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Antioxidant capacity of value-added sandwich cookie creams based on red palm olein during storage

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ABSTRACT: Red palm olein (RPOL) is naturally rich in antioxidants, yet changes in its antioxidant capacity during storage were uncertain, which limited the application of RPOL in fat-based food products. Therefore, this study incorporated shortenings composed of 0, 5, and 10% (w/w) RPOL into sandwich cookie creams of SCC-0, SCC-5, and SCC-10, respectively, and determined their antioxidant capacity during storage for six months at 20, 30, and 35 ºC. Both SCC-5 and SCC-10 exhibited higher carotene, tocopherol, tocotrienol, and total phenolic content (TPC) than that of SCC-0, yet all SCCs were comparable in their free fatty acid (FFA) and UV-total oxidation values. After six months, the SCCs exhibited low TPC and FFA, yet were high in DPPH scavenging activity. At 20 and 30 ºC, both SCC-5 and SCC-10 oxidized more slowly than that of SCC-0. These findings proved the antioxidant capacity of RPOL, which delayed oxidation reactions in the SCCs during storage.

KEYWORDS: *Functional food; Nutrition; Oxidation; Palm oil; Phytonutrient; Vitamin*

RESUMEN: ***Capacidad antioxidante de cremas para galletas tipo sándwich con valor agregado a base de oleína roja de palma durante el almacenamiento.*** La oleína roja de palma (RPOL) es naturalmente rica en antioxidantes, pero los cambios en la capacidad antioxidante durante el almacenamiento no estaban claros y limitaron la aplicación de RPOL en productos alimenticios a base de grasas. En este estudio se incorporó mantecas compuestas por 0, 5 y 10 % (p/p) de RPOL en las cremas de galletas tipo sándwich (SCC): SCC-0, SCC-5 y SCC-10, respectivamente, y se determinó su capacidad antioxidante durante el almacenamiento durante seis meses a 20, 30 y 35 ºC. Tanto SCC-5 como SCC-10 exhibieron un mayor contenido de caroteno, tocoferol, tocotrienol y fenoles totales (TPC) que SCC-0, sin embargo, todas las SCC fueron comparables en sus valores de oxidación total de ácidos grasos libres (FFA) y UV. Después de seis meses, las SCC exhibieron TPC y FFA más bajos, pero fueron más altos en la actividad de eliminación de DPPH. A 20 y 30 ºC, tanto SCC-5 como SCC-10 se oxidaron más lentamente que SCC-0. Estos hallazgos demostraron la capacidad antioxidante de RPOL que retrasó las reacciones de oxidación en las SCC durante el almacenamiento.

PALABRAS CLAVE: *Aceite de palma; Alimentos funcionales; Fitonutriente; Nutrición; Oxidación; Vitamina*

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1. INTRODUCTION

Red palm olein (RPOL) is a novel palm-based product which, upon refining, retained approximately 80% phytonutrient compounds from crude palm oil. The phytonutrients are mainly composed of carotenoids, tocopherols, and tocotrienols which are natural vitamin A and vitamin E in the human diet (Kannan and Gundappa, 2014; Teh and Lau, 2021). The carotenoids possess antioxidative properties which contribute to longer shelf life of the oil. Both α- and β-carotenes have been reported as phytochemicals of high value and reviewed in terms of their functionality in inhibiting lipid oxidation, besides being widely associated with improving human health (Teh and Lau, 2021). Specifically, β-carotene can effectively be converted into vitamin A and provitamin A, which contribute to resolving vitamin A deficiency among malnourished and poor populations suffering from severe complications such as blindness and stunted growth followed by death (Gurmu *et al.*, 2014). A number of studies have reported the potential of palm carotenoids to function synergistically with vitamin E as a super natural antioxidant in the oil (Loganathan *et al.*, 2020). Vitamin E is present naturally as tocotrienols and tocopherols in the form of several isoforms of α-, β-, γ-, and δ- (May and Nesaretnam, 2014). Vitamin E works together with carotenoids as antioxidants by neutralizing free radicals and reactive oxygen species directly attributed to the donation of hydrogen molecules (Peh *et al.*, 2016). Several human clinical studies proposed tocotrienols, especially α-tocotrienols, to exhibit outstanding anticancer activity (Meganathan and Fu, 2016; Peh *et al.*, 2016), and upon long-term supplementation, have lowered cardiovascular health risk, depending on the dose and subject matter (Meganathan and Fu, 2016).

The presence of beneficial compounds in RPOL is undisputable. Besides their nutritional and medicinal values, these compounds may also delay the oxidation reaction in a food's fat portion during storage and simultaneously enhance the product's shelf life (Ayu *et al.*, 2016; Kumar *et al.*, 2016). Fortification of RPOL in bakery and confectionery products was able to provide 15-200% of the recommended dietary allowances (RDA) for β-carotene per portion of product consumed (Benede, 2001). El-Hadad *et al.* (2010) and El-Hadad *et al.* (2011) found enhanced oxidative stability of biscuits and chocolate spread incorporated with RPOL during storage, respectively. Manorama (2014) proved the contribution of RPOL as a food supplement in combating against vitamin A-deficiency among children in developing countries. Nurkhuzaiah *et al.* (2015) have used RPOL for frying, which resulted in higher antioxidant content in fried chicken nuggets. Loganathan *et al.* (2020) reported enhanced heat stability and low oxidative degradation of RPOL as cooking oil upon heating using different cooking techniques. These studies highlighted the growing interest in the use of RPOL due to its phytonutrients which can act as natural antioxidants in food products comparable to the synthetic ones.

Among the fat-based food products of interest is sandwich cookie cream (SCC), also termed as biscuit cream or cream filling, a confectionery product applied in between two pieces of cookies or wafers to enhance palatability. Generally, SCC should possess good shelf-stability, high resistance to oxidation and a less greasy sensation in the mouth. These properties are mainly due to its fat portion as the major ingredient in an SCC formulation (Mat Yusoff *et al.*, 2013; Cruz Serna and Gonzalez, 2014). An SCC is composed of 20-40% fat and 20-22% moisture, which contribute to its desirable stability and palatability, besides having low water activity of 0.2-0.3 in ensuring its long shelf life. The fat portion is also important in providing a smooth and creamy texture at body temperature, and in developing good creaming, quick-setting, and firm structural properties of an SCC at room temperature (Mat Yusoff *et al.*, 2013; Biswas *et al.*, 2017). Despite these functional properties, the fat portions were always composed of high saturated fat which are undesirable in terms of consumer health, yet are important in imparting the desirable physicochemical properties of the SCC. Therefore, recent studies attempted to improve an SCC formulation by reducing the fat content, besides replacing the fat portion especially the saturated fat with those of plant-based fat replacers or fats with a lower degree of saturation. The focus of these studies was mainly to obtain newly-formulated SCCs which were similar to the original SCCs in terms of physicochemical properties (Cruz Serna and Gonzalez, 2014; Biswas *et al.*, 2017).

In addition to these previous attempts, it is noteworthy that the incorporation of RPOL can further contribute to the development of value-added SCCs with enhanced antioxidant capacity. However, the antioxidant compounds undergo oxidation themselves to preserve the RPOL quality. Due to this undesirable reaction, the stability of these antioxidants in different storage conditions is uncertain, which limited the incorporation of RPOL into fat-based food products. In addition, there are very few reports on the enhancement of the nutritional properties of SCC products. Therefore, in order to fill these research gaps, this study aimed to develop and determine the storage stability of SCCs formulated with shortenings composed of 0 (SCC-0), 5 (SCC-5), and 10% (SCC-10) RPOL (w/w). The storage stability was determined based on the antioxidant capacity of the extracted fat portions of SCCs during six months of storage at 20, 30, and 35 °C, which represented variation in storage conditions upon display in the hypermarket, keeping at room temperature at home, and during transportation.

2. MATERIALS AND METHODS

2.1. Materials

Blends of RPOL:PO:PS were prepared in shortenings at different ratios of SH-0 (0:50:50), SH-5 (5:45:50), and SH-10 (10:40:50) (Mohamad Shah *et al*., 2021). These fat portions and sunflower lecithin were provided by Sime Darby Plantation Research Sdn Bhd, Selangor, Malaysia. Other ingredients (Table 1) and plain black cookies were purchased from local bakery shops.

Table 1. Sandwich cookie cream formulation

Ingredients		Amount (% w/w)
Fat-based	Shortening	25.00
	Sunflower lecithin	1.50
Dry phase	Icing sugar	40.00
	Skim milk powder	18.00
	Wheat flour	10.00
	Salt	0.90
	Potassium sorbate	0.10
Wet phase	Vanilla extract	2.00
	Water	3.00

For the analysis methods, the chemicals were acetic acid, chloroform, Follin-Ciocalteu reagent, iso-octane, iso-propanol, methanol, n-heptane, n-hexane, petroleum ether (Merck, Darmstadt, Germany), phenolphthalein, potassium hydroxide, potassium iodide, starch, sodium carbonate, sodium thiosulphate (Fisher Scientific, Pittsburgh, PA), α-tocopherol, α-, β-, γ-, and δ- tocotrienol reagents (Davos Chemical Corp, New Jersey, USA), gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent (Sigma-Aldrich Chemical Co., St. Louis, MO).

2.2. Preparation and storage of sandwich cookie cream

The shortenings were further used to develop SCCs (SCC-0, SCC-5, and SCC-10) based on the formulation in Table 1 and the preparation method described by Mat Yusoff *et al.* (2013) with some modifications. Fat-based ingredients were mixed and beaten in a Hobart food mixer (N50 5-Quart Mixer, Ohio, USA) at low speed (1 min), while the dry and wet ingredients were mixed separately in different bowls. The dry ingredients were further mixed into the fat-based ingredients in the mixing bowl, followed by the wet ingredients. The mixture was continuously beaten at medium (5 min) and high (5 min) speeds until it became intact. During the mixing, the mixture was scrapped and folded for every 2 min interval. Lastly, the mixture was beaten at slow speed (1 min), forming SCC. Each SCC was layered between two cookie pieces of circle-shaped cookies to mimic the commercial SCC samples and packed into a single packaging. The single packages were packed into bulk-sealed packages before being placed into containers. A similar type of packaging material was used for all samples to minimize variations due to oxygen permeability. These containers were stored in FRIOCELL incubators to minimize light exposure (MMM Medcenter Einrichtungen, GmBH, Germany) for six months at 20, 30, and 35 °C (Figure 1). The 2-, 4-, and 6-month periods were used for sample collection and analysis based on our preliminary studies which showed that changes in fat properties took place slowly in food products. Therefore, a shorter storage period would not show any significant changes in fat properties. The shelf life of a commercial sandwich cookie cream product is up to one year. Therefore, the samples developed

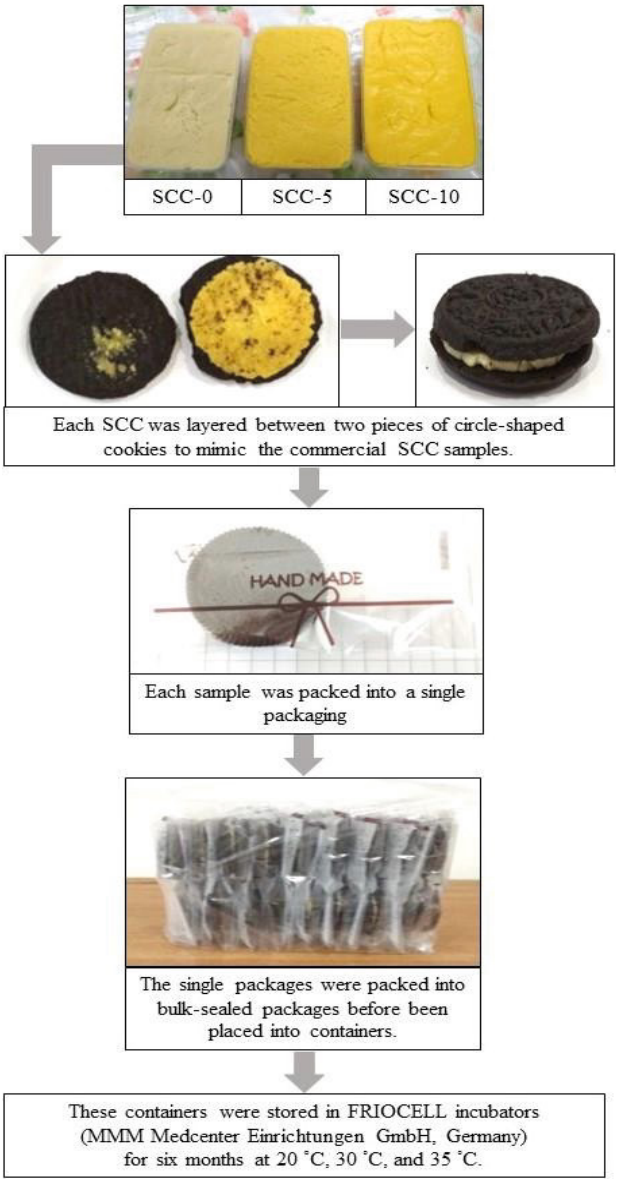

Figure 1. Preparation of sandwich cookie creams (SCC) for storage study

in this study were stored for six months only, since they were produced in the laboratory and not treated with any preservation method.

2.3. Fat extraction from sandwich cookie cream

After two, four, and six months of storage, the fat portions of the SCCs stored at all temperatures were extracted according to the MPOB Test Method (2005) with minor modifications. For each SCC, approximately 25 g were mixed with 100 ml petroleum ether and left at room temperature for 24 h. The mixture was further filtered into a round-bottom flask using Whatsman filter paper No. 1 (90 mm Ø), and the petroleum ether was removed from the mixture with a rotary evaporator (2422A0 RII Diagonal Rotary Evaporator System, Buchi, Flawil, Switzerland) at 50 °C. The flask containing the extracted fat was further heated in an oven (60 °C, 5 min) to remove the remaining petroleum ether. The extracted fat was transferred to a consumable tube and kept at 4 °C prior to the determination of antioxidant capacity.

2.4. Determination of carotene content and degree of bleachability index

The carotene content and degree of bleachability index (DOBI) (MPOB Test Method, 2005) were determined with a UV-VIS spectrophotometer (Varian Cary®, Agilent, USA). The oil sample (0.1 g) was weighed to the nearest of 0.001g into a 25-mL volumetric flask and dissolved with n-hexane. The diluted mixture was filled into a 10-mm cell and read at 446 nm for determining the carotene [Eq.1], and at 446 nm and 269 nm for the DOBI [Eq.2].

$$\text{Carotene content (ppm)} = \frac{25\ X\ a466\ X\ 10000}{W\ X\ 2610} \qquad \text{[Eq.1]}$$

a466 = absorbance reading of the oil sample
W = weight of the oil sample (g)

$$\text{DOBI} = \frac{\text{Absorbance at 446 nm}}{\text{Absorbance at 269 nm}} \qquad \text{[Eq.2]}$$

2.5. Determination of tocopherol and tocotrienol contents

Tocopherol and tocotrienol contents were determined using HPLC (MPOB Test Method, 2005). A melted sample of 0.02 g was dissolved into 5 mL n-heptane, followed by the injection of 20 µl of the mixture into the Agilent 1100 series HPLC equipped with a fluorescence detector (Agilent, USA). Emission and excitation wavelengths were set at 330 nm and 290 nm, respectively. The sample was eluted with a mobile phase consisting of heptane and isopropanol (99.5:0.5 v/v) at a flow rate of 1.4 mL/min and running time of 30 min. The column used was Luna® 5 µm silica of 250 mm length x 4.6 mm diameter (Phenomenex, California, USA) set at 35 °C, and a 95.5% tocols standard (Darvo Life Science, KLK OLEO) was used for the identification of compounds.

2.6. Extraction and determination of total phenolic content

The extraction of phenolic compounds was carried according to Abdullah *et al.* (2018) with minor alterations. The fat sample (5 g) was dissolved with n-hexane (5 ml) in a separating funnel and 10 ml methanol were added three times. For every methanol wash, the methanolic mixture was shaken for 5 min and rewashed with 10 ml n-hexane for another 1 min before being transferred to a round-bottom flask. The mixture was subjected to rotary evaporation (2422A0 RII Diagonal Rotary Evaporator System, Buchi, Flawil, Switzerland) at 50 °C for 5 min to remove the methanol and hexane. Then, the round-bottom flask was left in an oven at 40 °C for 3 hours to remove the remaining solvents. The phenolic extract was collected and kept in consumable tubes at -4 ºC prior to analysis.

The total phenolic content (TPC) was further determined using the Follin-Ciocalteu method according to Abdullah *et al.* (2018) with minor modifications. The calibration curves were prepared by using the gallic acid concentration at serial dilutions of 1000, 100, 50, 25, 12.5, and 6.25 ppm. The equation of plotted calibration curves was obtained as y = 0.007x + 0.0115, with $R^2 = 0.9985$ ($n = 6$). For sample preparation, the phenolic extracted previously was mixed with diluted Folin–Ciocalteu's reagent and sodium carbonate (Na_2CO_3). Methanol was used as blank solvent, while a mixture of methanol, Folin–Ciocalteu's reagent and Na_2CO_3 was used as a negative control. The prepared samples, standard, blank and negative control were loaded into a 96-well microplate and incubated for 30 min in a dark place, and then read at 765 nm using microplate readers (Synergy™ H1 microplate reader, BioTek Instruments, Inc, USA). The TPC was calculated according to Eq.3.

$$\text{TPC (mg GAE/g)} = \frac{(\text{absorbance}-\text{blank})-c}{m} \qquad \text{[Eq.3]}$$

c = 0.0115 (y-intercept obtained from the gallic acid standard equation)
m = 0.007 (gradient obtained from the gallic acid standard equation)

2.7. Determination of DPPH scavenging assay

A scavenging assay of DPPH was used to determine the free radical-scavenging activity of the phenolic extract according to Antoniewska *et al.* (2018) with minor alterations. For sample preparation, 50 µL of the phenolic extract were mixed with a 100 µL diluted DPPH solution. For the blank sample, 50 µL of the phenolic extract were diluted with 100 µL methanol. A mixture of methanol and diluted DPPH solution (1:2) was used as the blank solvent, while a mixture of gallic acid and diluted DPPH solution (1:2) was used as the negative control. Each mixture was loaded into a 96-well microplate which was further incubated in a dark place at room temperature for 30 min. The readings were taken at 517 nm with a microplate reader (Synergy™ H1 microplate reader, BioTek Instruments, Inc, USA) and were further used to calculate the scavenging activity according to Eq.4, expressed as mg GAE/100 g phenolic extract.

$$\%\ \text{DPPH Scavenging Activity} = \frac{(\text{BSO})-(\text{SA}-\text{BSA})}{(\text{BSO})} X\ 100 \qquad \text{[Eq.4]}$$

SA = absorbance of sample dilution (nm)
BSA = absorbance of blank sample dilution (nm)
BSO = blank solvent dilution of phenolic extract of the SCCs

2.8. Determination of free fatty acid

Free fatty acid (FFA) was determined according to the AOCS Official Method Ca 5a-40 (2003) using the titration method with potassium hydroxide until the solution turned pink in color. The % of FFA was calculated using the following equation:

$$\%\ \text{of FFA} = \frac{v\ X\ N\ X\ 256}{10\ X\ W} \qquad \text{[Eq.5]}$$

V = Volume of potassium hydroxide (ml);
N = Normality of potassium hydroxide (eq./ml);
W = Weight of the fat sample (g);
256 = Molecular weight of palmitic acid (g/mol)

2.9. Determination of peroxide value

Peroxide value (PV) was determined according to the AOCS Official Method Ca 8-53 (2003) using the titration method with 0.01 N sodium

thiosulphate until the solution turned clear. The PV was calculated by using the following equation:

$$\text{PV (meq } O_2/\text{g)} = \frac{1000\ (v1-v2)N}{W} \quad \text{[Eq.6]}$$

v1 = Volume of sodium thiosulphate without fat sample (ml);
v2 = Volume of sodium thiosulphate used to titrate the blank sample (ml);
N = Normality of sodium thiosulphate (0.01 N) (eq./ml);
W = Weight of fat sample used (g)

2.10. Determination of UV-total oxidation (UV-TOTOX) value

UV-TOTOX was determined with a UV-VIS spectrophotometer (Varian Cary®, Agilent, USA) at 233 and 269 nm and was calculated by using Eq.7-10 (MPOB Test Method, 2005).

$$\text{Volume of sample mixtures} = \frac{\text{Volume of iso-octane (ml)}}{100\ \text{X weight of extracted fats (g)}} \quad \text{[Eq.7]}$$

$$\text{EC269} = \frac{[\text{volume of sample mixtures (ml) X Absorbance 269}]-(0.18)}{383\ \text{X carotene content (ppm)}} \quad \text{[Eq.8]}$$

$$\text{EC233} = \frac{[\text{volume of sample mixtures (ml) X Absorbance 233}]-(0.06)}{383\ \text{X carotene content (ppm)}} \quad \text{[Eq.9]}$$

$$\text{UV TOTOX} = \text{EC269} + \text{EC233} \quad \text{[Eq.10]}$$

2.11. Statistical analysis

All results were expressed as mean ± standard deviation (n=6) using MINITAB™ Statistical Software (MINITAB® 14.12.0, New York, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparison test at 95% confidence level was used to determine significant differences among three or more sets of data, while a two-sample t-test was used for two sets of data.

3. RESULTS AND DISCUSSION

3.1. Effect of storage conditions on the antioxidative properties of the fat portions in sandwich cookie creams.

On day 0, the carotene content in SCC-10 (84.75 ± 1.22 ppm) was significantly higher than those of SCC-5 (50.32 ± 7.94 ppm) and SCC-0 (18.47 ± 0.42 ppm) (Table 2(a)). This trend took place throughout the storage period and was in line with El-Hadad *et al.* (2011), who reported an increase in carotene from 10.0 to 148.0 ppm as RPOL increased from 0 to 20% in chocolate spread. Changes in the carotene were clearly observed after 4 and 6 months. At 20 °C, a significant decrease ($p < 0.05$) in the carotene took place only after 6 months in all SCCs. At 30 and 35 °C, the carotene started to decrease faster in SCC-0 after 4 months, and in SCC-10 after 2 months. At 35 °C, the carotene in SCC-5 started to decrease significantly ($p < 0.05$) after 4 months. These findings concluded faster carotene degradation at higher temperatures, which also occurred due to lipid oxidation. Therefore, a simultaneous decrease and increase occurred in the DOBI (Table 2(b)) and UV-TOTOX (Table 3(c)), respectively.

DOBI indicates oil stability and quality through the measurement of bleaching earth required to refine the oil, which further depends on the oil's carotene content. Greater DOBI values indicate better oil quality (Tan *et al.*, 2017). However, for RPOL, its DOBI ranged below one (< 1) due to the fact that RPOL did not undergo an intense bleaching step during the refining process (Kannan and Gundappa, 2014). Based on Table 2(b), DOBI of the developed SCCs on day 0 ranged between 0.01-0.44. Throughout the storage period, SCC-10 was significantly higher ($p < 0.05$) in DOBI, which proved its greater quality due to its higher RPOL, followed by SCC-5 and SCC-0. Despite this finding, the DOBI of both SCC-5 and SCC-10 significantly decreased ($p < 0.05$) after 2 months and remained unchanged for up to 6 months at all temperatures. The sudden decrease indicated rapid oxidation in food incorporated with RPOL, as DOBI is more affected by the oxidation level of oil and the presence of antioxidants and contaminants besides the carotene content (Corley and Tinker, 2003). After 2 and 4 months, the different temperatures insignificantly affected ($p > 0.05$) the DOBI in all SCCs, yet the

Table 2. Antioxidant properties of red palm olein-based sandwich cookie creams (SCC) during storage for six months at different temperatures as described by their (a) carotene, (b) degree of bleachability index, (c) tocopherol, (d) tocotrienol, (e) total phenolic content, and (f) DPPH scavenging activity.

(a) Carotene content

Storage temperature (ºC)	Storage month	SCC-0	SCC-5	SCC-10
-	0	18.47 ± 0.42^{cA}	50.32 ± 7.94^{bA}	84.75 ± 1.22^{aA}
	2	18.31 ± 6.02^{cA}	48.09 ± 1.31^{bAB}	72.35 ± 0.12^{aABC}
20	4	10.58 ± 0.82^{cABC}	47.06 ± 7.73^{bAB}	73.36 ± 6.31^{aAB}
	6	5.10 ± 0.60^{cC}	21.72 ± 0.65^{bCD}	42.62 ± 0.26^{aDE}
	2	11.01 ± 1.15^{cABC}	40.97 ± 2.71^{bAB}	67.05 ± 3.12^{aABC}
30	4	8.43 ± 1.18^{cBC}	42.20 ± 3.90^{bAB}	64.07 ± 2.72^{aABC}
	6	6.07 ± 1.23^{cBC}	35.24 ± 2.19^{bBCD}	56.55 ± 3.54^{aCD}
	2	14.89 ± 7.48^{bAB}	44.62 ± 9.55^{aAB}	65.35 ± 8.22^{aABC}
35	4	7.26 ± 0.20^{cBC}	36.36 ± 1.74^{bABC}	64.98 ± 2.33^{aABC}
	6	5.80 ± 0.54^{cC}	20.66 ± 3.30^{bD}	40.73 ± 5.64^{aE}

(b) Degree of bleachability index (DOBI)

Storage temperature (ºC)	Storage month	SCC-0	SCC-5	SCC-10
-	0	0.01 ± 0.01^{cA}	0.25 ± 0.02^{bA}	0.44 ± 0.00^{aA}
	2	0.01 ± 0.00^{cA}	0.06 ± 0.00^{bB}	0.08 ± 0.00^{aBC}
20	4	0.01 ± 0.00^{cA}	0.06 ± 0.00^{bB}	0.09 ± 0.01^{aB}
	6	0.03 ± 0.02^{bA}	0.06 ± 0.01^{aB}	0.08 ± 0.00^{aBC}
	2	0.01 ± 0.00^{cA}	0.04 ± 0.00^{bB}	0.07 ± 0.00^{aC}
30	4	0.02 ± 0.01^{cA}	0.04 ± 0.00^{bB}	0.07 ± 0.00^{aC}
	6	0.02 ± 0.01^{bA}	0.06 ± 0.01^{aB}	0.07 ± 0.00^{aC}
	2	0.02 ± 0.01^{cA}	0.06 ± 0.00^{bB}	0.09 ± 0.01^{aB}
35	4	0.01 ± 0.01^{cA}	0.04 ± 0.01^{bB}	0.07 ± 0.01^{aBC}
	6	0.01 ± 0.00^{bA}	0.05 ± 0.01^{aB}	0.07 ± 0.00^{aC}

(c) Tocopherol content (ppm)

Storage temperature (ºC)	Storage month	SCC-0	SCC-5	SCC-10
-	0	142.67 ± 2.08^{bA}	140.33 ± 1.53^{bA}	150.33 ± 3.06^{aA}
	2	144.00 ± 7.00^{aA}	143.00 ± 1.73^{aA}	145.33 ± 4.04^{aA}
20	4	117.67 ± 1.16^{cCD}	125.33 ± 1.15^{bBC}	128.33 ± 0.58^{aA}
	6	106.33 ± 1.53^{cE}	114.00 ± 2.65^{bF}	120.33 ± 1.53^{aA}
	2	123.33 ± 2.08^{aBC}	129.00 ± 1.73^{aB}	168.67 ± 54.88^{aA}
30	4	112.67 ± 4.73^{bDE}	121.67 ± 1.53^{aCD}	121.33 ± 2.08^{aA}
	6	109.00 ± 0.00^{cDE}	114.67 ± 0.58^{bEF}	123.67 ± 2.08^{aA}
	2	131.33 ± 0.58^{bB}	125.67 ± 1.53^{cBC}	136.00 ± 0.00^{aA}
35	4	113.00 ± 1.00^{bDE}	119.3 ± 1.53^{abDE}	121.00 ± 4.36^{aA}
	6	114.00 ± 3.61^{aDE}	120.67 ± 2.89^{aCD}	124.67 ± 5.77^{aA}

(d) Tocotrienol content (ppm)

Storage temperature (ºC)	Storage month	SCC-0	SCC-5	SCC-10
-	0	425.00 ± 4.36^{aA}	431.00 ± 1.00^{aA}	399.67 ± 54.50^{aABC}
	2	439.00 ± 24.76^{aA}	428.00 ± 2.65^{aA}	437.33 ± 16.50^{aA}
20	4	348.00 ± 3.46^{cC}	363.67 ± 1.53^{bC}	370.33 ± 0.58^{aBCD}
	6	311.33 ± 4.04^{cD}	326.33 ± 6.66^{bE}	344.00 ± 2.00^{aD}
	2	386.00 ± 7.00^{aB}	393.00 ± 4.36^{aB}	399.67 ± 10.07^{aABC}
30	4	334.67 ± 13.32^{bCD}	362.33 ± 10.21^{aCD}	349.33 ± 5.69^{abCD}
	6	320.33 ± 1.53^{cCD}	328.67 ± 1.15^{bE}	355.67 ± 4.62^{aBCD}
	2	387.67 ± 2.89^{bB}	380.67 ± 6.11^{bB}	406.67 ± 1.53^{aAB}
35	4	332.33 ± 1.53^{aCD}	348.33 ± 4.16^{aCD}	346.33 ± 12.74^{aCD}
	6	335.00 ± 9.85^{aCD}	347.33 ± 7.77^{aD}	356.00 ± 16.17^{aBCD}

(e) Total phenolic content (mg GAE/g)

Storage temperature (ºC)	Storage month	SCC-0	SCC-5	SCC-10
-	0	9.99 ± 8.08^{aA}	14.13 ± 2.65^{aA}	15.08 ± 0.40^{aA}
	2	6.71 ± 0.12^{aA}	7.14 ± 0.80^{aAB}	7.67 ± 0.08^{aCD}
20	4	4.07 ± 0.50^{aA}	4.55 ± 0.46^{aAB}	5.27 ± 1.29^{aCDE}
	6	3.08 ± 0.33^{bA}	4.08 ± 0.46^{abB}	4.79 ± 0.48^{aF}
	2	5.21 ± 0.74^{aA}	7.05 ± 0.66^{aAB}	7.86 ± 4.27^{aB}
30	4	3.83 ± 0.22^{aA}	4.37 ± 1.07^{aAB}	5.06 ± 0.11^{aC}
	6	2.13 ± 0.54^{aA}	2.94 ± 0.36^{aAB}	4.04 ± 11.29^{aEF}
	2	4.21 ± 0.48^{bA}	6.14 ± 0.47^{aAB}	6.64 ± 0.30^{aB}
35	4	3.63 ± 0.37^{bA}	4.50 ± 0.46^{abAB}	4.79 ± 0.16^{aC}
	6	1.94 ± 0.17^{aA}	2.56 ± 0.08^{aAB}	3.04 ± 0.76^{aDEF}

(f) DPPH scavenging activity (mg GAE/100g)

Storage temperature (˚C)	Storage month	SCC-0	SCC-5	SCC-10
-	0	25.88 ± 13.07^{aAB}	30.68 ± 6.56^{aA}	30.90 ± 3.88^{aA}
	2	25.64 ± 2.00^{aAB}	23.23 ± 0.14^{aABCD}	24.89 ± 0.29^{aA}
20	4	17.10 ± 1.65^{aB}	16.82 ± 0.39^{aE}	17.10 ± 0.46^{aB}
	6	30.90 ± 0.68^{bA}	27.41 ± 0.11^{cBCD}	33.48 ± 0.97^{aAB}
	2	24.81 ± 0.90^{abAB}	24.64 ± 0.52^{bABCD}	26.31 ± 0.00^{aA}
30	4	$14. 77 \pm 0.23^{bB}$	18.56 ± 1.72^{aDE}	19.47 ± 0.57^{aB}
	6	23.46 ± 1.46^{aAB}	22.95 ± 1.32^{aBCD}	25.14 ± 0.34^{aAB}
	2	26.48 ± 1.53^{aAB}	27.48 ± 0.80^{aAB}	28.14 ± 0.76^{aA}
35	4	15.46 ± 0.60^{aB}	16.93 ± 1.95^{aCDE}	17.35 ± 0.26^{aB}
	6	22.82 ± 2.74^{aA}	24.24 ± 2.63^{aABC}	26.70 ± 7.18^{aA}

MINITAB™ Statistical Software (MINITAB® 14.12.0, New York, USA) was used for the one-way analysis of variance (ANOVA) and Tukey's multiple comparison test at 95% confidence level to determine significant differences between three or more sets of data. Means ± standard deviation ($n = 6$) with different small letters in each row, and means ± standard deviation ($n = 6$) with different capital letters in each column, are significantly different ($P < 0.05$). SCC-0 (0:50:50), SCC-5 (5:45:50), and SCC-10 (10:40:50) are SCCs composed of different ratios of red palm olein: palm oil: palm stearin

DOBI of SCC-10 significantly decreased ($p < 0.05$) at 30 °C and 35 °C after 6 months, which further proved the greater oxidation rate at higher storage temperature (Leonardis *et al.*, 2016).

With reference to Table 2(c), on day 0, SCC-10 (150.33 ± 3.06 ppm) was significantly higher ($p < 0.05$) in tocopherol content followed by SCC-0 (142.67 ± 2.08 ppm) and SCC-5 (140.33 ± 1.53 ppm). At 20 °C, the tocopherol in both SCC-0 and SCC-5 decreased significantly ($p < 0.05$) after 4 months, while no significant changes ($p > 0.05$) took place in SCC-10. At 30 °C, both SCC-0 and SCC-5 started to decrease ($p < 0.05$) in tocopherol sooner, after 2 months, and further decreased ($p < 0.05$) after 4 months, while in SCC-10, it started to decrease ($p < 0.05$) later, after 4 months. These findings highlighted the decreasing tocopherol in the SCCs due to prolonged storage time, which took place faster at higher temperatures. Similar trends were observed in the case of tocotrienol (Table 2(d)). The results also revealed 15-20% loss in total tocopherol and tocotrienol contents in all SCCs, which was most likely due to oxidative activity in the fats throughout the storage period. The antioxidant compounds, including tocopherol and tocotrienol had to self-immolate in order to scavenge free radicals prior to lipid oxidation (Ayu *et al.*, 2016), thus their amount decreased with storage.

The DPPH scavenging activity (Table 2(f)) was determined based on reactivity of TPC (Table 2(e)) as an antioxidant to resist rapid lipid oxidation. SCC-10 (15.08 ± 0.40 mg GAE/g) was significantly higher ($p < 0.05$) in TPC than those of SCC-5 (14.13 ± 2.65 mg GAE/g) and SCC-0 (9.99 ± 8.08 mg GAE/g), and this trend continued throughout the storage period. On the other hand, the scavenging activities were insignificantly different ($p > 0.05$) from each other (25.88-30.90 mg GAE/100g) on day 0, and were insignificantly affected ($p > 0.05$) by temperature after 2 and 6 months. At all temperatures, the scavenging activity of the SCCs at month 4 were significantly lower ($p < 0.05$), while those of month 6 were insignificantly different ($p > 0.05$) from those of day 0. These findings concluded that the scavenging activity of the SCCs were significantly affected by storage time, but not the temperatures. In relation to the TPC after 6 months, the TPC was significantly lower ($p < 0.05$), whilst the scavenging activity was higher ($p < 0.05$). These trends indicated that the phenolic compounds underwent scavenging activity, and thus their values decreased as the scavenging activity increased (Kumar *et al.*, 2016), and the changes were more obvious after 6 months. Moreover, both TPC and scavenging activity were insignificantly affected ($p > 0.05$) by temperature. Fluctuating scavenging activity and antioxidant content during storage were also reported in blends of groundnut and sunflower oils (Sunil *et al.*, 2015), which in the latter case was most likely dependent on the rate of lipid oxidation and reactivity of antioxidant compounds.

Table 3 summarizes the antioxidants present in the SCCs in comparison with other RPOL-based food products. The presence of 5% (w/w) and 10% (w/w) RPOL in the shortenings contributed to 1.25% (w/w) and 2.50% (w/w) RPOL in the SCC-5 and SCC-10, respectively. These small amounts of RPOL contributed to higher tocopherol contents as compared to biscuits (El-Hadad *et al.,* 2010) and chocolate spread (El-Hadad *et al.*, 2011), and approximately similar tocotrienol content to that of biscuits. Moreover, the carotene content in the SCCs was comparable to the lower amount of carotene present in chicken nuggets, comprising 50-100% (w/w) RPOL (Nurkhuzaiah *et al*., 2015). These findings highlighted the potential of SCC as another value-added food product to be developed based on RPOL.

3.2. Effect of storage conditions on the oxidative properties of the fat portions in sandwich cookie cream

The determination of FFA (Table 4(a)) is important for developing fat-based food products because it indicates the level of fat deterioration in the food (Leonardis *et al.*, 2016). On day 0, there was no significant difference ($p > 0.05$) between the FFA (0.05-0.06%) of SCC-0, SCC-5, and SCC-10, which were lower than the acceptable FFA for fresh palm-based products (< 5.0%) (Codex Alimentarius, 2017), thus indicating their low oxidation state. However, the values significantly increased ($p < 0.05$) after 2 and 4 months at all temperatures due to lipid oxidation as most likely affected by the storage conditions. Oxidative degradation still took place after 6 months, yet in unsaturated fatty acids such as linoleic and linolenic acids, which increased levels of hexanal

Table 3. Antioxidant compounds in sandwich cookie creams (SCC) composed of red palm olein-based shortenings (SH), and other red palm olein-based food products

	Sandwich cookies creams			Red palm olein-based food products		
	SCC-0	**SCC-5**	**SCC-10**	**Chicken nuggets (Nurkhuzaiah *et al.*, 2015)**	**Chocolate spread (El-Hadad *et al.*, 2011)**	**Biscuits (El-Hadad *et al.*, 2010)**
Red palm olein (% w/w)	0 (0% in SH-0)	1.25 (5% in SH-5)	2.50 (10% in SH-10)	50-100	0-20	40-60
Antioxidant compounds						
Carotene (ppm)	18.47 ± 0.42^{c}	50.32 ± 7.94^{b}	84.75 ± 1.22^{a}	53-505	148.0	173.0-188.0
Tocopherol (ppm)	142.67 ± 2.08^{b}	140.33 ± 1.53^{b}	150.33 ± 3.06^{a}	> 200	60.8	93.0-116.0
Tocotrienol (ppm)	425.00 ± 4.36^{a}	431.00 ± 1.00^{a}	399.67 ± 54.50^{a}	> 600	221.2	387.3-462.2
Total phenolic content (mg GAE/g)	9.99 ± 8.08^{a}	14.13 ± 2.65^{a}	15.08 ± 0.40^{a}	nd	nd	nd

nd: not determined. MINITAB™ Statistical Software (MINITAB® 14.12.0, New York, USA) was used for the one-way analysis of variance (ANOVA) and Tukey's multiple comparison test at 95% confidence level to determine significant differences between three or more sets of data. For the SCC, means ± standard deviation ($n = 6$) with different small letters in each row are significantly different ($P < 0.05$).

but not the FFA (Heydanek and McGorrin, 1988), thus the FFA significantly decreased ($p < 0.05$). After 4 and 6 months, the FFA in all SCCs increased with increasing temperature, which highlighted the significant effect of high temperature in promoting oxidation in the SCCs.

In addition to TPC, the trend in changes in DPPH scavenging activity (Table 3(d)) from day 0 to month 6 was also in line with the trend in changes in FFA at most temperatures. On day 0, the scavenging activity was the highest while FFA was the lowest, which indicated a low oxidative state of the fat portions. After 4 months, the scavenging activity decreased significantly ($p < 0.05$) and was the lowest, while the FFA significantly increased ($p < 0.05$) and was the highest, signifying increased oxidation as storage progressed. However, after 6 months, both TPC and FFA significantly decreased ($p < 0.05$) while the scavenging activity significantly increased ($p < 0.05$) which indicated the effectiveness of TPC in decreasing the oxidation reaction.

Peroxide value (PV) indicates the initial stage of oil deterioration in terms of peroxides and hydroperoxides as primary oxidation products (Tan *et al.*, 2017). The standard limit for the PV of fresh fats and oils is less than 10.0 meq O_2/g (Codex Alimentarius, 2017). Low PV may also indicate the degradation of primary oxidation products into secondary oxidation products with greater oxidation reaction (Tan *et al.*, 2017). As shown in Table 4(b), on day 0, the PV of SCC-0 was 0.88 ± 0.20 meq O_2/g. The presence of RPOL most likely lowered the oxidation rate of both SCC-5 and SCC-10, thus their PVs were too low and could not be determined. At all temperatures, the PV of all SCCs significantly increased ($p < 0.05$), indicating that all SCCs continuously underwent oxidation throughout storage, with the highest PVs ($p < 0.05$) recorded after 6 months. Due to absence of RPOL, SCC-0 showed the highest values compared to both SCC-5 and SCC-10. In particular, after 4 months, SCC-10 exhibited insignificantly different ($p > 0.05$) PV at all temperatures. However, after 6 months, SCC-10 exhibited higher PV at 20 °C compared to 30 °C ($p > 0.05$) and 35 °C ($p < 0.05$). This finding was most likely due to the higher oxidation rate at higher temperatures (30-35 °C), which led to the degradation of primary oxidation products into secondary oxidation products and thus lowered the PV (Tan *et al.*, 2017). Furthermore, after 6 months, except for SCC-10, the PV of the SCCs (11.71-17.21 meq O_2/g) had exceeded the limit of PV for fresh-produced fats and oils (<10.0 meq O_2/g) (Codex Alimentarius, 2017). Yet, in most cases, commercial SCCs have a shelf life of at least 1 year (Daglioglu *et al.*, 2004). According to Gotoh and Wada (2006), the threshold value for PV in fat-based food should be not more than 30 meq O_2/g. Thus, it can be concluded that the SCCs produced in

Table 4. Oxidative properties of red palm olein-based sandwich cookie creams (SCC) during storage for six months at different temperatures as described by their (a) free fatty acid, (b) peroxide value, and (c) UV-total oxidation (TOTOX) value.

(a) Free fatty acid (%)

Storage temperature (˚C)	Storage month	SCC-0	SCC-5	SCC-10
-	0	0.05 ± 0.01^{aE}	0.06 ± 0.01^{aE}	0.06 ± 0.01^{aE}
	2	0.96 ± 0.04^{bD}	1.10 ± 0.04^{aC}	1.08 ± 0.04^{aCD}
20	4	1.32 ± 0.02^{aB}	1.31 ± 0.03^{aB}	1.30 ± 0.02^{aBC}
	6	0.94 ± 0.07^{aD}	0.88 ± 0.01^{aD}	0.85 ± 0.03^{aD}
	2	1.14 ± 0.04^{aC}	1.14 ± 0.10^{aC}	1.07 ± 0.07^{aCD}
30	4	1.50 ± 0.02^{cA}	1.64 ± 0.03^{aA}	1.56 ± 0.03^{bAB}
	6	1.16 ± 0.03^{aC}	1.23 ± 0.06^{aBC}	1.05 ± 0.17^{aCD}
	2	1.12 ± 0.07^{aC}	1.12 ± 0.06^{aC}	1.12 ± 0.00^{aCD}
35	4	1.59 ± 0.04^{bA}	1.69 ± 0.01^{aA}	1.61 ± 0.02^{bA}
	6	1.06 ± 0.04^{aCD}	1.32 ± 0.09^{aB}	1.18 ± 0.19^{aCD}

(b) Peroxide value (meq O_2/g)

Storage temperature (˚C)	Storage month	SCC-0	SCC-5	SCC-10
-	0	0.88 ± 0.20^{aB}	Null^{bC}	Null^{bE}
	2	4.48 ± 1.93^{aB}	1.41 ± 0.14^{bBC}	1.24 ± 0.00^{bDE}
20	4	4.66 ± 0.11^{aB}	4.70 ± 0.20^{aB}	3.88 ± 0.23^{bCDE}
	6	15.86 ± 0.09^{aA}	11.71 ± 0.56^{bA}	15.22 ± 0.90^{aA}
	2	3.81 ± 0.15^{aB}	3.07 ± 0.14^{bBC}	2.82 ± 0.14^{bDE}
30	4	4.86 ± 0.17^{aB}	4.52 ± 0.37^{aB}	4.71 ± 0.54^{aBCD}
	6	16.89 ± 0.45^{aA}	13.46 ± 2.76^{aA}	8.83 ± 0.83^{bABC}
	2	4.43 ± 3.59^{aB}	3.79 ± 2.21^{aB}	3.57 ± 1.81^{aDE}
35	4	4.66 ± 0.16^{aB}	4.83 ± 0.55^{aB}	4.61 ± 0.82^{aBCD}
	6	17.21 ± 4.90^{aA}	12.14 ± 1.33^{abA}	8.45 ± 1.87^{bB}

(c) UV-Total oxidation value (UV-TOTOX)

Storage temperature (˚C)	Storage month	SCC-0	SCC-5	SCC-10
-	0	1.86 ± 0.03^{bC}	2.03 ± 0.10^{aC}	1.79 ± 0.03^{bC}
	2	4.81 ± 0.09^{aB}	4.85 ± 0.04^{aB}	4.85 ± 0.03^{aB}
20	4	5.14 ± 0.19^{aAB}	5.05 ± 0.12^{aAB}	5.03 ± 0.15^{aAB}
	6	5.33 ± 0.09^{aAB}	4.89 ± 0.10^{bB}	5.32 ± 0.14^{aA}
	2	5.02 ± 0.01^{aAB}	4.91 ± 0.14^{aB}	4.87 ± 0.10^{aB}
30	4	5.38 ± 0.49^{aA}	5.22 ± 0.10^{aAB}	5.19 ± 0.15^{aAB}
	6	5.36 ± 0.00^{aA}	5.13 ± 0.12^{bAB}	5.29 ± 0.10^{abA}
	2	5.07 ± 0.09^{aAB}	4.93 ± 0.14^{aB}	4.93 ± 0.15^{aB}
35	4	5.50 ± 0.14^{aA}	5.42 ± 0.07^{aA}	5.38 ± 0.13^{aA}
	6	5.48 ± 0.06^{aA}	5.24 ± 0.31^{aAB}	5.13 ± 0.14^{aAB}

MINITAB™ Statistical Software (MINITAB® 14.12.0, New York, USA) was used for the one-way analysis of variance (ANOVA) and Tukey's multiple comparison test at 95% confidence level to determine significant differences between three or more sets of data. Means ± standard deviation ($n = 6$) with different small letters in each row, and means ± standard deviation ($n = 6$) with different capital letters in each column, are significantly different ($P < 0.05$). SCC-0 (0:50:50), SCC-5 (5:45:50), and SCC-10 (10:40:50) are SCCs composed of different ratios of red palm olein: palm oil: palm stearin

this study were still acceptable in the market after 6 months at all temperatures tested.

Table 4(c) shows the UV-TOTOX values of the SCCs in different storage conditions which indicate the level of lipid oxidation, similar to DOBI (Tan *et al.*, 2017). On day 0, SCC-5 (2.03 ± 0.10) was significantly higher ($p < 0.05$) in UV-TOTOX, followed by SCC-0 (1.86 ± 0.03) and SCC-10 (1.79 ± 0.03). After 2 months, all SCCs significantly increased ($p < 0.05$) in UV-TOTOX, yet no critical changes were further observed afterwards for up to 6 months at all temperatures. These findings were inversely correlated with the trends observed in their DOBI. On day 0, both SCC-5 and SCC-10 were significantly higher ($p < 0.05$) in DOBI and were significantly lower in UV-TOTOX. After 2 months, the DOBI significantly decreased ($p < 0.05$), while the UV-TOTOX significantly increased, which indicated rapid oxidation at the early stage of storage as described earlier. However, all values were insignificantly affected ($p > 0.05$) by the different storage time and temperatures afterwards. Different observations were made in SCC-0, where the DOBI values remained unchanged, yet its UV-TOTOX significantly increased ($p < 0.05$) after 2 months and onwards. Such findings are common to the oxidation of vegetable oils with lower or minute amounts of carotene (Table 2(a)), such as the shortening used in the SCC-0, which was not incorporated with RPOL. In this case, oxidation produced oxidative products which led to higher UV-TOTOX after 2 months, yet the DOBI were not critically affected due to the lower amount of carotene compared to those of SCC-5 and SCC-10.

4. CONCLUSIONS

The development of value-added SCCs incorporated with RPOL was carried out. The carotene, tocopherol, tocotrienol, and TPC were higher in SCC-5 and SCC-10 compared to SCC-0, yet both FFA and UV-TOTOX were comparable in all SCCs. Due to storage, the beneficial compounds underwent degradation which in most cases was reflected by increased DOBI and decreased PV and FFA. The lowest TPC after 6 months was reflected by the highest DPPH scavenging activity and the lowest FFA in the SCCs. In addition to these findings, the degradation rate in SCC-5 and SCC-10 was lower than that of SCC-0 at 20 and 30 ºC. Overall, higher RPOL enhanced the antioxidant capacity and assisted in delaying oxidation reactions in the SCCs in all storage conditions tested, with SCC-10 showing the best results.

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