J Am Oil Chem Soc (2010) 87:295–304
DOI 10.1007/s11746-009-1497-x

ORIGINAL PAPER

Red Palm Olein: Characterization and Utilization in Formulating Novel Functional Biscuits

Nesma El-Hadad · Hany Aly Abou-Gharbia · Mohammed Hamadi Abd El-Aal · Mohammed Mohamoud Youssef

Received: 19 January 2009 / Revised: 15 October 2009 / Accepted: 19 October 2009 / Published online: 17 November 2009

Abstract Functional biscuits were formulated by replacing white shortening (WS) by red palm olein (RPOL) at 20, 40, 60, 80 and 100%. Sensory evaluation of fresh biscuits indicated that all RPOL levels were significantly as acceptable as or superior to the control. Consequently, two superior RPOL levels (40 and 60%) were chosen for further investigation along with the control. Biscuits made from 40% WS + 60% RPOL exhibited significantly the lowest values regarding water loss during baking, volume before baking, specific volume, specific lightness, water activity and shearing force. Triacylglycerol and fatty acid composition of formulated biscuits resembled their counterparts for RPOL. These biscuits contained 1.8 times more tocopherols and tocotrienols and 10.4–14.8 times more carotenes than the control. Meanwhile, packaged biscuits were able to be stored at room temperature in the light for not less than 6 months without any deterioration in their quality.

Keywords Physical properties · Chemical composition · Triacylglycerol · Fatty acid composition · Tocopherols · Tocotrienols · Carotenes · Sensory evaluation · Storage stability

N. El-Hadad · H. A. Abou-Gharbia · M. H. Abd El-Aal · M. M. Youssef (✉)
Food Science and Technology Department, Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria 21545, Egypt
e-mail: m_m_youssef@yahoo.com

H. A. Abou-Gharbia
e-mail: hanyagharbia@hotmail.com

Introduction

Palm oil is becoming increasingly important worldwide. Palm oil and its fractions (olein and stearin) are used in different food applications, such as a cooking oil for various type of dishes, frying oil and manufacturing shortening and margarine [1, 2].

A novel process for refining crude palm oil has been applied, retaining about 80% of the nature goodness in the form of carotenoids and vitamin E in the original crude oil [3]. This novel health-promoting oil is known as red palm oil (RPO). The characteristic red color of RPO is due to the multi-carotenoids present in the oil, totaling about 575 ppm with 90% as the provitamin A carotenoid, especially β-carotene and α-carotene. Meanwhile, tocopherols (vitamin E) and tocotrienols (provitamin E) are powerful antioxidants that confer oxidative stability to RPO as well as help to keep the carotenoids and other quality parameters of the oil stable [4, 5].

The RPO can be processed into several fractions (olein and stearin) with different physicochemical properties, thereby facilitating its use in a wide range of food applications. The RPO supplementation has been used successfully to elevate vitamin A content in human diet, such as utilizing RPO in cakes, biscuits, bread, cookies, rusks and red shortening [6–9].

Nowadays, people are becoming more health conscious and are seeking foods with functional properties that may positively affect their health. For the last three decades, vitamin A deficiency has been recognized as a major public health problem in the developing countries. However, one of the most effective and sustainable ways to overcome vitamin A deficiency is through a food-based strategy, which has become a way of life. Some inexpensive vitamin A enriched foods are available in poor communities, but

due to lack of knowledge and improper use, the deficiency is still prevailing. RPO has been shown to increase retinol levels in populations with marginal vitamin A deficiency. It is worth mentioning that vitamin A deficiency is still considered a major nutritional problem in the developing countries [9, 10].

Consequently, the present work was carried out to study the physicochemical and functional properties of red palm olein (RPOL). Moreover, the work aimed to utilize RPOL as a source of natural antioxidants to formulate biscuits. The sensorial properties and storage stability of the RPOL-formulated biscuits were also evaluated.

Materials and Methods

Materials

A representative sample (40 kg) of RPOL was kindly acquired from the Carotino SDN BHD Company, Malaysia. All the ingredients used in the present work were purchased from a local market, in Alexandria, Egypt.

Technological Methods

Recipes (Table 1), procedures and processing conditions applied in industry (Fig. 1) were followed in the present work but on a pilot-scale. The white shortening (WS) made from vegetable oil and produced by Integral Oils Industries Company, Suez, Egypt was used in the manufacture of conventional biscuits (control) and replaced by RPOL at the following levels: 20, 40, 60, 80 and 100%.

Physical Methods

Samples of RPOL were heated at 70 °C until they had melted completely and were then homogenized before taking a portion for a test.

Table 1 The recipe of biscuit manufacturing

Ingredients	Amount (g)
Wheat flour (72% extraction)	200
Sugar powder	45
White shortening	40
High fructose corn syrup (HFCS)	13
Skimmed powdered milk	3
Ammonium bicarbonate	1.25
Sodium bicarbonate	3.75
Water	62.5
Vanillin	0.1

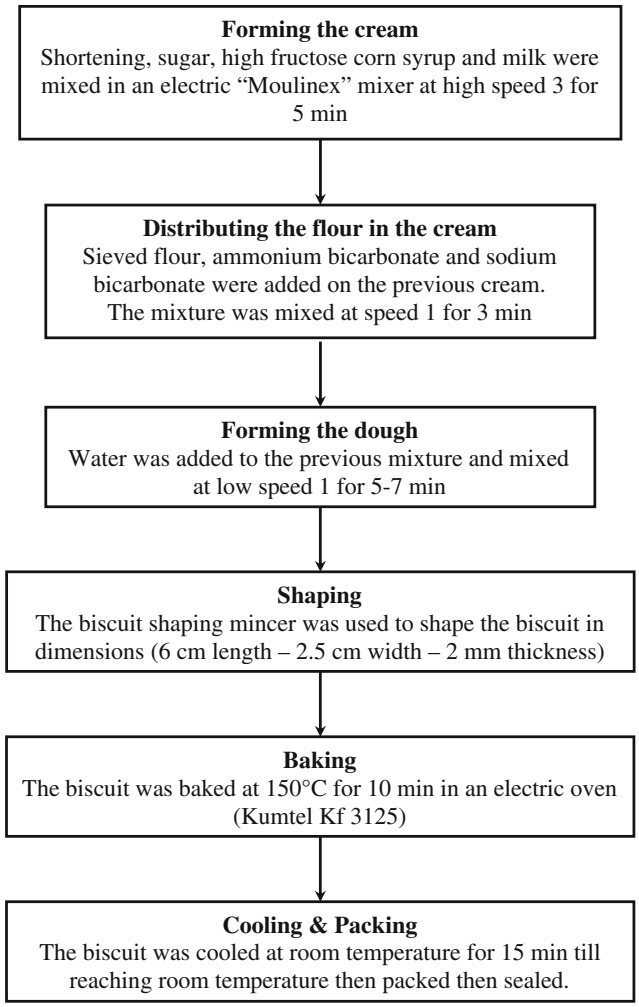

Fig. 1 Flow sheet of biscuit manufacturing

Oil impurities were determined according to ISO/FDIS [11]. A Lovibond Tintometer (Model F, Interscience Son, BHD, No. 1383) was used for measuring the color of oil samples, that were melted at 60 ± 5 °C prior to the test and poured into the sample glass cells (5¼ inch) as outlined in ISO/FDIS [11].

A refractometer (Atago IT, Japan, No. 53825) was used to measure the refractive index of the oil [12]. The cloud point of the oil was measured according to the AOCS method [13].

Oil slip melting point was measured according to the AOCS method [13]. Specific gravity of the oil was determined by means of pycnometer (Ca 50 ml capacity, Kimble Glass Inc. No. 15123-50) as described in the AOAC Official Methods [14].

The loss of water during baking of biscuits was calculated as the difference of the weight of seven biscuits before and after baking [15]. Biscuit volumes were measured by the displacement method as outlined in AACC

Methods [16]. Biscuits specific lightness and specific volume were determined as follows [17]:

$$\text{Specific lightness (cc/g)} = \frac{\text{Volume of 7 biscuits}}{\text{Baked weight of 7 biscuits}} \times 20$$

$$\text{Specific volume (cc/g)} = \frac{\text{Volume of 7 biscuits}}{\text{Dough weight of 7 biscuits}} \times 20$$

where the value 20 is an empirical constant.

Water activity (a_w) of biscuits was determined using a water activity Meter (Decagon Devices, Pullman, Washington, 99163, USA, No. 0990405) according to Topuz [18]. The biscuit texture was measured using the TAXT2 Texture Analyzer (Stable micro systems, 1996, 96 A 0406 M, UK) according to Tyagi et al. [19].

Chemical Methods

The lipids in the biscuits were extracted by the Folch method [20], using a methanol: chloroform mixture (2:1 v/v).

The iodine value (IV) and saponification value (SV) were determined according to ISO/FDIS [21] and to ISO/FDIS [12]. Unsaponifiable matter (method No. Ca 6b-53), peroxide value (method No. 28.023) and free fatty acids/acid value (method No. 28.030) were determined according to AOAC Official Methods[14]. The *p*-anisidine value was determined according to IUPAC [22], method No. 2-504.

Fatty Acid Composition by GLC

Fatty acid methyl esters (FAME) were prepared using the BF_3 method as outlined in the AOCS Official Methods [13]. Fatty acid composition was analyzed as FAME using an HP Hewlett Packard 6890 GC equipped with a Supelco SP^{TM}-2340, fused silica capillary column (60-m length, 0.25-mm diameter and 0.2-μm thickness), The carrier gas initial flow was 0.8 ml/min, pressure 162.78 kPa, average velocity 20 cm/s and the run time 50 min. Oven temperature was initially 150 °C and ramped to 210 °C after 30 min. Injection port and FID detector temperatures were set at 240 °C. The split flow rate of the helium carrier gas was 83.3 ml/min. The injection volume of the sample was 1 μl. Identification of FAME was based on the comparison of their retention times with those of FAME standard mixture (Supelco FAME Mix RM-6, Supelco 07631-1AMP). Quantification was performed by computer control using area normalization [13].

Triacylglycerol Composition by RP-HPLC

The triacylglycerol composition was determined using Reverse Phase (RP)-HPLC. Samples were prepared by dissolving 10% of reactant in acetone (HPLC-grade), then filtering through a 0.45 μm nylon membrane filter to remove impurities. Ten microliters of the sample were then injected into a SUPELCO SIL^{TM} LC-18, USA (5 μm × 250 mm × 4.6 mm) using a Waters 2695 Separations Module HPLC (Waters Corporation, USA), equipped with an auto injector Waters SM7 "No. C055M7882 M" column (Waters Corporation, USA) and a refractive index detector Waters 2414 "No.: J04214474 M"; Waters Corporation, USA) under isocratic conditions. The mobile phase comprised a mixture of acetone and acetonitrile (50:50 v/v) and was set at a flow rate of 2 ml/min. The oven temperature was maintained at 40 °C. Standards of commonly found fatty acids and acylglycerol moieties in palm olein (palmitoyl, oleoyl, linoleoyl and stearoyl) were used to determine the retention times at which these compounds were eluted. The contents of FFA and acylglycerols, namely, mono (MAG), di (DAG) and tri (TAG) acylglycerols were expressed as wt% of the total weight of the sample. Quantification was performed by the computer using area normalization [13].

Tocopherols and Tocotrienols Content by HPLC

A 0.1-g sample was weighed and transferred into a 10-ml volumetric flask and made up to volume with *n*-hexane. The solution was filtered under vacuum through filter paper and 20 μl of filtrate were injected into a Genesis silica column 25 cm length × 4.6 mm inside diameter × 4.6 in outside diameter (Jones Chromatography, UK) at 30 °C. A Waters 2695 Separations Module (Waters Corporation, USA) equipped with an auto injector (Aglient Technologist G 1321A, DE 14903748, UK) was used for the analysis. The mobile phase comprised a mixture of hexane: isopropanol (99.5: 0.5 v/v) was set at a flow rate of 1.4 ml/min and the run time of 22 min. Pure tocopherols (Sigma St. Louis, MO, USA) and tocotrienols (95.4%) developed by MPOB were used as standard references (These tocotrienols were extracted from palm oil and were traceable to Merck individuals α, β, γ and δ tocotrienols). The standard solutions prepared by taking 0.1 ml from each standard into a 10-ml volumetric flask and were made up to volume with *n*-hexane to achieve 100 ppm. Calibration curves (1, 3 and 5 mg/kg) were prepared. The content of tocopherols and tocotrienols were expressed as mg/kg of the total weight of the sample. Quantification was performed by computer control using area normalization [13].

Total Carotenes Content

The total carotenes content was determined by a spectrophotometric method at 446 nm using 1E-UV visible, Varian, No. 94071244, UK, as described by the AOAC [14]. Total carotenes content of oil as β-carotene, was calculated using Cary Windows UV Software No. 8510162500.

Sensory Evaluation

Sensory evaluation of the biscuits was carried out one day after baking. Samples were placed on a white foam plate provided with a glass of warm tap water to clean their palates before and after tasting each sample. Each panelist was asked to give a number from 1 (Extremely dislike) to 9 (Extremely like). Hedonic scales for color, hardness, air pores, flavor, taste and overall acceptability were performed [23].

Storage Stability

Using conventional (control) and functional packaging in polyethylene tetra metallized packs, the biscuits were stored at room temperature in the light for 12 months. Water activity, fat stability and the most abundant natural antioxidant composition were monitored at 0, 6 and 12 months.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to separate the treatment means as outlined by Steel and Torrie [24]. The analysis was computed using the SAS program.

Results and Discussion

Red Palm Olein (RPOL)

Physicochemical properties of RPOL are presented in Table 2. Data given here with specific gravity (0.903 at 50 °C), slip point (23.8 °C), cloud point (8.5 °C) and refractive index (1.455) are in accordance with numerous research papers [5, 25–27].

Moisture (0.021%), iodine value (56.7), saponification value (209.0), unsaponifiable matter (1.3%), peroxide value (1.5 mequiv O_2/kg), anisidine value (0.02), acid value (0.25) and FFA (0.12%) are in a good agreement with other researchers [5, 26–29].

The point of interest is that RPOL exhibited a low content of impurities (0.48%). It is likely that impurities, such as gums, phospholipids and trace metals act as crystal promoters and thereby elevate the cloud point of the oils [25].

Triacylglycerol and Fatty Acid Compositions

Data given in Table 3 indicate that 1,3 dipalmitoyl-2-oleoylglycerol (POP) and 1-palmitoyl-2,3 dioleoylglycerol (POO) are the most predominant triacylglycerols present in RPOL, since they comprised 54.25% of the total triacylglycerols. Other TAG include 1,3-dipalmitoyl-2-linoleoylglycerol (PLP) and 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL) which exhibited 9.97% and 10.86%, respectively of the TAG. Data presented here are in a good agreement with published data [27, 30, 31].

The TAG composition is an important indicator from a nutritional point of view. Moreover, such a composition is responsible for some of the quality attributes of oils. In this respect, POP and POS were identified as the major TAG causing the clouding of palm olein stored at low temperature (12.5 °C) for up to 24 h. It was obvious that the increase in POP and POS concentrations was concomitant with a decrease in the content of POO. The least amount of POO was also obtained in clouds collected between 15 and 18 h of storage compared to the original oil sample [30].

It was obvious that palmitic acid ($C_{16:0}$) is the most abundant saturated fatty acid in RPOL being 39.3% out of 45.1% for the total saturated fatty acids. Notwithstanding, oleic acid ($C_{18:1}$) was found to be the most predominant unsaturated fatty acid present in RPOL (43.7%) out of 43.9% for the total monounsaturated fatty acids. Linoleic acid ($C_{18:2}$) comprised 10.5% out of 11.0% for the total polyunsaturated fatty acids. The saturated:unsaturated fatty acids ratio in RPO is 1:1.22 (Table 3). Data presented here regarding fatty acid composition of RPOL are in accordance with those published by many authors [5, 25, 27, 32].

Table 2 Physicochemical properties of red palm olein

Properties	Value	Properties	Value
Specific gravity (at 50 °C)	0.903 ± 0.153	Saponification value	209.0 ± 1.72
Slip point (°C)	23.8 ± 0.03	Unsaponifiable matter (%)	1.3 ± 0.05
Cloud point (°C)	8.5 ± 0.12	Peroxide value (mequiv peroxide/kg)	1.5 ± 0.22
Refractive index	1.455 ± 0.00	Anisidine value	0.02 ± 0.00
Color	50R-20Y	Acid value	0.25 ± 0.03
Moisture %	0.021 ± 0.008	FFA %	0.12 ± 0.02
Iodine value	56.7 ± 0.42	Impurities (%)	0.48 ± 0.00

Results are mean values of three determinations ± standard deviation (SD)

Table 3 Triacylglycerols (TAG) and fatty acid compositions of red palm olein

Triacylglycerols		Fatty acid composition	
TAG	%	Fatty acid	%
POP	28.93	Lauric ($C_{12:0}$)	0.2
POO	25.32	Myristic ($C_{14:0}$)	0.9
PLP	9.97	Palmitic ($C_{16:0}$)	39.3
POL	10.86	Margaric ($C_{17:0}$)	0.1
POS	5.10	Stearic ($C_{18:0}$)	4.2
OOO	4.15	Arachidic (C_{20}:0)	0.4
SOO	2.90	TSFA	45.1
PLL + MOL	2.37	Palmitoleic ($C_{16:1}$)	0.2
OOL	1.94	Oleic ($C_{18:1}$)	43.7
PPP	0.55	TMUFA	43.9
MLP + MOM	0.65	Linoleic ($C_{18:2}$)	10.5
OLL	0.50	Linolenic ($C_{18:3}$)	0.5
MMP	0.24	TPUFA	11.0
Unknown DAG	5.11	Others	0.2
Unknown MAG & FAA	0.17	S/U	1: 1.22

L Linoleic acid, *P* palmitic acid, *M* myristic acid, *S* stearic acid, *O* oleic acid, *DAG* diacylglycerols, *MAG* monoacylglycerols, *TSFA* total saturated fatty acids, *TMUFA* total monounsaturated fatty acids, *TPUFA* total polyunsaturated fatty acids, *S/U* saturated fatty acid:unsaturated fatty acids

Table 4 Composition of the most abundant antioxidants present in red palm olein

Antioxidant	Content (ppm)
α-Tocopherol	173
α-Tocotrienol	254
β-Tocotrienol	266
γ-Tocotrienol	261
δ-Tocotrienol	104
Total α-tocopherol and tocotrienols	820
Carotenes	580

Natural Antioxidants in Red Palm Olein

RPOL is considered as the vegetable oil with the richest content of natural antioxidants, namely carotenes (provitamin A); tocopherols (vitamin E); tocotrienols (provitamin E) and sterols. The α-tocopherol amounted to 173 ppm, while tocotrienol contents can be placed in ascending order as follows: β-tocotrienol (266 ppm); γ-tocotrienol (261 ppm); α-tocotrienol (254 ppm) and δ-tocotrienol (104 ppm). Total α-tocopherol and tocotrienols amounted to 820 ppm in RPOL (Table 4). Carotenes amounted to 580 ppm. These data are quite comparable to the published data [27, 33].

The RPO is an unconventional oil produced from crude palm oil (CPO) through a new process in which the deacidification and deodorization are carried out using molecular distillation under milder conditions. This preserves more than 80% of each of the carotenoids, tocopherols and tocotrienols [34], unlike in conventional refining where all the carotenoids are destroyed. RPO is therefore the first physically refined vegetable oil rich in natural carotenoids, tocopherols and tocotrienols [33].

Functional Biscuits

In the present study, white shortening (WS) and red palm olein (RPOL) were utilized either individually or in blends to manufacture a novel functional biscuit. Biscuit samples made by replacing WS with RPOL (at levels of 20, 40, 60, 80 and 100%), were comparable to the control made from 100% WS as judged by panelists (Table 5). The biscuit color scores were more highly desirable for all biscuit treatments formulated with RPOL as compared to control biscuits formulated with white shortening. Panel comments explored that elevation of RPOL percent in biscuits formula resulted in an improvement in both color and texture. The color of RPOL biscuits ranged from yellow to orange. Meanwhile, RPOL biscuits possessed smaller and more desirable air pores, especially for biscuits containing 40 and 60% RPOL. It was obvious that these chosen two levels of RPOL produced significantly superior biscuits based on overall acceptance (Table 5).

Physical Properties

Data given in Table 6 indicate that biscuit samples made from WS and RPOL blends were significantly different from the control in terms of their physical properties. It was obvious that biscuit sample made from 40% WS + 60% RPOL significantly exhibited the lowest values regarding water loss during baking, volume before baking, specific volume, specific lightness, water activity and shearing force.

Triacylglycerol Composition

Dipalmitoyl-2-oleoylglycerol (POP) and 1-palmitoyl-2,3 dioleoglycerol (POO) were the highest triacylglycerols content in biscuit samples (Table 6). Notwithstanding, 1,3-dipalmitoyl-2-linoleoylglycerol (PLP) and 1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (POL) possessed a content ranging from 8.11 to 9.47% in the investigated biscuit samples. Such a composition resembles its counterpart for RPOL given in Table 3. (Table 7)

Table 5 Sensory evaluation of biscuits made from 100% white shortening (WS) and by replacing it with red palm olein at different levels

Biscuit samples	Organoleptic properties					
	Color	Crispness	Air pores	Odor	Taste	Overall acceptability
100% WS (control)	5.88^{c}	7.75^{a}	6.38^{b}	7.88^{a}	8.13^{a}	5.23^{c}
80% WS + 20% RPOL	6.88^{b}	7.88^{a}	6.63^{b}	7.63^{a}	7.63^{a}	5.11^{c}
60% WS + 40% RPOL*	7.69^{ab}	8.75^{a}	5.63^{b}	8.00^{a}	7.13^{a}	7.58^{a}
40% WS + 60% RPOL*	8.25^{a}	7.69^{a}	8.13^{a}	7.75^{a}	7.63^{a}	7.92^{a}
20% WS + 80% RPOL	7.69^{ab}	7.81^{a}	6.88^{ab}	7.63^{a}	7.81^{a}	6.80^{b}
100% RPO	8.06^{a}	7.88^{a}	6.13^{b}	7.00^{a}	7.69^{a}	6.40^{b}

* Samples that were chosen for further investigation

Hedonic scale of sensory evaluation: means ($n = 10$) in a column not sharing the same superscript are significantly different at $P < 0.05$

Table 6 Physical properties of biscuits made using 100% white shortening (WS) and by replacing it with 40 and 60% red palm olein

Physical properties	Biscuit samples		
	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60 RPOL
Water loss during baking (%)	1.23 ± 0.54^{a}	1.27 ± 0.58^{a}	1.17 ± 0.43^{b}
Volume before baking (cc)	8.27 ± 0.78^{b}	9.28 ± 1.55^{a}	7.86 ± 0.52^{c}
Volume after baking (cc)	13.5 ± 0.97^{a}	10.00 ± 1.12^{c}	11.43 ± 0.78^{b}
Specific volume	37.51^{a}	26.37^{b}	20.00^{c}
Specific lightness	45.98^{a}	33.50^{b}	31.65^{b}
Water activity (21 °C)	0.26 ± 0.07^{a}	0.23 ± 0.07^{a}	0.22 ± 0.14^{a}
Shear force (g)	1033.4 ± 23.9^{b}	1107.6 ± 14.6^{a}	810.8 ± 10.5^{c}

Results are mean values of three determinations ± SD

Means ($n = 3$) in a row not sharing the same superscript are significantly different at $P < 0.05$

Table 7 Triacylglycerol (TAG) composition of biscuits made from 100% white shortening (WS) and by replacing it with 40 and 60% red palm olein

TAG %	Biscuit samples		
	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL
POP	29.41	28.25	28.07
POO	19.41	21.11	22.08
PLP	8.92	9.03	9.24
POL	8.11	8.96	9.47
POS	5.21	4.92	4.93
PPP	5.09	4.43	4.32
OOO	3.31	3.66	3.71
SOO	2.24	2.40	2.56
PLL + MOL	2.23	2.44	2.57
OOL	1.57	1.69	1.78
PPS	1.19	1.13	0.86
OLL	0.72	0.71	0.72
MMP	0.67	0.61	0.50
MLP + MOM	0.55	0.58	0.58
Unknown DAG	6.39	5.71	5.31
Unknown MAG & FAA	4.04	2.43	2.33

Triacylglycerol (TAG) composition: one analysis

L Linoleic acid, *P* palmitic acid, *M* myristic acid, *S* stearic acid, *O* oleic acid, *DAG* diacylglycerols, *MAG* monoacylglycerols

Table 8 Fatty acid composition of biscuits made from 100% white shortening (WS) and by replacing it by 40 and 60% red palm olein

Fatty acids %	Biscuit samples		
	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL
Lauric ($C_{12:0}$)	0.1	0.13	0.14
Myristic ($C_{14:0}$)	1.0	0.97	0.98
Palmitic ($C_{16:0}$)	46.3	43.55	42.24
Margaric ($C_{17:0}$)	0.1	0.11	0.14
Stearic ($C_{18:0}$)	4.9	4.59	4.47
Arachidic (C_{20}:0)	0.4	0.38	0.38
TSFA	52.8[a]	49.73[b]	48.35[c]
Palmitoleic ($C_{16:1}$)	0.2	0.18	0.19
Oleic ($C_{18:1}$)	36.5	38.91	39.02
TMUFA	36.7[c]	39.09[b]	40.11[a]
Linoleic ($C_{18:2}$)	9.6	10.47	10.78
Linolenic ($C_{18:3}$)	0.3	0.42	0.45
TPUFA	9.9[c]	10.89[b]	11.23[a]
Others	0.3	0.29	0.34
S/U	1:1.13	1:0.99	1:0.94

TSFA Total saturated fatty acids, *TMUFA* total monounsaturated fatty acids, *TPUFA* total polyunsaturated fatty acids, *S/U* saturated fatty acid:unsaturated fatty acids

Fatty Acid Composition

Table 8 shows the fatty acid composition of the control biscuit along with two biscuit samples made from 60% WS + 40% RPOL and 40% WS + 60% RPOL.

Palmitic acid represented the most abundant saturated fatty acid in the aforementioned biscuit samples occurring in a range from 42.2 to 46.3%. Meanwhile, the total saturated fatty acids varied significantly being 52.8, 49.7 and 48.4% for the control, and biscuits made from 60% WS + 40% RPOL and 40% WS + 60% RPOL, respectively.

Oleic acid values ranged between 36.5 and 39.02% for the three biscuit samples. Total monounsaturated fatty acids varied significantly in the following descending order: 40.1% (biscuit made from 40% WS + 60% RPOL), 39.1% (biscuit made from 60% WS + 40% RPOL) and 36.7% for the control (Table 8).

Data in Table 8 reveal that linoleic acid varied from 9.6 to 10.8% in biscuit samples under study. The PUFA varied significantly as follows: control (9.9%), biscuit made from 60% WS + 40% RPOL (10.89%) and biscuit made from 40% WS + 60% RPOL (11.2%).

The saturated/unsaturated fatty acids ratio (S/U) were found to be as follows: 1:1.13 (control); 1:0.99 (biscuit made from 60% WS + 40% RPOL), and 1:0.94 (biscuit made from 40% WS + 60% RPOL) as shown in Table 8.

Storage Stability

The biscuit samples investigated in the present study were stored at room temperature for 12 months. Changes in stability indices and antioxidants were monitored at regular intervals (zero, 6 and 12 months).

Stability Indices

Data presented in Table 9 indicate that the water activity of biscuit samples did not vary significantly within the same storage period. However, water activity increased gradually as the storage period proceeded.

Significant differences in peroxide value (PV) within the same storage period (Table 9) were observed. All PV values were less than 10.0 mequiv O_2/kg for all biscuit samples at all storage periods, with sample made from 100% WS and stored for 12 months being the only exception (PV = 10.0). Consequently, functional biscuits formulated in the present study could be stored for more than 6 months without incident of oxidative rancidity. This point deserves further investigation to assure that the auto-oxidation curve did not go through multiple cycles during the 6 months intervals.

The anisidine values given in Table 9 indicate that they increased as the storage period proceeded. The point of interest is that biscuits made from 40% WS and 60% RPOL possessed the lowest anisidine values after storage for 6 and 12 months.

Table 9 Effect of storage at room temperature on some quality attributes of biscuits made from 100% white shortening (WS) and by replacing it by 40 and 60% red palm olein

Properties	Storage period								
	Zero time			6 Months			12 Months		
	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL
Water activity (21 °C)	0.26 ± 0.07^{a}	0.23 ± 0.07^{a}	0.22 ± 0.14^{a}	0.29 ± 0.12^{a}	0.29 ± 0.09^{a}	0.29 ± 0.14^{a}	0.32 ± 0.17^{a}	0.32 ± 0.13^{a}	0.39 ± 0.17^{a}
Peroxide value (mequiv peroxide/kg)	1.0 ± 0.44^{b}	1.5 ± 0.59^{a}	1.7 ± 0.23^{a}	6.5 ± 0.52^{a}	5.3 ± 0.37^{b}	4.2 ± 0.27^{c}	10.0 ± 0.46^{a}	9.8 ± 0.52^{b}	7.3 ± 0.35^{c}
Anisidine value	1.5 ± 0.04^{a}	0.7 ± 0.04^{b}	0.72 ± 0.12^{b}	1.6 ± 0.06^{a}	1.3 ± 0.07^{b}	1.0 ± 0.07^{c}	1.8 ± 0.04^{a}	1.8 ± 0.03^{a}	1.2 ± 0.15^{b}
Acid value	0.15 ± 0.08^{a}	0.17 ± 0.05^{a}	0.17 ± 0.07^{a}	1.44 ± 0.45^{a}	1.53 ± 0.52^{a}	1.5 ± 0.18^{a}	1.7 ± 0.44^{a}	1.72 ± 0.31^{a}	1.8 ± 0.2^{a}
FFA (%)	0.07 ± 0.05^{a}	0.08 ± 0.03^{a}	0.08 ± 0.05^{a}	0.67 ± 0.31^{a}	0.71 ± 0.38^{a}	0.7 ± 0.11^{a}	0.79 ± 0.32^{a}	0.80 ± 0.17^{a}	0.84 ± 0.14^{a}

RPOL Red palm olein

Means ($n = 3$) in a row within the same storage period not sharing the same superscript are significantly different at $P < 0.05$

Table 10 Effect of storage at room temperature on the natural antioxidant content (ppm) of biscuits made from 100% white shortening (WS) and by replacing it by 40 and 60% red palm olein

Properties	Storage period								
	Zero time			6 Months			12 Months		
	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL	100% WS (Control)	60% WS + 40% RPOL	40% WS + 60% RPOL
α-Tocopherol	51.2	93.0	116.0	48.9	66.7	95.0	36.7	53.3	83.6
α-Tocotrienol	69.9	126.4	158.0	69.5	94.0	123.4	53.5	75.0	115.7
β-Tocotrienol	88.0	85.2	85.7	60.0	84.0	89.6	62.0	67.0	80.6
γ-Tocotrienol	64.3	123.0	154.3	71.0	112.5	136.0	71.0	91.0	132.0
δ-Tocotrienol	32.5	52.7	63.2	27.0	53.0	58.0	31.0	43.0	58.7
Total α-tocopherol + tocotrienols	306.0^{c}	480.0^{b}	577.0^{a}	267.0^{c}	410.0^{b}	502.0^{a}	254.0^{c}	329.0^{b}	471.0^{a}
Carotenes	18.0^{b}	173.0^{a}	188.0^{a}	13.0^{b}	172.0^{a}	184.0^{a}	12.0^{c}	126.0^{b}	177.0^{a}

Means in a row within the same storage period not sharing the same superscript are significantly different at $P < 0.05$

No significant differences could be detected regarding the acid value and FFA% of biscuits within the same storage period for biscuit samples under investigation (Table 9). On the other hand, a longer storage period resulted in increased acid values in biscuit treatments.

Antioxidants

Data presented in Table 10 show the antioxidant contents of biscuit samples stored at room temperature for 6 and 12 months. It was obvious that biscuit samples made from 40% WS + 60% RPOL exhibited the highest antioxidant contents at zero time, 6 and 12 months of storage.

Total α-tocopherol and tocotrienols were found to decrease as the storage period was extended. For biscuit sample made from 40% WS + 60% RPOL, contents of the aforementioned antioxidants exhibited the following values: 577, 502 and 471 ppm after 0, 6 and 12 months of storage, respectively (Table 10). On the other hand, control biscuit (made from 100% WS) possessed the lowest contents of total α-tocopherols and tocotrienols. Utilizing RPOL resulted in almost doubling (1.8 fold) the total α-tocopherol and tocotrienol content of the biscuits.

The carotene content was significantly elevated (10.4–14.8 fold) as a result of utilizing RPOL in formulating biscuits applied in the present study. Carotene contents were 188, 172 and 177 ppm for biscuit samples made from 40% WS + 60% RPOL that were stored for zero, 6 months and 12 months, respectively (Table 10).

Conclusions

High quality functional biscuits were able be produced by replacing white shortening by RPOL at 40% and 60% levels. Formulated biscuits exhibited 1.8 times more tocopherols and tocotrienols and 10.4–14.8 times more carotenes than the control.

Mass production of such biscuits can be considered as one of the most effective and sustainable ways of overcoming vitamin A deficiency. This has been recognized as a major public health problem, especially for children in developing countries. Meanwhile, formulated biscuits contain high concentrations of natural antioxidants. It goes without saying that such components possess health benefits that have been extensively reported in literatures.

Acknowledgments The authors are grateful and indebted to the Malaysian Palm Oil Board (MPOB) for valuable help given to Mrs. Nesma N.M. El-Hadad for traveling to Malaysia and use of all the technical facilities available in MPOB's laboratories. Obviously, without such help, this work would not have been possible.

References

1. Nor Aini I, Miskandar MS (2007) Utilization of palm oil and palm products in shortenings and margarines. Eur J Lipid Sci Technol 109:422–432
2. Wai Lin S (2007) Chemical and physical characteristics of palm and palm kernel oils and their crystallization behaviour. In: Proceeding of 27th palm oil familiarization programme organized by Malaysian Palm Oil Board, August 14–24, 2007, Kuala Lumpur, Malaysia
3. Mayamol PN, Balachandran C, Samuel T, Sundareson A, Arumughan C (2007) Process technology for the production of micronutrient rich red palm olein. J Am Oil Chem Soc 84:587–596
4. Lin C, Koh CS (2000) Red palm oil healthful addition to human diet, nutrition briefs. 8th Issue Publisher MPOC, KDN 1505 (10310)
5. Koh CS, Wan L (2003) Red palm oil nutrition briefs. 10th Issue MPOC, KDN 1505 (10310)
6. Stuijvenberg ME, Benade AJS (2000) South Africa experience with the use of red palm oil to improve the vitamin A status of primary school children. Food Nutr Bull 21:212–214
7. Al-Hooti SN, Sidhu JS, Al-Saqer JM, Al-Amiri HA, Al-Othman A, Mansour IB, Minal J (2002) Developing functional foods using red palm olein in pan bread and sugar snap cookies. Adv Food Sci 24:138–143
8. Al-Saqer JM, Sidhue JS, Al-Hooti SN, Al-Amiri HA, Al-Othman A, Haji LA, Ahmed N, Mansour IB, Minal J (2004) Developing functional foods using red palm olein tocopherols and tocotrienols. Food Chem 85:579–583
9. Butt MS, Rasool J, Sharif K (2006) Preparation and characterization of cake rusks by using red palm oil fortified shortening. Food Sci Tech Int 12:85–90
10. Hekmat S, Hains J (2003) Comparison of the effects of Carotino and canola oils on the sensory properties of various foods. Nutr Res 23:1211–1219
11. ISO/FDIS (1998) Animal and vegetable fats and oils: determination of insoluble impurities. International Standard References, Switzerland
12. ISO/FDIS (2002) Animal and vegetable fats and oils: determination of refractive index. International Standard References, Switzerland
13. AOCS (2001) Official methods and recommended practices of the American Oil Chemist Society, Champaign, IL, USA
14. AOAC (2000) Official methods of analysis of AOAC international, 17th edn. Gaitherburg, Maryland
15. Wade P (1972) Flour properties and the manufacture of semi-sweet biscuits. J Sci Food Agric 23:737–744
16. AACC (1970) American Association of Cereal Chemists. Cereal Laboratory Methods. American Association of Cereal Chemists, St Paul
17. Elling HR, Millner M (1951) Influence of wheat variety, malt and shortening on the characteristics of chemical leavened biscuits. Cereal Chem 28:207–218
18. Topus AA (2008) A novel approach for colour degradation kinetics of paprika as a function of water activity. LWT Food Sci Technol 41:1672–1677
19. Tyagi SK, Manikantan MR, Oberoi HS, Kaur G (2007) Effect of mustard flour incorporation on nutritional, textural and organoleptic characteristics of biscuits. J Food Eng 80:1043–1050
20. Folch J, Lees M, Stanley S (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
21. ISO/FADIS (1996) Animal and vegetable fats and oils: determination of iodine value. International Standard References, Switzerland

22. IUPAC (1979) Standard methods for the analysis of oils, fats and derivatives, 7th edn. International Union of Pure and Applied Chemistry Commission on Oils, Fats and Derivatives. Blackwell, UK
23. Annonymous (1981) Sensory evaluation guide for testing food and beverage production. Food Technol 35:50–59
24. Steel RGD, Torrie TH (1980) Principles and procedures of statistics. McGraw Hill, USA
25. Nor Aini I, Hanirah H, Siew WL, Yusoff MSA (1988) Cold stability of red palm oleins. J Am Oil Chem Soc 75:749–751
26. O'Brien RD (2004) Fats and oils formulating and processing for applications, 2nd edn. CRC Press, UK
27. Gee PT (2007) Analytical characteristics of crude and refined palm oil and fractions. Eur J Lipid Sci Technol 109:373–379
28. Nowkolo E, Smart J (1995) Food and feed from legumes and oilseeds. Chapman & Hall, London, pp 318–325
29. Chiew Let C (2007) An introduction to production, processing and quality of palm and palm kernel products. In: Proceeding of 27th palm oil familiarization programme. Organized by Malaysian Palm Oil Board, August 14–24, 2007, Kuala Lumpur, Malaysia
30. Swe PZ, CheMan YB, Ghazali HM, Wei LS (1994) Identification of major triacylglycerols causing the clouding of palm olein. J Am Oil Chem Soc 71:1141–1144
31. CheMan YB, Harayati T, Ghazali HM, Asbi BA (1999) Composition and thermal profile of crude palm oil and its products. J Am Oil Chem Soc 76:237–242
32. Tan CP, CheMan YB (2000) Differential scanning calorimetric analysis of edible oils: Comparison of thermal properties and chemical composition. J Am Oil Chem Soc 77:143–155
33. Bonnie TYP, Choo YM (2000) Valuable minor constituents of commercial red palm olein: Carotenoids, vitamin E, ubiquinones and sterols. J Oil Palm Res 12:14–24
34. Choo YM, Ma AN, Yap SC, Ool CK, Basiron Y (1996) Production and application of deacidified and deodorized red palm oil. Palm Oil Dev 19:30–34