

VITAMIN E CONTENTS AND OXIDATIVE STABILITY OF RED PALM OILS BLENDED CHICKEN NUGGETS DURING FROZEN STORAGE

(Kandungan Vitamin E dan Kestabilan Oksidatif Nugget Ayam Campuran Minyak Sawit Merah Semasa Penyimpanan Beku)

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Abstract

Red Palm Oil (RPO) has a high oxidative stability and contains high levels of natural antioxidants, such as vitamin E and carotenoids. In this study, Vitamin E contents and lipid oxidation of chicken nuggets blended with red palm oil consist of NVRO, NVRO-100 and NVRO-50 were compared against the control chicken fat treatment, each containing 10% fat. Vitamin E contents, thiobarbituric acid (TBA) values and peroxide values (PV) for all samples were measured throughout 4 months of storage at -18°C. All the vitamin E homologues were decreased. α -tocopherol and α -tocotrienol decreased faster meanwhile δ -tocopherol decreased slower than other homologues. Besides that, Vitamin E content in NVRO and NVRO-100 was significantly decreased ($p < 0.05$) from 767.15 to 482.14 $\mu\text{g/g}$ and 842.73 to 672.36 $\mu\text{g/g}$ respectively. TBA and PV values for all samples chicken nuggets increased throughout 3 months of frozen storage but started to decrease thereafter. However, chicken nuggets formulated with NVRO, NVRO-100 and NVRO-50 significantly reduced ($p < 0.05$) TBA and PV values compared with chicken fat treatments. This study showed that frozen storage influence vitamin E stability and the potential of utilization of red palm oils in improving nutritional quality and reducing lipid oxidation of chicken nugget.

Keywords: Vitamin E; oxidative stability; red palm oil; chicken nugget

Abstrak

Minyak sawit merah mempunyai kestabilan oksidatif yang tinggi dan mempunyai kandungan antioksidan semulajadi yang tinggi seperti vitamin E dan karotenoid. Dalam kajian ini, kandungan Vitamin E dan pengoksidaan lipid nugget ayam campuran minyak sawit merah yang terdiri daripada NVRO, NVRO-100 dan NVRO-50 telah dibandingkan dengan kawalan sampel yang mengandungi lemak ayam, masing-masing mengandungi 10 % lemak. Kandungan vitamin E, nilai asid tiobarbiturik (TBA) dan peroksida (PV) bagi kesemua sampel dinilai sepanjang 4 bulan penyimpanan pada suhu -18°C. Kesemua kandungan homolog vitamin E menunjukkan penurunan. α -tokoferol dan α -tokotrienol berkurang dengan kadar lebih cepat manakala δ -tokoferol berkurang dengan kadar perlahan berbanding homolog lain. Selain itu, kandungan vitamin E pada sampel NVRO dan NVRO-100 menunjukkan penurunan signifikan ($p < 0.05$) masing-masing daripada 767.15 kepada 482.14 $\mu\text{g/g}$ dan 842.73 kepada 672.36 $\mu\text{g/g}$. Nilai TBA dan PV bagi semua sampel nugget ayam meningkat pada 3 bulan pertama penyimpanan beku dan kemudian mula menunjukkan penurunan. Walau bagaimanapun, nugget ayam yang mengandungi NVRO, NVRO-100 dan NVRO-50 menunjukkan nilai TBA dan PV lebih rendah ($p < 0.05$) berbanding dengan sampel yang mengandungi lemak ayam. Kajian ini menunjukkan bahawa penyimpanan beku mempengaruhi kestabilan vitamin E dan juga potensi penggunaan minyak sawit merah dalam meningkatkan nutrisi dan mengurangkan pengoksidaan lipid dalam nugget ayam.

Kata kunci: Vitamin E; kestabilan oksidatif; minyak sawit merah; nugget ayam

Introduction

Animal fats are added to meat products because of its texture, flavour and juiciness. However, it is also has high calories and saturated fatty acid content [1]. With the purpose of generating products with reduced calories, animal fats should be substituting with other sources of fat containing unsaturated fatty acids such as vegetables oils. Unlike animal fats, plant fats and oils are higher in unsaturated fatty acids but have low cholesterol. Palm oils (POs) and red palm oils (RPOs) have been reported to have balance proportion of saturated and unsaturated fatty acids. In spite of its level of saturated fatty acid content (50%), red palm oil has not been found to promote atherosclerosis and/or arterial thrombosis. This is probably due to the ratio of its saturated fatty acid to unsaturated fatty acid content and its high concentration of antioxidants such as beta-carotene, tocotrienols and tocopherols [2]. A lot of research has been carried out to study the effect of partially or completely replaced animal fat with palm fat on emulsion stability, nutritional composition, texture and sensory quality of meat products [1-4]. Babji et al. [5] and Hsu and Yu [6] have reported that there were no significant differences in the cooking loss, texture, juiciness, aroma, oiliness and overall acceptance between burgers prepared with palm fats and the conventional ones with beef fat.

On the other hands, RPO is not only rich in β -carotene, but it is also an excellent source of vitamin E, which are fat-soluble antioxidants. Vitamin E occurs as a mixture of tocopherols (~30%) and tocotrienols (~70%) in PO and RPO. It has eight naturally occurring forms α , β , γ and δ tocopherols (α -T, β -T, γ -T, δ -T) and α , β , γ and δ tocotrienols (α -T3, β -T3, γ -T3, δ -T3) [7]. In nature, carotenoids are mainly responsible for red, yellow and orange colours and have potential health benefit in maintaining human health. Chicken meat is widely used in the production of a variety meat products and one of it is chicken nuggets. Nuggets are a restricted meat product with batter and coater to retain the quality [8]. Nuggets are a ready to cook and ready to eat products with simple preparation; this makes them a popular choice for a quick meal among consumers. Meat products containing palm fats that are naturally rich in tocopherols and tocotrienols are believed to be healthy and better. Wan Rosli et al. [4] has reported that potential of utilizing red palm oils as animal fat analogues in improving vitamin E, reducing cholesterol but not carotene in beef frankfurters.

The low oxidative stability of meat and meat products is a problem for all those involved in the meat production chain and thus, understanding and controlling the processes which lead to lipid oxidation is a major challenge for meat scientists. Vitamin E could be an effective way to prevent lipid oxidation in food products and the consequent obtaining of meat products with extended shelf-life. Freezing is one of the most important preservation methods for meat; it leads to a minimal loss of quality during long-term storage and to retard undesirable biochemical reactions in meat. Nutritional quality in term of cholesterol content, texture and fatty acid composition of palm fat substituted chicken nuggets has been reported to have beneficial effect by Alina et al. [9]. However, reports are lacking on a shelf life study of chicken nugget blended with red palm oils at frozen storage (-18°C).

Therefore, the objective of this study is to determine the vitamin E content and lipid stability of chicken nugget blended with red palm oils which contain high levels of carotene, tocopherols and tocotrienols over 4 months of frozen storage (-18 °C).

Materials and Methods

Sample Preparation

Red palm oils known as Natural Vitamin Rich Oil (NVRO, NVRO-100 and NVRO-50) were supplied by the Carotino Sdn Bhd, Pasir Gudang, Johor, Malaysia. Certificate of analysis for NVRO, NVRO-100 and NVRO-50 are shown in Table 1. Chicken meat (whole chicken) and fat were purchased from the wet market in Kajang. Dry ingredients (Table 2) such as black pepper, onion, garlic, salt, wheat flour and potato starch were purchased from the Giant supermarket in Kajang. Isolated soy protein (ISP) and sodium triphosphate (STPP) were purchased from Lucky Food Processing Sdn. Bhd., Pulau Pinang, Malaysia. Four chicken nugget formulations were compared. Each formulation contained 10% fat from chicken (control), NVRO (505 ppm carotenes), NVRO-100 (113 ppm carotenes) and NVRO-50 (53 ppm carotenes).

Table 1. Certificate of Analysis for NVRO, NVRO-100 and NVRO-50

Parameters	Red palm oils		
	NVRO	NVRO-100	NVRO-50
Free Fatty Acids, %	0.049	0.050	0.059
Iodine Value	51.6	51.6	51.7
Moisture & Impurities, %	0.04	0.03	0.03
Slip Melting Point, ° C	37.0	37.0	37.0
Carotenes, ppm	505	113	53
Tocopherols & Tocotrienols, ppm	>800	>800	>800

Source: Carotino Sdn.Bhd, Pasir Gudang, Johor,Malaysia

Table 2. Chicken nugget formulations

Ingredient	Percentage
Spent hen	51.5
Fat (chicken fat, NVRO, NVRO-100, NVRO-50)	10
Ice water	22
Black pepper	0.5
Onion	1.5
Garlic	1.0
ISP	3.0
STPP	0.5
Salt	1.0
Wheat flour	3.0
Potato starch	6.0
TOTAL	100

Method of production of chicken nuggets

Chicken meat from whole chicken part was ground by using ORIMAS Meat Chopper Model TBS 200. The meat chunks were blended with fat for 2.5 min. Meat and fat were subsequently mixed for another 1.5 min with salt, STPP and ISP. Then, other dry ingredients such as black pepper, onion, garlic, wheat flour and potato starch were mixed with ice water before adding them to the mixer and blending was continued at low speed for another 2 min. The finished chicken nugget batter was shaped with each nugget weighing around 20 grams. Then, the nuggets were coated with wheat flour, egg and breadcrumbs. The samples then were vacuum packed and stored at -18 ± 1 °C until further analysis. The samples were stored for 4 months.

Fat Extraction

Vitamin E analysis

Fat extraction was carried out with a method from Kinsella et al. [10]. In brief, the sample was blended and mixed with methanol and chloroform at the ratio of 1:2:1 for 2 min before it was added with 90 ml chloroform and 90 ml distilled water. The mixture is homogenized for another 30 seconds and filtered using filtered paper Whatman No. 1. Then, bottom layer of the filtrate was taken out and evaporated at 60°C by using vacuum rotary. The extracted fats were stored at -18°C for further analysis.

Peroxide value

The oil of the samples for PV test was extracted by using cold extraction, method from Alina et al. [11] with slight modification. Petroleum ether (bp 40-60 °C) was used for the extraction reagent to trap the oil. The ratio of the quantity of nugget sample to petroleum ether used was 1:3 respectively. In this extraction, 40 g of samples were ground and homogenized with 120 ml of petroleum ether and kept overnight. The flasks used were wrapped by aluminum foil to avoid the samples from being exposed to the light. Then the mixture of sample and petroleum ether was filtrated using vacuum filtration through a filter paper (Whatman No.1) in Buchner funnel to remove the grounded nugget from a solvent. The filtrate then was transferred into round bottom flask and attached to rotary evaporator. Discharging the petroleum ether solution process was done at 40 °C. The extracted fat was kept in the flask and wrapped in aluminum foil to avoid exposure to the light before being used in the next analysis.

Tocopherols and tocotrienols analysis

Tocopherols and tocotrienols were analysed using HPLC with stainless steel Agilent Lichrosorb normal phase column (250 nm ×4.6 mm×5 µm) according to the method of AOCS Ce 8-89 [12]. 20µl of samples were injected, and peak responses of tocopherols and tocotrienols were measured using fluorescence detector with excitation and emission wavelength at 290nm and 330nm respectively. The solvent system was hexane: isopropyl alcohol (99:1, v/v) at a flow rate of 1.0 ml/min. Tocopherols and tocotrienols analysis of chicken nuggets were duplicated.

Calculation of percentage reduction of vitamin E during storage

The percentage reduction of vitamin E during storage at 0 month was calculate based on the values of vitamin E in extracted fats from nuggets at 0 month, subtracted with values of vitamin E content from initial raw fat materials, divided by the initial values of vitamin E detected from initial raw fat materials. On the other hand, for percentage reduction of vitamin E during storage at 4 months, it was calculate based on the values of vitamin E in extracted fats from nuggets at 4months, subtracted with values from vitamin E in extracted fat at 0 month , divided by the vitamin E content in 0 month. The raw fat materials (chicken fat, NVRO, NVRO-100, NVRO-50) are the fats used before incorporate them into the sample in the nugget-making.

Determination of Peroxide Value

The peroxide value was determined according to the AOAC test method [13], where the freed iodine was titrated against a solution of sodium thiosulphate. Peroxide value (PV) was expressed in miliequivalents of peroxide per kilogram of fat sample.

Determination of Thiobarbituric Acid (TBA) Value

TBA value was tested by using the method of Buege and Aust [14] with slight modification. A 0.5g sample of sausage was homogenated and mixed with 2.5 ml of 0.375% TBA solution, 15% TCA and 0.25 N of hydrochloric acid (HCl). The mixture was heated for 10 minutes at boiling temperature (100 °C) to allow the formation of pink colour. Then, the solution was cooled and 1 mL of chloroform was added into the solution. The solution was centrifuged at 5500 rpm speed for 25 minutes. The absorption of supernatant was determined by using a spectrophotometer at wavelength of 532 nm. TBA value was expressed in milligrams of malonaldehyde (MA) equivalents per kilogram sample. The TBA value calculated based on the following formula:

$$\text{TBA Value} = \text{Reading at } 532\text{nm} \times 2.77 \quad (1)$$

Statistical Analysis

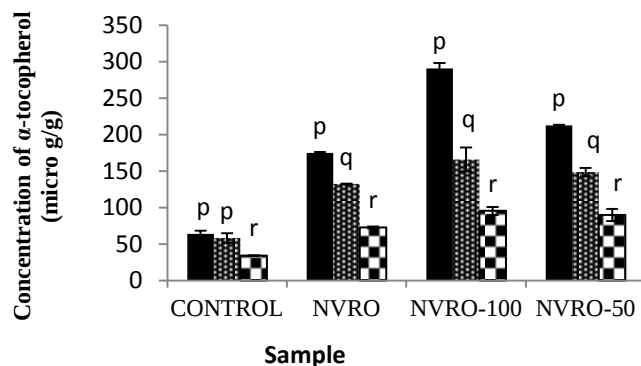
Data obtained were tested for significance using one-way analysis of variance (ANOVA). Duncan's multiple range test were carried out to resolve the difference among the treatment means. All the statistical evaluation of the results was performed using the IBM SPSS Statistics 20. Significance was established at $p < 0.05$ unless otherwise indicated.

Results and Discussion

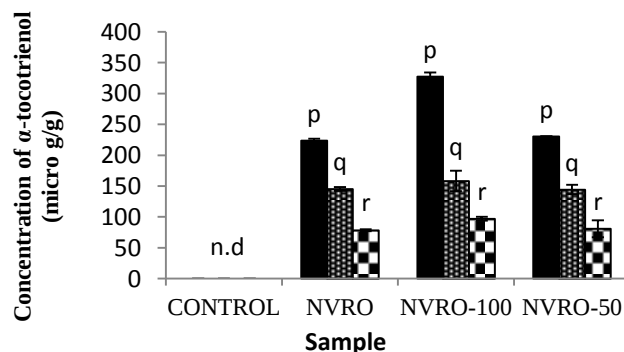
Vitamin E Contents of Chicken Nugget Blended with Red Palm Oils

α -tocopherol in all samples significantly decreased ($p < 0.05$) after 4 months of storage. The concentration of α -tocopherol significantly decreased ($p < 0.05$) to a range of 34.3-95.5 $\mu\text{g/g}$ (39-45% loss) during storage at -18°C after 4 months (Figure 1a and Table 3). A similar trend of a reduction was also detected in α -tocotrienol (38-46% loss), γ -tocopherol (6-30% loss), γ -tocotrienol (2-33% loss), δ -tocopherol (9-23% loss) and δ -tocotrienol (15-38% loss) in all samples except in the control sample as these homologues were not presented (Figure 1b-f). Chicken nugget containing NVRO showed the highest percentage loss for all vitamin E homologues in comparison with NVRO-100 and NVRO-50 after 4 months of storage as shown in Table 3. Total vitamin E concentration after 4 months of storage in the control chicken nugget was the lowest. On the other hands, NVRO showed the lowest total vitamin E concentration while NVRO-100 showed the highest among chicken nugget samples blended with red palm oils (Figure 1 g).

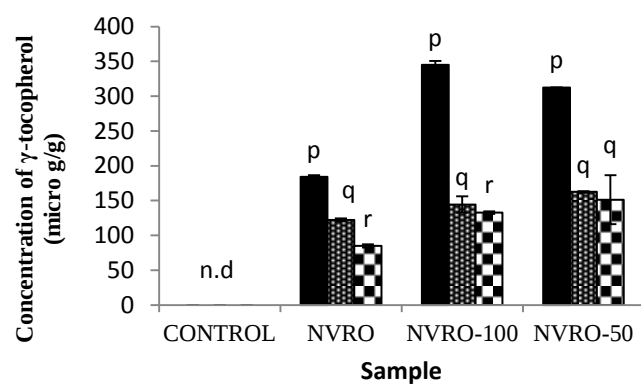
(a)



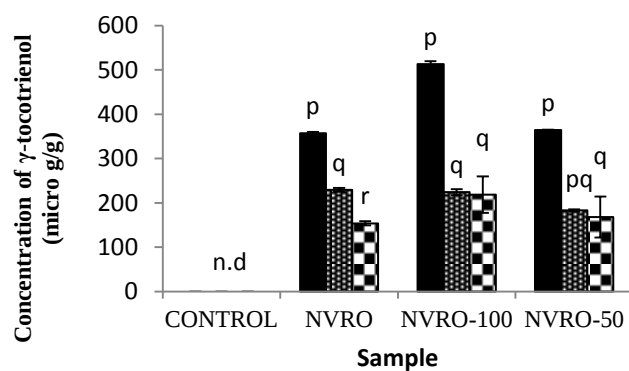
(b)



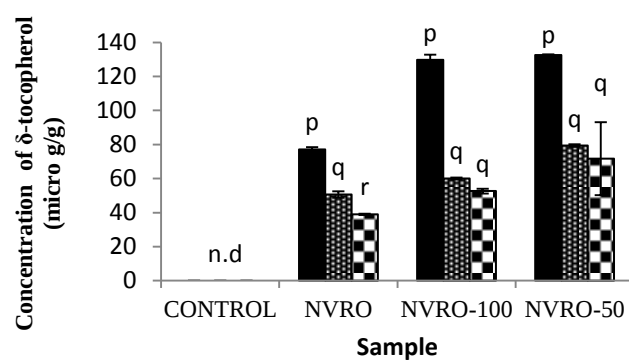
(c)



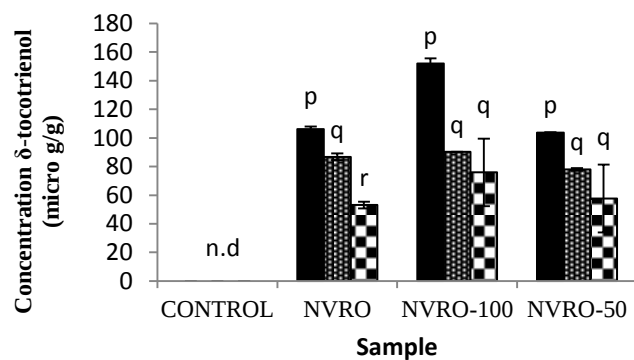
(d)



(e)



(f)



(g)

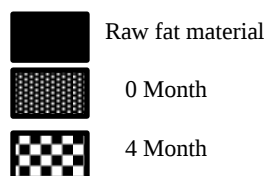
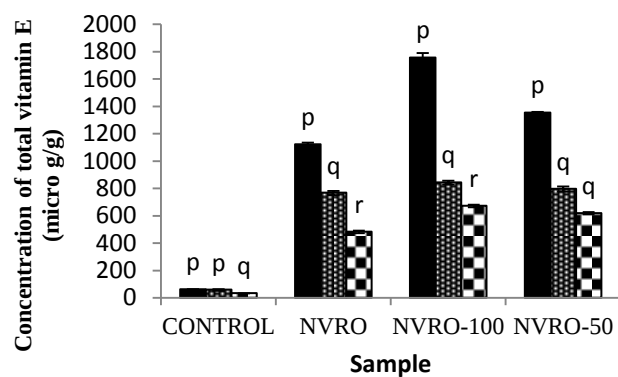


Figure 1. Mean $\pm$ standard deviation of concentration of (a) α -tocopherol, (b) α -tocotrienol, (c) γ - tocopherol, (d) γ - tocotrienol , (e) δ -tocopherol, (f) δ -tocotrienol and (g) Total Vitamin E for nugget blended with red palm oils at 0 month, 4 month and in fat raw material.

Table 3. Percentage reduction of vitamin E homologues for chicken nugget blended with red palm oils during storage for 4 months.

Vitamin E homologue	Percentage reduction (%) ^a				
	Storage time	Chicken fat (control)	NVRO	NVRO-100	NVRO-50
α -tocopherol	0 month	9.06	24.43	42.99	30.05
	4 month	41.25	45.00	42.40	39.53
α -tocotrienol	0 month	n.c	34.99	51.69	37.24
	4 month	n.c	46.08	38.70	44.05
β -tocopherol	0 month	n.c	n.c	n.c	n.c
	4 month	n.c	n.c	n.c	n.c
β -tocotrienol	0 month	n.c	n.c	n.c	n.c
	4 month	n.c	n.c	n.c	n.c
γ -tocopherol	0 month	n.c	33.59	58.19	47.98
	4 month	n.c	30.46	7.99	6.88
γ -tocotrienol	0 month	n.c	35.78	56.32	49.83
	4 month	n.c	33.03	2.52	8.20
δ -tocopherol	0 month	n.c	34.24	53.74	40.15
	4 month	n.c	23.01	12.39	9.68
δ -tocotrienol	0 month	n.c	18.15	40.63	24.89
	4 month	n.c	38.83	15.85	25.96
Total vitamin E	0 month	9.06	31.72	69.37	41.31
	4 month	41.25	37.15	20.11	22.18

^a For month 0, calculations are based on the values of extracted fats from nuggets at 0 month, subtracted from values for initial fat raw materials, divided by the initial values of fats detected from fat raw materials.

For month 4, calculations are based on the values of extracted fats from nuggets at 4 months, subtracted from values for month 0, divided by the fats detected from month 0.

^{n.c.} Percentage reduction of vitamin E in chicken nuggets was not calculated since all vitamin E homologues except tocopherol are absent in chicken fat material.

^{n.d.} Vitamin E homologues are not detected/absent.

Vitamin E was reduced or degraded after processing and during storage in chicken nuggets. The loss of vitamin E during storage may possibly be due to the formation of peroxides during lipid oxidation as shown in TBARS and PV analyses (Figure 2 and 3). Peroxides were degradable at higher temperatures but were stable at temperatures below 0°C; as a consequence, these peroxides can react with the vitamin E [15]. Processing of chicken nuggets cause a huge loss of vitamin E and the loss may have occurred during mixing the meat batters in the presence of oxygen [4].

γ -tocotrienol in all samples recorded the highest concentrations in raw fat materials and after 4 months of storage (Figure 1d). δ -tocopherol recorded the lowest percent loss of vitamin E after 4 months of storage (9-23 % loss), thus

δ -tocopherol was the most stable component in chicken nugget followed by γ -tocopherol (6-30% loss), γ -tocotrienol (8-33% loss), δ -tocotrienol (15-38% loss), α -tocotrienol (38-46% loss) and lastly α -tocopherol (39-45% loss).

This study showed that both α -tocopherol and α -tocotrienol decreased faster than other homologues due to their high percentage loss. The fast reduction of α -tocopherol in this study is possibly due to α -tocopherol better antioxidant activity than the other homologues. The presence of more methyl substituents in the phenolic ring of the α -tocopherol and α -tocotrienol does not only enhance their antioxidant activity, but also increase their lipophilic properties, making the α -homologues the most soluble vitamin E in lipid substrates [16]. Besides that, α -tocopherol is the most frequently used lipid-soluble free radical scavenger, and it could scavenge the free radical on the surface of cell membrane efficiently and finally terminate the lipid oxidation [17].

The differences of carotene content between samples blended with NVRO, NVRO-100 and NVRO-50 were not directly related with the vitamin E content of the samples. This means that highest carotene content in NVRO does not necessarily contained the highest vitamin E and vice versa as shown in Figure 1g. The small amount of α -tocopherol in the control sample was detected probably due to the chicken being fed with α -tocopherol supplemented diet. In order to improve the oxidative stability and increase the shelf life of the meat, antioxidants especially Vitamin E has been added to animal feeds. α -tocopherol content in poultry meat increased linearly as the dietary α -tocopherol supplementation increased [18]. This type of diet is generally given to the poultries in order to reduce or inhibit lipid oxidation. This is important as a few studies showed the presence of α -tocopherol in lipid extracted from chicken meat [7, 18-19].

Lipid Oxidation of Chicken Nugget Blended With Red Palm Oils

Hydro peroxides, are primary products of oxidative reaction [20]. Peroxide value indicates the initial stages of lipid oxidation. The peroxide values (PV) of the chicken nugget were shown in Figure 2. PV of the chicken fat (control sample) was the highest throughout the 4 months of frozen storage as shown in Figure 2. This similar with the result reported by Alina et al. [11], where higher degree of unsaturation in chicken fat compared to red palm oil made it less stable against oxidation. Frozen storage can slow down the oxidation process, but it cannot stop the oxidation from occurring. However, frozen storage is better in slowing down the oxidation of meat products than chilled storage as compared with study done by Alina et al. [11]

Thiobarbituric acid (TBA) value measures secondary lipid oxidation products, which responsible for the rancid taste developed during storage [21]. The TBA values of the chicken nugget during frozen storage were shown in Figure 3. Like PV, the TBA values also increased for the initial storage and started to slow down during the latter part of storage because of malanaldehyde (MA) decomposition and polymerization [22]. Increasing in TBA values is indicator of the development of rancidity [20]. TBA of the control sample containing chicken fat was the highest during 4 months of frozen storage (Figure 3). Meanwhile, chicken nuggets incorporation with red palm oils have lower TBA values than control sample especially at 0, 3 and 4 months. This showed that, utilization of red palm oil was able to slow down the secondary lipid oxidation in chicken nuggets.

Blended red palm oil chicken nuggets between NVRO, NVRO-100 and NVRO-50 showed no significant difference in both PV and TBARS results. Based on the values recorded for both TBA and PV in this study, it can be concluded that red palm oil and frozen storage have strong effects on the susceptibility of chicken nuggets on lipid oxidation. However, NVRO-50 can be said as suitable fat replacement because vitamin E content of NVRO-50 did not loss significantly after 4 months, good oxidative stability and less carotene content (101 ppm) which makes the colour of chicken nugget less reddish in comparison with NVRO and NVRO-100.

The relationship between vitamin E and β -carotene and their combine effects on oxidative stress may be quite complex. In vitro studies suggest that α -tocopherol and β -carotene have complementary roles relative to the varying O_2 tension in biological membranes and synergistically protective relationship between the two vitamins [23]. In contrast, a study also showed that the antioxidant capacity of β -carotene combined with α -tocopherol was inferior to α -tocopherol alone because its auto-oxidation weakened the effect of α -tocopherol [24].

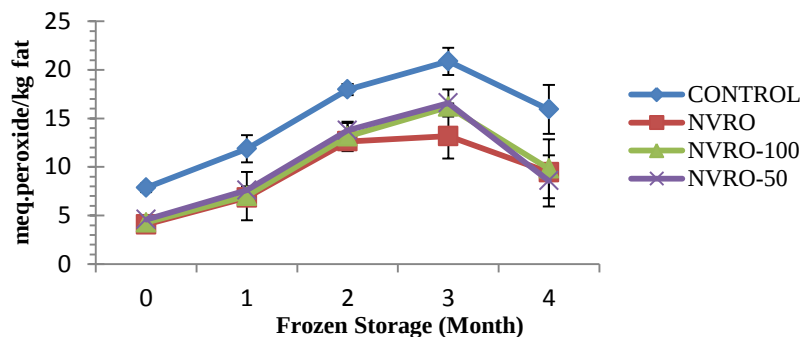


Figure 2. Peroxide value (PV) for chicken nugget samples during 4 months of frozen storage

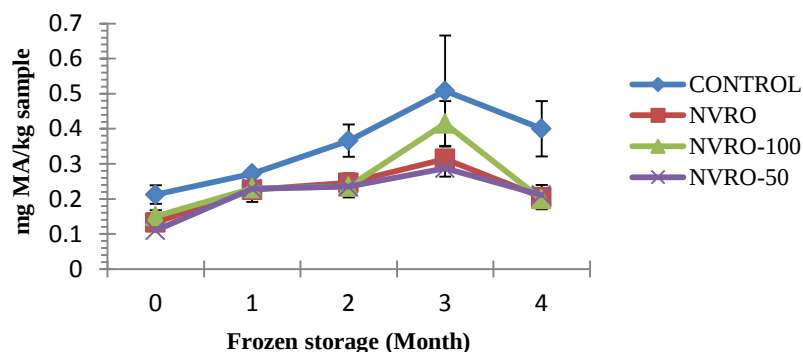


Figure 3. Thiobarbituric acid values (TBA) for chicken nugget samples during 4 months of frozen storage

Conclusion

During the storage period at -18°C , all the vitamin E homologues were decreased with δ -tocopherol was quite stable in chicken nuggets blended with red palm oils. α -tocopherol and α -tocotrienol decreased rapidly compared with other homologues. Vitamin E content in NVRO and NVRO-100 samples resulted in a significant loss ($p < 0.05$) after 4 months of frozen storage. Samples blended with red palm oils significantly ($p < 0.05$) showed the lower PV and TBARS values. This study showed that frozen storage and types of fat used could influence vitamin E stability and the potential of utilization of red palm oils in improving nutritional quality and reducing the oxidation of lipid in chicken nugget and other processed meat products.

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