modified to create multiplevariations with the addition of flavors (smell and taste) to be tested in this study. Formula emulsions were made with three flavors (melon, vanilla and citrus). The results of the hedonic test were then used to produce an emulsion to be used in the efficacy trials. The emulsions were made by using a homogenizer rotated at a speed of 250 rpm; therefore, they have the appearanceof an orange-yellowcolor, soft texture, they taste sweet and oily and have a smell in accordance with the flavor used, namely melon,vanilla or citrus.

Hedonic test: A test was used to determine the acceptance and consumer preferences toward the product emulsions made with the addition of flavor variations and smells. The number of panelists participating in the test was as many as 30 people. The panelists involvedwere children who do not suffer from colds and coughs and they consisted of 2 men (6.67%) and 28 women (93.33%). Hedonic test results of the product emulsions are presentedin Table 1.

The results of the assessment by the panelists show a mean value of scores between 2.49 (less preferred) to 4.03 (love). These results indicate that the emulsions made using the melon flavor were preferable because that flavor has the highest mean score (4.03), while emulsions prepared using the vanilla flavor and citrus flavor are less favored. Based on the test results, it can be concluded that the emulsions with the melon flavor should be subsequently produced for the purposes of the intervention process.

Test results based on the organoleptic acceptance of the emulsion productswere conductedto determinethe consumer acceptance of the product in terms of smell, texture, color and taste, as presented in Table 2.

Table 2 showed that the average quality score for the smell of red palm oil emulsions ranged between 2.13 and 4.03. A statistical test using the Kruskal Wallis test showedthat there is a significantdifference betweenthe scores of the panelists' acceptance of the three types of flavors used (p = 0.000). The emulsions using the melon flavor had the highest mean score of 4.03 compared with the emulsions made using vanilla and citrus. This means that the most favored emulsion is an emulsion made using a melon flavor. The same results were found based on texture, color and taste, so the most preferredemulsion is made using melon flavor.

Effect of red palm oil emulsionon the plasma retinal levels: A total of 45 students were enrolled, but only 36 students agreed to participate in this study; they were divided into three groups, each with 12 people. Each group was given a red palm oil emulsion of different doses for one month. The first group consumed 5 ml emulsion/day, the second group consumed 10 ml/day, while the third group consumed 15 ml/day. At the beginning of the study, the local clinic medical doctors (Patingaloang Puskesmas) carried out an examination of the children, which includedthe clinical state, weight, height and blood sampling for plasma retinol levels, but the unhealthy children did not undergo blood sampling.

Table 1: Average score of panelists' test results for emulsions based on flavor used

	---Type of flavor---			
Score of panelist test results	Melon	Vanilla	Citrus	p-value
Average	4.03	2.59	2.49	0.000

Table 2: Mean score of organoleptic quality test of red palm oil emulsion based on flavor used

Flavor	Smell	Texture	Color	Taste
Melon	4.03	4.00	4.10	4.00
Vanilla	2.47	2.63	3.20	2.07
Citrus	2.13	2.80	2.87	2.17
p-value	0.000	0.000	0.000	0.000

Table 3: Retinal plasma levels before and after giving the red palm oil emulsion to state primary school children in Cambaya, District Ujung Tanah, Makassar 2014

		- Retinal plasma levels (µg/dl)-			
Doses		Before	After	% of change	p-value
5 mUday	Minimum	3.19	8.24	61.29	0.059'
	Maximum	23.48	29.52	20.46	
	Average	8.82	11.60	23.97	
10 mUday	Minimum	2.28	7.50	69.60	0.019'
	Maximum	11.11	9.75	-13.95	
	Average	6.12	8.64	29.17	
15 mUday	Minimum	2.99	8.13	63.22	0.003'
	Maximum	9.90	15.17	34.74	
	Average	5.68	10.08	43.65	
	p-value	0.179'	0.050'		

'Wilcoxon Testm, 'Paired t-test, 'ANOVA

Blood sampling was then performed after consuming red palm oil emulsion for one month. The red palm oil emulsion was given by the classroom teachers during school break time, which was at approximately 10 am.

In the implementation of the study, there were three children who dropped out, two students in the first group and one student in the third group because their post• test or blood sample after the intervention process could not be found. Therefore, the number of samples at the end of the study was 33 people. The results of the measurementsof plasma retinol levels before and after the interventionare presented in Table 3.

Table 3 showsthat overall, with no regard to group types of intervention, before giving red palm oil emulsion, the lowest plasma retinol level was 2.28 g/dl and the highest was 23.48 ug/dl. After giving the red palm oil emulsion for one month, the lowest plasma retinol concentrationwas 7.50 mg/di and the highestwas 29.52 µg/dl. For all treatments applied, there was an increase in the average plasma retinol levels of students after the intervention compared to before and the highest increase occurred in the administration of the emulsion at a dose of 15 ml/day, which was an increase of 43.65% and the lowest was in the administration of a dose of 5 ml/day.

The results of the statistical test using analysis of Variance (ANOVA) showed that prior to the administration of red palm oil emulsions (pre-test), there was no significant difference betweenthe mean plasma retinol levels of children based on the dose applied (p = 0.179). Meanwhile, after the administration of the red palm oil emulsion (post-test), there were significant differences between the mean plasma retinol levels of children based on the dose applied (p = 0.050). Further test results using the least significant difference test showed that the different treatment groups were group II (dose of 10 ml/day) and group III (15 ml/day). In providing red palm oil emulsionwith a dose of 5 ml/day, there was an increase in the average plasma retinol levels of 23.97%, from 8.82 ug/dl before interventionto 11.60 mg/di after the intervention. Statistical test results usingthe Wilcoxon sign rank test showed that there was no significant difference between plasma retinol levels beforeand afterthe intervention (p = 0.059). This means that there is no effect of the red palm oil emulsionwith a dose of 5 ml/day on plasma retinol levels of children.

Unlike the administration of the red palm oil emulsion with a dose of 5 ml/day, the administration of the red palm oil emulsion with a dose of 10 ml/day may increase the plasma levels of retinol, as shown in Table 3.Here, an increase in average plasma retinol levels of 29.17%was observedafter the interventioncomparedto before, from 6.12 µg/dl before the interventionto 29.17 ug/dl after the intervention. Statistical test results using a paired t-test showed that there was a significant difference between the plasma retinol levels of the children before and after the administration of the red palm oil emulsion with a dose of 10 ml/day for one month (p = 0.019). This means that there is an effect of the red palm oil emulsion with a dose of 10 ml/day on the plasma retinol levels of the children.

The provision of the red palm oil emulsion with a dose of 15 ml/day also increasedthe plasma levels of retinol, as shown in Table 3, where an increase in the average plasma retinol levels of 43.65% was observed after the intervention compared to before, from 5.68 μg/dl before the intervention to 10.08 μg/dl after the intervention. Statisticaltest results using the Wilcoxon test showed that there was a significant difference betweenthe mean plasma retinol levels of the children before and after the administration of the red palm oil emulsion with a dose of 15 ml/day for one month ($p = 0.003$). This meansthat there is an effect of the red palm oil emulsion with a dose of 15 ml/day on the plasma retinol levels of the children.

DISCUSSION

Red palm oil production: The manufactureof red palm oil was performed by using the optimum conditions obtained in previous studies (Masni, 2013) that produced red palm oil with carotene concentrations of 10,121 ppm. This concentrationis 6.4 times higher than the concentration of carotenoids from crude palm oil (CPO), which is 157 4 ppm. This suggeststhat efforts to increasethe concentrationof carotenoids by passingthe red palm oil through column chromatographyusing rice hull ash as an absorbent material is quite efficient. The concentrationof carotene obtained in this study is higher than that obtained by Najamuddin (2009),who produces pure red palm oil by way of a direct skip crude palm oil (CPO) in the chromatography column, which also uses rice husk ash as an absorbent material, without going through the stages of previous filtration. The increasing concentrations of carotene obtained in this study were lower than those using synthetic absorbent materials (Latip *et al.*, 2000). The use of synthetic materials may provide a higher concentrationof carotenoids, but has a greater cost because using synthetic materials requires elution materials, such as hexane or methanol, which are expensive. Compared with the absorbent material used in this study, the price of rice hull ash isvery cheap. The use of rice hull ash as an absorbent material, besides being cheap, can also serve as an attempt to increase the economic value of rice hull ash, which is usually only used as an ash by housewives. Another aspect that also needs consideration regardingthe use of synthetic absorbent material is the fact that using typical elution material, where in the elution substance is toxic chemicals (methanol or hexane), will cause problems when consumed. We do dry a sample under a streamof nitrogen gas, but we cannot be sure whether the methanol or hexane is removed.

Hedonic test: The hedonic test or test acceptance and preference for a food product needs to be conductedto determine the acceptance or consumer preferencesfor the products made. The test results and acceptance of the red palm oil emulsion products made with three variations of additions of flavoring and smell (Flavor) indicate that the product is liked and accepted best as an emulsion that uses the melon flavor. The assessment reviews based on the scores given by the panelists involved showing that acceptance scores for emulsion products that use the melon flavor are higher than for emulsion productsthat use the vanilla or citrus flavor.

Additional flavor in red palm oil emulsion preparation needs to be performed in an attempt to cover up the smell of palm oil, which is very specific and could affect people who consume it. Given their taste and smell, emulsion products could be more easily given to children. As in the research conducted by the Research Institute of Nutrition Bogor (1991), for the prevention of vitamin A deficiency (VAD), as much as one tablespoon of palm oil should be provided every day to children. From this research, it was revealedthat it is very hardto givethis oil to children so that they will consume palm oil every day, but the administration was carried out by force.

Other efforts that have been made to reduce the smell and taste typical of red palm oil by the research of Permaesih (1991) include extracting carotenoids from palm oil using acetone: hexane (1:3) to obtain concentrated carotene. These concentrates are then mixedwith milkto give to children. However, this method is certainly more costly than the methods for this study because it uses materialsfor extraction and it could also pose a danger ifthe evaporationof the extractor material was not perfect. The addition of red palm oil to biscuits as shortening has also been tested by Martha and Benade (2000) in South Africa in an attempt to cover up the smell of palm oil. In that study, Benade compared biscuits enriched with a synthetic carotene and biscuits enrichedwith natural carotenoidsfrom red palm oil.

Effect of red palm oil emulsion on plasma retinol levels: The studywas conductedat the State Elementary School District of Ujung Tanah Cambaya Makassar, an elementary school located in a coastal area with environmental conditions a little rundown. At the beginning of the study, we carried out an examinationof the children including clinical state, weight, height and blood sampling for plasma retinol level examination. After that, the children were administered red palm oil emulsion of 5 ml/day in group I, 10 ml/day in group II and 15 ml/day in group III. The doses given were based on the results of previous studies by Oey *et al.* (1967) and Karyadi (1968), which indicate that giving one teaspoon of crude palm oil (CPO) per day can prevent VAD and giving one tablespoon of crude palm oil every day can cure xerophthalmia.

The measurement results of the plasma retinol concentration in the whole sample before giving red palm oil emulsions (pre-test) showed that 27 of the 36 children involved in the study (75%) had plasma retinal levels of less than 10 μg/dl. After giving the red palm oil emulsion for one month, the number who suffered severe VAD was reduced to 69%. This indicates that the majority of children in the study site suffered severe VAD, the rest are VAD mild and only one person does not suffer from VAD. The statistical analysis results showed that in the pre-test, there was no significant difference between the mean plasma retinal level in each treatment group ($p>0.05$), which means that the administration of the intervention in each group can be continued and can be compared because each group has the same starting condition.

After the children consumed the palm oil emulsion for one month at a dose of 5 ml/day, there was a decline in the prevalence of children who suffer from severe KVA from 60 to 30%. This is observed in the 23.97% increase in mean plasma retinal levels when comparing the values before and after the administration of the red palm oil emulsion; however, based on the results of a statistical test, this difference was not significant ($p>0.05$). The percentage change obtained in this study is higher than the results of the study by Karyadi (1968), which only showed a change of 19.5%. This can be understood because these studies gave children crude palm oil, as opposed to this study.

In the provision of the red palm oil emulsion with a dose of 10 ml/day, severe VAD prevalence fell by 16.4% (83 to 66.6%). This result is in line with the increase in the average value of plasma retinal levels after administration of the red palm oil emulsion (6.12 to 8.64 g/dl), where there is a change of 29.17%. In other words, the plasma retinal levels of the children who get red palm oil emulsions of 10 ml/day were significantly higher ($p<0.05$) than those of the children before they received the emulsion. The percentage change obtained at this higher dose is greater than that of the emulsion given with a dose of 5 ml/day. This can be understood, as the amount of palm oil contained in the emulsions given at 10 ml/day as higher and thus there was also a greater carotene intake.

Just as in the provision of emulsion at a dose of 10 ml/day, giving an emulsion of 15 ml/day also provided a significant effect on the plasma retinal levels of the children. As we have observed in the results in Table 6, the prevalence of severe VAD decreased by 27.3%, which is higher than the decrease achieved in the administration of a dose of 10 ml/day. This was confirmed again by an increase in the mean plasma levels of retinal after the intervention, where there was a change of 43.65%.

The overall results of the intervention in this study showed that the largest percentage change of the plasma retinal levels was obtained in the provision of the red palm oil emulsion of 15 ml/day. The occurrence of this increase can be understood, as a higher amount of red palm oil administration caused an increase in the carotene intake.

Some research that showed similar results as the results of this study showed the effect of palm oil on plasma retinal levels. The research of Martha and Benade (2000) in South Africa on elementary school children compared the plasma retinal levels of the children who were given biscuits containing red palm oil or natural carotenoids with vitamin A-containing biscuits and crackers without any synthetic vitamin A as a control. The results showed that the plasma retinal levels of the children who ate the biscuits increased and either pure or synthetic carotenoids increased the plasma retinal levels significantly compared with the control group after consuming biscuits for three months. Although the results of Martha and Benade (2000) showed no significant differences between the biscuits that were fortified with natural carotenoids and synthetic carotenoids, the results of this study suggest that using the addition of red palm oil is cheaper, it also naturally contains carotene and vitamin E and palm oil does not contain trans fatty acids.

The research of Zeba *et al.* (2006) with elementary school children in Burkina Faso, Africa compared a group given red palm oil mixed into the child's diet and a group given vitamin A capsules three times a week for one year. The results showed that palm oil given in small amounts and on a regular basis provides more effective results in reducing the incidence of vitamin A deficiency. Likewise, the research results of Rukmini (1994) and Perdani (2012) showed that the consumption of crude palm oil may increase the plasma levels of retinal. The results also showed that the CPO has an antioxidant activity that can improve health.

The time of intervention used in this study was very brief, only one month. Other studies generally use an intervening time of a minimum of three months or up to one year. With a short intervention time, we still obtained good results, shown by a significantly decreasing prevalence of heavy KVA with each treatment applied. This indicates that the administration of a MSM sufficient emulsion has the potential to be developed, but it is necessary to study the dose and duration of administration that is most efficient to resolve the case KVA or, at a minimum, reduce KVA.

An overview of the concentration of retinal or Vitamin A from the emulsion produced in this study in the results of laboratory analyses showed that the concentration of carotene in the red palm oil emulsion is approximately 2476 mg/l or 2476 mg/1000 ml. This means that 100 ml of emulsion contained 247.6 mg of carotene and 15 ml of emulsion contained 37.05 mg of carotene or carotenoids at 37050 mg/15 ml emulsion. If this value is converted to the equivalent value of retinal, then by taking 15 ml of the emulsion, we will obtain retinal in the amount of 6175 g (6 g ~-carotene equivalent to mgretinol), which is six times greater than the RDA recommended for children aged 10-12 years, i.e. 1035 g. This indicates that the beverage emulsion obtained from this study has the potential to be used in the prevention of VAD.

According to the results of the intervention and the results of the measurements of the plasma retinol levels in children after taking the red palm oil emulsions for a month, it appears that the decrease in the prevalence of VAD, as measured from the mean percentage change in the plasma retinol concentrations before and after the intervention is less than 50%. This is understandable because many factors can influence the occurrence of these changes, for example, the presence or absence of infectious diseases and the intake of food consumed each day.

One limitation of this study was that we did not control the intake of vitamin A obtained by children from their daily diet. Dietary recall was performed, but the results were not perfect because the study was conducted during school hours. Research outside of school hours is difficult to do and it is hard to bring children in on holiday because there are some children who help their parents to sell fish at the market.

Conclusions:

a: The obtained concentration of carotene in pure red palm oil was 10,121 ppm, which was 6.4 times its original CPO concentrations (1574 ppm to 10,121 ppm) or 3.5 times that of the carotene concentrations of the red palm oil filtering results (2,865 ppm to 10, 121 ppm)

b: The most preferred emulsion product is an emulsion of red palm oil using melon flavor

c: The concentration of retinol (vitamin A) of the emulsion product is 6175 mg/15 ml emulsion

d: There is an effect of the red palm oil emulsion on the plasma retinol levels of primary school children. Administration of the emulsion at a dose of 10 and 15 ml/day provides a significant effect, while giving the emulsion at a dose of 5 ml/day did not have a significant effect

Recommendations: Considering that this study was only carried out for a month, further research is needed to assess the dose and the duration of administration of red palm oil emulsion that could resolve KVA.

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