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TOCOPHEROL AND TOCOTRIENOL CONTENTS OF CHICKEN NUGGETS BLENDED WITH RED PALM OILS BEFORE AND AFTER FRYING

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ABSTRACT

Tocopherols and tocotrienols content before and after frying in chicken nuggets blended with red palm oils NVRO, NVRO-100 and NVRO-50 was compared against the chicken fat treatment used as control. The lowest total tocopherols and tocotrienols content after frying was observed in control samples and the highest was in NVRO-100. Control samples showed significant increase of total tocopherols and tocotrienols from 34.32 ug g^{-1} before frying to 429.29 ug g^{-1} after frying due to oil uptake during frying where the cooking oil used was palm oil with inherent vitamin E precursors. The NVRO-50 showed significant decrease from 795.72 µg g^{-1} before frying to 690.87 µg g^{-1} after frying. Chicken nuggets blended with NVRO-100 were more heat stable followed by chicken nuggets blended with NVRO and NVRO-50. There was a significant loss of γ-T and δ-T in all samples after frying. This study showed the potential of utilising natural vitamin rich red palm oils as animal fat analogues in improving the nutritional quality of meat products.

Keywords: frying, tocopherol, tocotrienol, chicken nugget, red palm oil.

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INTRODUCTION

Animal fats are common raw materials added in emulsion-type meat products for reasons of texture, flavour and juiciness but they also contain high calories (Bañón *et al.*, 2008; Ghidurus *et al.*, 2010; Keeton, 1991; Youssef and Barbut, 2011). The high contents of saturated fats and cholesterol have been a major problem, resulting in meat products becoming the subject of scrutiny by nutritional, medical and consumer group. As consumers become more informed about the risks of having high saturated fat food in meat products, they tend to choose foods that are low fat or low saturated fat in an attempt to reduce risk factors. Palm oil has low concentrations of polyunsaturated fatty acid (PUFA) (Eder *et al.*, 2005) as well as free from cholesterol, unlike animal fat (Alina *et al*., 2009). Examples of those interesting oils used for this purpose are palm oils (PO) and red palm oils (RPO).

As palm oil has been found to be a reasonable replacement for partially hydrogenated oils which contain *trans* fatty acids, a lot of research have been carried out to study the effect of partially or completely replacing animal fat with palm fat on emulsion stability, nutritional composition, texture and sensory quality of meat products (Youssef and Barbut, 2011; Alina *et al*., 2009; 2012; Tan *et al*., 2006; Wan Rosli *et al*., 2010). RPO is not only rich in β-carotene, but is also an excellent source of vitamin E, which are fat-soluble antioxidants (Andreu-

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Sevilla *et al.,* 2009). These natural antioxidants are preferable compared to synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) due to carcinogenic risk of these synthetic antioxidants (Wanasundara and Shahidi, 1998; Tan *et al.,* 2006).

In nature, carotenoids are mainly responsible for red, yellow and orange colours, besides providing potential health benefit to humans. Carotenoids are commonly found in yellow, orange and green coloured fruits and vegetables. Of the vegetable oils that are widely consumed, palm oil contains the highest known concentration of agriculturally derived carotenoids (Tan, 1989). In fact, crude palm oil is the world's richest natural plant source of carotenes in terms of retinol equivalent. It contains about 15 to 300 times as many retinol equivalents as carrots, leafy green vegetables, and tomatoes, which are considered to have significant quantities of provitamin A activity (Tan, 1987). In food, in addition to their function as the natural pigments and provitamin A, these compounds can be used as food additives for colouring (Britton, 1995). The β-carotene is one of the most important and widely studied carotenoids. Thus, its recovery from palm oil and its by-products is important.

Vitamin E occurs as a mixture of tocopherols (~30%) and tocotrienols (~70%) in PO and RPO (Ooi *et al.,* 1996). It has eight naturally occurring forms, *i.e.* 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). Vitamin E is effective in inhibiting lipid oxidation in foods and biological systems (Van Acker *et al.,* 1993). Therefore, meat products such as sausage, nugget and burger containing palm fats that are naturally rich in tocopherols and tocotrienols are believed to be healthy and better (Wan Sulaiman *et al.,* 2001; Wan Rosli *et al.,* 2010).

Chicken meat is widely used in a variety of meat products and one of it is the chicken nuggets. Nuggets are a restricted meat product with batter and coater to retain the quality (Ismed *et al*., 2009). 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. Nutritional quality in terms of cholesterol content, texture and fatty acid composition of palm fat substituted chicken nuggets has been reported to be positive by Alina *et al*. (2009). However, comparison in vitamin E contents of chicken nuggets with different carotene contents in samples before and after frying has not been reported.

Frying is a traditional heat processing method for food preparation throughout the world and it is the main method of cooking for chicken nuggets. During frying, a complex series of chemical reactions takes place such as hydrolysis, polymerisation and thermoxidation. Frying oil decomposes to form a variety of volatile compounds as well as monomeric and polymeric products (Fritsch, 1981; Rehab and El Anany, 2012). There are many studies reporting on the effect of frying on vitamin E in frying oil (Rossi *et al.,* 2007; Simonne and Eltenmiller, 1998) but reports are lacking on the effect of frying on tocopherols and tocotrienols in processed meat products.

Therefore, this study looked into the content of tocopherols and tocotrienols before and after frying in chicken nuggets blended with red palm oils of different carotene contents.

MATERIALS AND METHODS

Materials

Red palm oils, the NVRO, NVRO-100 and NVRO-50 were supplied by the Carotino Company (Carotino Sdn Bhd, Johor, Malaysia). Certificate of analysis for NVRO, NVRO 100 and NVRO-50 is shown in *Table 1*. Chicken meat and fat were purchased from the wet market. Dry ingredients (*Table 2*) such as black pepper, onion, garlic, salt, wheat flour and potato starch were purchased from the supermarket. Isolated soya protein (ISP) and sodium triphosphate (STPP) were purchased from Lucky Food Processing Sdn Bhd, Pulau Pinang, Malaysia. Hexane and isopropanol used for HPLC are HPLC grade.

Sample Preparation

Four chicken nugget formulations were compared. Chicken nugget samples consisted of (1) 10% chicken fat (control); (2) 10% NVRO (505 ppm carotenes); (3) 10% NVRO-100 (113 ppm carotenes) ; and (4) 10% NVRO-50 (53 ppm carotenes).

Method of Production of Chicken Nuggets

Chicken meat 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 was shaped to resemble a bone (5 cm × 3 cm × 1.5 cm) with each nugget weighing around 20 g. Then, the nuggets were coated with wheat flour, egg and breadcrumbs. The samples were then vacuum packed and kept at -18 ± 1°C until further analysis.

Fat Extraction

Nuggets were fried at 180 ± 1°C in a Graes deep fryer (N-50, Canada) for 3 min with PO-based frying

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.

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	16.5
Onion	
Garlic	
ISP	
STPP	
Salt	
Wheat flour	
Potato starch	
Total	100

Note: ISP – isolated soya protein. STPP – sodium triphosphate.

oil. The ratio between nuggets to frying oil used was 1:40. Fat extraction was carried out following the method of Kinsella *et al.* (1977). The extracted fats were stored at -18°C for further analysis and comparisons were made on tocopherols and tocotrienols content between the extracted fats of nugget before and after frying.

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 (1992). Samples of 20 μl were injected, and peak responses of tocopherols and tocotrienols were measured using fluorescence detector with excitation and emission wavelength at 290 nm and 330 nm, respectively. The solvent system was hexane: isopropyl alcohol (99:1, v/v) at a flow rate of 1.0 ml min^{-1}. Tocopherols and tocotrienols analyses of chicken nuggets were replicated twice.

Statistical Analyses

Data obtained were tested for significance using analysis of variance (ANOVA) and the Duncan multiple range test. Significance was established at $P \leq 0.05$ unless otherwise indicated. The results of before and after frying of samples were compared using Paired t-test using mean and standard deviation with 95% confidence interval and analysed by IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Figure 1 and *Table 3* show the concentrations of tocopherols and tocotrienols in samples before and after frying and percentage of increase and decrease of vitamin E homologues after frying chicken nuggets containing either chicken fat, RPO NVRO, NVRO-100 or NVRO-50. After frying, α-T in chicken nuggets increased in control, NVRO and NVRO-100 samples but decreased in NVRO-50 samples (*Figure 1a*).

The same trend was observed for α-T3 as shown in *Figure 1b*. The β-T was not present in all chicken nugget samples, both before and after frying. The β-T3 could only be detected in control samples after frying with concentration of 25.731 μg g^{-1} (*Figure 1c*).

Concentrations of γ-T in NVRO, NVRO-100 and NVRO-50 samples decreased after frying (*Figure 1d*). The γ-T could not be detected in control samples both before and after frying. As for γ-T3, its concentration increased after frying in NVRO, NVRO-100 and NVRO-50 (*Figure 1e*). For the control samples, γ-T3 was absent before frying but 156.52 μg g^{-1} was detected after frying.

Concentrations of δ-T for NVRO, NVRO-100 and NVRO-50 decreased after frying and no δ-T was

TABLE 3. PERCENTAGE INCREASE OF VITAMIN E HOMOLOGUES AFTER FRYING OF CHICKEN NUGGETS BLENDED WITH RED PALM OIL

Vitamin E homolog	Percentage of increase/decrease of vitamin E after frying (%)[a]			
	chicken fat	NVRO	NVRO-100	NVRO-50
α-Tocopherol	+216.79	+14.01	+17.49	-6.32
α-Tocotrienol	n.c	+13.17	+20.17	-2.46
β-Tocopherol	n.d	n.d	n.d	n.d
β-Tocotrienol	n.c	n.d	n.d	n.d
γ-Tocopherol	n.d	-32.65	-31.70	-41.48
γ-Tocotrienol	n.c	+16.35	+16.59	+9.15
δ-Tocopherol	n.d	-42.19	-43.46	-46.67
δ-Tocotrienol	n.c	+1.65	+6.98	-5.42

Note: [a] Calculations are based on the values of extracted vitamin E concentrations before frying subtracted from values of extracted vitamin E concentrations after frying, divided by the values of extracted vitamin E concentrations before frying.
n.d Vitamin E homologues are not detected/absent.
n.c Percentage of increase/decrease of vitamin E homologue after frying was not calculated since vitamin E homologue is absent before frying.
(+) Increase in percentage of vitamin E content.
(-) Decrease in percentage of vitamin E content.

detected in control samples both before and after frying (*Figure 1f*). In the case of δ-T3, its concentration increased in NVRO and NVRO-100 but decreased in NVRO-50 after frying. For control samples, δ-T3 was absent before frying but 50.763 μg g^{-1} of δ-T3 was detected after frying (*Figure 1g*).

For total tocopherols and tocotrienols content, control samples showed significant increase ($p < 0.05$) after frying while NVRO-50 showed significant decrease. Total tocopherols and tocotrienols content increased slightly in NVRO and NVRO-100 after frying but they were not significantly different ($p > 0.05$) (*Figure 1h*).

Decreasing content of carotenes on formulation was not able to give consistent effect on the content of α-T, α-T3, δ-T3 and total vitamin E compared to the content of γ- T, γ- T3 and δ-T in samples before frying. This may due to the carotenes content in each formulation did not represent the content of vitamin E homologues. In other words, the highest carotenes content in NVRO did not represent the highest content of vitamin E in NVRO, and *vice versa.*

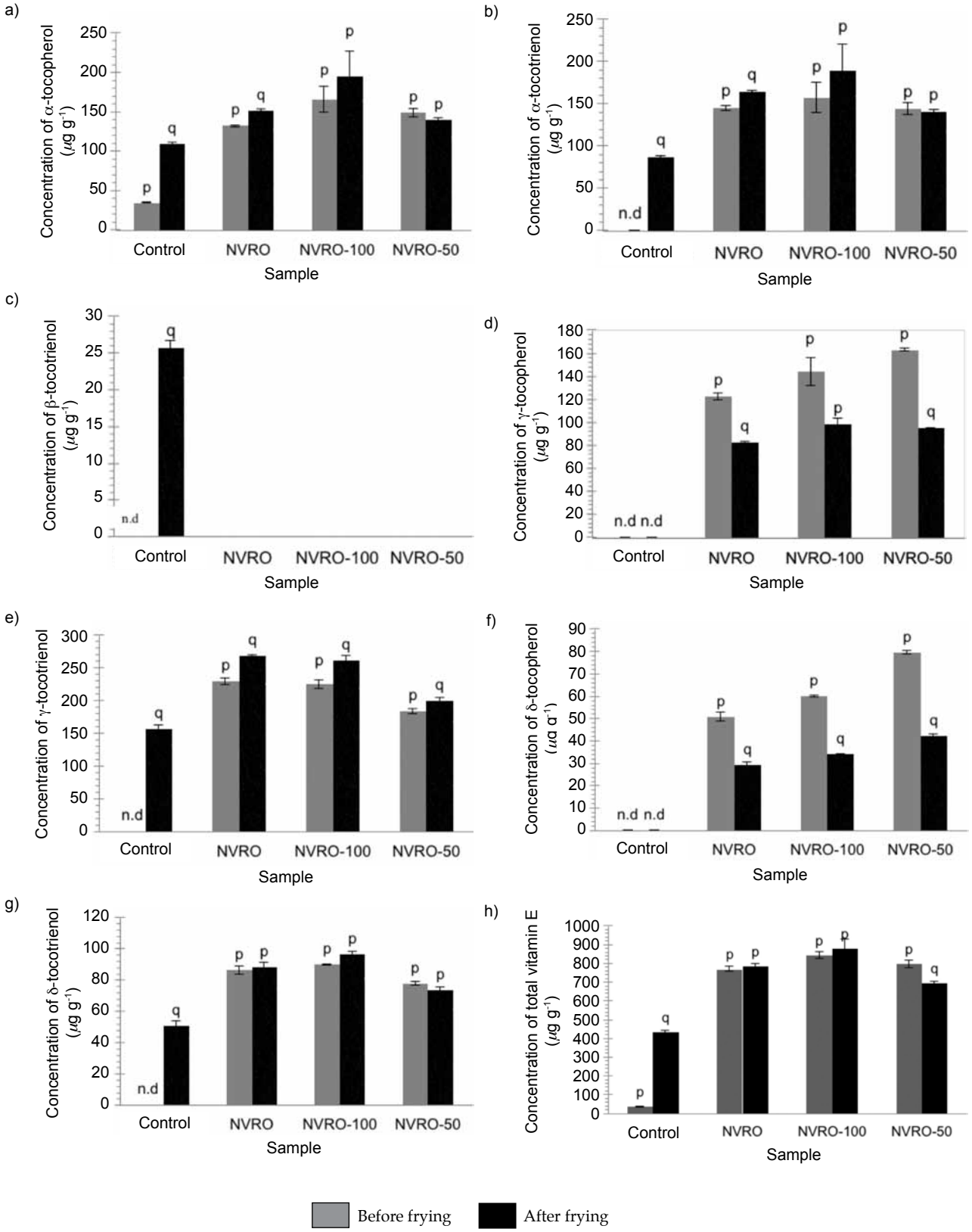
The type of frying oil used does affect the concentration of tocopherols and tocotrienols in chicken nugget samples formulated with RPO. During frying, oxygen is depleted and vitamin E is lost along with the oxidation of unsaturated fatty acids (Yi *et al.*, 2011). Frying oil is absorbed by the food during cooking and the absorbed quantity depends on the quality of the cooking oil, which affects the net intake of vitamin E (Andrikopoulos *et al.*, 2002). Vegetables oils are excellent sources of tocopherols and tocotrienols.

In the present study, due to oil uptake and their fats content, fried nuggets were enriched with considerable amounts of the tocopherols and tocotrienols. For example, in control samples, concentrations of α-T, α-T3, β-T3, γ-T3 and δ-T3 increased after frying compared to before frying. On the other hand, no β-T, γ-T and δ-T were detected in samples before and after frying. From the results, the detection of α-T3, β-T3, γ-T3 and δ-T3 after frying in control samples can be assumed to have come from the frying oil used, which was palm oil-based. The concentration of each homologue in the frying oil is shown in *Table 4*. This frying oil is characterised by the presence of α-T, α-T3, β-T3, γ-T3 and δ-T3 where γ-T3 was the most abundant.

An unexpected observation was the presence of α-T in control samples which contained chicken fat before frying. This was probably due to the chicken being fed with α-T supplemented diet. In order to improve the oxidative stability and thus increase the shelf life of meat, antioxidants especially vitamin E, has been successfully added to animal feeds. The α-T content in poultry meat increased linearly as the dietary α-T supplementation increased (Bou *et al.*, 2001). This type of diet is given to most poultry for reducing or inhibiting lipid oxidation. Results from a number of studies showed the presence of α-T in

TABLE 4. CONCENTRATIONS OF VITAMIN E HOMOLOGUES DETECTED IN FRYING OIL

Vitamin E homolog	Concentration (μg g^{-1})
Alpha T	225.548 ± 3.53
Alpha T3	264.914 ± 4.38
Beta T3	19.775 ± 0.27
Gamma T3	432.308 ± 5.08
Delta T3	127.869 ± 0.77



Note: p–q Bars with different letters within the same sample indicate significant differences ($p < 0.05$).
n.d. - Vitamin E homologues are not detected / absent.

Figure 1. Mean ± standard deviation of concentration of (a) α-tocopherol, (b) α-tocotrienol, (c) β- tocotrienol, (d) γ- tocopherol, (e) γ- tocotrienol , (f) δ-tocopherol, (g) δ-tocotrienol and (h) total vitamin E for nugget before and after frying blended with red palm oils.

lipid extracted from chicken meat (Bou *et al.,* 2001; Hewavitharanan *et al.,* 2004; O'neill *et al.,* 1998).

The β-T and β-T3 were absent in NVRO, NVRO-100 and NVRO-50 samples either before or after frying. However, frying oil used in this study contained a small amount of β-T3 as shown in *Table 4*, and this homolog might have been absorbed in a very small amount into the sample during frying. If this was the case, β-T3 would have a very small peak which overlapped with δ-T and thus its presence was camouflaged. Tocopherols are found in lipid soluble fractions of biological systems (Kurilich *et al.,* 1999). In general, tocopherol homologues, except α-T, have rapid loss than tocotrienol homologues. Their rapid loss is due to the degradation to oxidation products particularly in the less unsaturated oil such as PO (Mara *et al.,* 2009). This could explain the significant decrease of γ-T and δ-T in all samples after frying as shown in *Figures 1d* and *1f.* The γ-T and δ-T showed a significant decrease with the highest percentage loss in all samples after frying. This observation shows that γ-T and δ-T are less stable toward heat compared to other homologues.

The γ-T3 recorded the highest concentrations in all samples except control samples before and after frying (*Figure 1e*) . After frying, percentage of increase for γ-T3 showed the highest while δ-T3 showed the lowest (*Table 3*). This is remarkable since γ-T3 was present at high concentrations and the most abundant in the chicken nuggets and also the frying oil used. This could influence the final intake of γ-T3 and its high concentrations in all samples after frying.

Control nugget samples containing chicken fat had the least total tocopherols and tocotrienols content before and after frying. Among NVRO, NVRO-100 and NVRO-50, nugget samples formulated with NVRO-50 had less total tocopherols and tocotrienols concentration after frying than the other two samples. After frying, while the concentration of α-T, α-T3, and δ-T3 decreased in NVRO-50, their concentrations increased in NVRO and NVRO-100 as shown in *Table 3*. This could be due to differences in heat stability of the samples. This observation suggests that NVRO-50 has low heat stability compared to NVRO and NVRO-100 in terms of protecting those homologues during frying. Low heat stability of NVRO-50 also can be observed in γ-T and δ-T where γ-T and δ-T in NVRO-50 showed the highest percentage loss compared to γ-T and δ-T in NVRO and NVRO-100. The NVRO and NVRO-100 showed a better stability toward heat during frying as the concentration of α-T, α-T3 and δ-T3 increased after frying while decrease in NVRO-50. However, NVRO-100 showed better protection than NVRO as the percentage increase of those homologues in this sample was higher than NVRO. This was also possibly due to the high concentrations of α-T, α-T3 and δ-T3 in samples of NVRO-100 compared to NVRO before frying.

The findings of this study can be compared to that reported by Carlson and Tabacchi (1986) who found no significant change in the vitamin E content of the French fries during four days of commercial frying due to the increase in the fat intake of the fries after frying. This fat intake compensated for the vitamin E reduction and resulted in no significant change in the vitamin E content. Comparison can also be made with the study carried out by Simonne and Eitenmiller (1998) about changes of vitamin E content of chicken nuggets and breaded shrimps during frying in soyabean and corn oils. They observed an increase in total vitamin E after frying these samples (Simonne and Eitenmiller, 1998).

CONCLUSION

Tocopherols and tocotrienols content of vitamin E in chicken nuggets blended with RPO before and after frying were influenced by frying oil used which was PO-based, and also on the stability of each homologue and carotenoid contents. The γ-T and δ-T in all samples showed significant loss after frying. The γ-T3 showed the highest concentration in chicken nuggets both before and after frying. Chicken nuggets blended with NVRO-100 were more heat stable followed by chicken nuggets blended with NVRO and NVRO-50. This study showed the potential of utilising RPO as animal fat substitutes in improving the nutritional quality (vitamin E) of meat products.

Being one of the biggest producers and exporters of PO, Malaysia has an important role to play in fulfilling the growing global need for oils and fats sustainably. Results from this study could help increase consumers' awareness on the roles of RPO toward increasing nutritional values of meat products.

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