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Pilot scale production of functional foods using red palm olein: Antioxidant, vitamins' stability and sensory quality during storage



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ABSTRACT

The objective of this research work was to produce acceptable quality functional foods, namely, extruded snacks, digestive biscuits and pan bread, on a pilot scale, using vitamin E and β -carotene-rich red palm olein (RPOL) and red palm shortening (RPS). These products were evaluated for their chemical composition and sensory quality along with the antioxidants and vitamin contents during the six months of storage at room temperature (22 ± 1 °C). Extruded snacks and digestive biscuits prepared with RPOL and RPS were found to be good sources of these antioxidant vitamins. The average β -carotene content of the control and test snacks at the end of six months of storage ranged from 26.8 to 56.1 mg/kg fat, and from 430.9 to 468.9 mg/kg fat, respectively. The total vitamin E content in control and test snacks made in Plant No. 1 decreased after six months of storage from 786.1 to 704.4 mg/kg fat, and from 765.1 to 695.4 mg/kg fat, respectively. As expected, the total tocotrienol content was four to five times higher than the total tocopherols in control biscuits. The RPOL containing 600–750 ppm of carotenes (mainly α - and β -carotenes), 710–774 ppm of vitamin E, was found to be suitable for industrial application in producing acceptable guality pan bread, digestive biscuits and snacks. These functional foods contained significant amounts of β -carotene and total vitamin E, indicating the possibility of producing such foods rich in these two of the important antioxidant vitamins coming from a natural source. The research findings strongly indicate that good-quality pan bread, extruded snacks and digestive biscuits can successfully be produced to offer healthier eating choices to the consumers of this region, thereby promoting better health and productivity among the population.

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1. Introduction

The Malaysian Palm Oil Board has developed a process to produce red palm olein (RPOL) and red palm shortening (RPS) rich in natural carotenes and vitamin E, which has been adopted on a

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commercial scale by a Malaysian company (Choo et al., 1996; Abd Rashid et al., 2021; Nur Silihatimarsyila et al., 2020). The RPOL is reported to contain 600–750 ppm of carotenes (mainly α - and β carotenes), 710–774 ppm of vitamin E, 325–365 ppm of sterols and 18–25 ppm of ubiquinone (Ricaurte et al., 2018; Phoon et al., 2018). Because of their ability to scavenge free radicals in the environment, antioxidants such as carotenes, tocopherols, tocotrienols and ubiquinones are highly reactive compounds, which bring about the possibility that one or more of these compounds may be depleted with time during processing and storage when exposed to different conditions. Heat is the catalyst for the formation of free radicals leading to higher losses of these antioxidant vitamins (Teh and Lau, 2020; Gonzalez-Diaz et al., 2021).

The vitamin E content of palm oil is unique, represented mainly as tocotrienols (70%) rather than as tocopherols (30%). α tocopherol and γ -tocotrienol account for the major portions of the total tocopherols and tocotrienols. Natural tocopherol,

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especially α -tocopherol, is a superior radical chain-breaking antioxidant compared with synthetic ones (Teh and Lau, 2020). These natural antioxidant vitamins have been suggested to provide protection against several diseases involving free radical reactions (Kritchevsky et al., 2000; Absalome et al., 2020). Kumar et al. (2020) have reported that alpha- and gamma-tocopherols intakes by pregnant mother gave better lung functions in their offsprings during mid-childhood. The higher oxidative stress in this part of the world that is caused by strong sun radiations, vehicle exhaust, lack of greenery etc. increases our requirements for these antioxidant phytonutrients. Consumption of foods rich in these antioxidant vitamins would, therefore, provide health benefits to the consumers (Idris et al., 2014; Gonzalez-Diaz et al., 2021). The presence of this natural vitamin E in palm oil ensures a longer shelf life for palm-oil-based food products (Meng et al., 2020). Another advantage of palm oil is that it can be used without hydrogenation. thereby, eliminating the possibility of adverse effects of dietary trans fatty acids to the consumers of such food products.

The bakery shortening usually consists of a blend of hydrogenated fats and liquid vegetable oils. However, during hydrogenation process, it is known that *trans* fatty acid isomers are produced, which are currently the subject of controversy because of their ability to increase the risk of coronary heart disease (CHD). Several shortening formulations using unhydrogenated fats are now available from the Malaysian Palm Oil Board. Meng et al. (2020) have investigated the use of L-ascorbyl palmitate as a potential fat crystallization modifier for bakery shortening to be used in bread, as it gave a softer texture.

RPOL and RPS, as source of valuable phytonutrients (Kua et al., 2016; Riyadi et al., 2016; Iftikhar et al., 2017; Lee et al., 2018), have been used for the production of acceptability quality pan bread and sugar-snap cookies in a laboratory as well as on a pilot scale. Chemical composition, sensory quality and consumer acceptability of these laboratory prepared products have already been reported (Al-Hooti et al. 2002; Sidhu et al. 2003). The major objective of this study was to explore the possibility of producing shelf-stable nutritious products in two commercial plants by scaling up the laboratory results to a pilot scale as well as to investigate the effect of storage time on the chemical composition, retention of antioxidant vitamins and sensory quality of pan bread, extruded snacks, and digestive biscuits.

2. Materials and methods

2.1. Materials

The RPS and RPOL used in this study were obtained from the Carotino Company of Malaysia. For the pilot-scale production of these products, white wheat flour (72% extraction), wholegrain wheat flour, fine wheat bran, fine granulated sugar, common salt, regular bakery shortening, chemical leavening agents, instant dry yeast, nonfat dry milk, raw materials for extruded snacks (corn grits, soy grits, cheese, colorings, flavorings etc.) and the required packaging materials were obtained from the participating companies participating in the pilot scale trials. The total vitamin E contents of control palm oil shortening, RPS and RPOL used in this study were 456.5, 463.6 and 717.8 mg/kg fat, respectively.

2.2. Pilot scale production

2.2.1. Extruded snacks

Corn-grits-based extruded snacks were produced in two commercial plants. The control snacks were prepared using refined bleached deodorized palm oil (RBDPO), whereas in the experimental snacks, RPOL was used in place of RBDPO. The corn grits were

extruded under high shear force in a single-screw extruder (Lallesse Maschinebau, Arnhen, the Netherlands) and then dried to suitable moisture content (1–3%). A slurry consisting of palm oil, cheese, flavoring agents, table salt and coloring matter was sprayed onto these extruded snacks at a temperature of 22 ± 1 °C, and the snacks were then packaged in BOPP metalized film (about 20 g/package) using automatic packing machines. In experimental samples, coloring matter was not used, as the RPOL had sufficient yellow coloring of its own due to the presence of β -carotenes. The prepared and packaged snacks were transported to KISR's laboratories for chemical and sensory analyses during the storage period of six months at room temperature (22 ± 1 °C). The various parameters during extrusion were: screw speed, 350 rpm; moisture content of feed, 22%; feed (corn/soy grits, 96:4) mixture; flow rate, 4.4 g/s; cutter speed, 140 rpm; extrusion temperature, 127 °C. The extruded product characteristics were: expansion ratio, 3.25; bulk density. 0.14 g/cc.

2.2.2. Digestive biscuits

Three types of digestive biscuits were prepared in a commercial biscuit plant using wholegrain wheat flour (45 parts) and biscuit flour (55 parts). Other ingredients like hydrogenated palm oil (regular bakery shortening), butter, sugar, liquid glucose, malt flour, whey powder, emulsifier (soy lecithin), table salt, sodium bicarbonate and ammonium bicarbonate, were used on a total flour weight basis. Control palm oil shortening was completely replaced with RPS in Sample No. 2, whereas in Sample No. 3, equal parts of RPS and RPOL were used in place of control shortening. Biscuits were deposited on a conveyor belt using a rotary molder, baked in an electrically heated oven for about 8 min at a temperature gradient of $195 \rightarrow 225 \rightarrow 215 \rightarrow 195 \,^{\circ}C$ during the four baking temperature zones. Biscuits were cooled to room temperature and packaged in small units of two biscuits (19.5 g) as well as larger packs of 18 biscuits (165 g) using biaxially oriented polypropylene (BOPP) metalized film. The prepared biscuit samples were transported to KISR's laboratories for chemical and sensory analyses during the storage period of six months at room temperature (22 ± 2 °C).

2.2.3. Pan bread

Pan bread was produced in a commercial bakery using white wheat flour as well as wholegrain wheat flour, instant yeast, sugar, regular bakery shortening, salt, supergama as an improver (Zeelandia, Holland) and sodium strearoyl-2-lactylate (SSL) as an emulsifier (American Ingredients Co., Kansas City, USA). The pan bread was produced as per the method described earlier by Al-Hooti et al. (2002). The water contents (on a flour basis) for white flour bread, brown bread and wholegrain wheat bread were 55, 57 and 57%, respectively. The water level was adjusted by feeling the dough's handling quality. These ingredients were mixed to a final dough temperature of 30 °C. The mixed dough was rested at room temperature $(22 \pm 1 \circ C)$ for 45 min, and then divided into 580-g pieces, shaped and put into pans. It was proofed for about 45 min at 35 °C and 75% relative humidity. After proofing, the bread was baked in a rotary oven at 200 °C for 50 min. The bread was cooled to room temperature, sliced, and packaged in polyethylene bags. These bread samples were transported to laboratories at the Kuwait Institute for Scientific Research (KISR) for further chemical and sensory analyses during the storage period of five days at room temperature.

2.3. Chemical analysis

The bread samples were freeze-dried and powdered in a Falling Number Mill (Model 3100, Sweden) to pass through a 100-mesh sieve and stored in airtight plastic containers in a freezer (-18 °C) for further chemical analysis. The digestive biscuits and extruded snacks were directly ground, as they had low moisture contents (1-3%) and stored in airtight containers for further chemical analysis.

Lipids were extracted by the method of Bligh and Dyer (1959) and were analyzed, in duplicate, for β -carotene, peroxide value (PV) and free fatty acids (FFA). The β -carotene content was estimated as per the Palm Oil Research Institute of Malaysia's procedure (1995). FFA and PV were determined using the American Oil Chemists' Society's (AOCS's) Methods Aa 6-38 and Cd 8-53, respectively. Vitamin E was determined using the AOCS Method Ce 8-89 (1997). Vitamin E isomers and β-carotene pure standards were obtained from Sigma Chemicals, USA. All chemical reagents were of analytical grade. Vitamin E isomers were determined by the HPLC method as reported earlier in detail by Al-Sager et al. (2004). The concentration of each isomer was determined by standard method (AOCS, Ce 8-89). The experimental conditions for vitamin E estimation using high-pressure liquid chromatography (HPLC) (Shimadzu, Japan, model LC6A) were as follows:

- Column: Vario Valco LiChrosorb Si 60-5, 250×4.6 mm.
- Detector: Spectrophotometric, 290 mm Ex, 330 Em and 8 Attenuation
- Solvent system: Hexane (1000 ml) Fluka; tetrahydrofuran (60 ml) BDH; and isopropanol (3 ml) BDH.
- Standards: 10 ppm.
- \bullet Sample size: Approximately 0.1 g /ml of hexane, and injection volume: 20 μl
- Flow rate for mobile phase: 1.0 ml/min

2.4. Sensory analysis

The pan bread (for five days only), digestive biscuits and extruded snack samples were subjected to sensory analysis on a nine-point hedonic scale for crumb color, texture, flavor and overall acceptability, using a semi-trained panel of 12 judges from among KISR's employees, initially and after six months of storage at room temperature $(22 \pm 1 \circ C)$ as per the procedure reported earlier by Al-Hooti et al. (2002). Each panelist was served a control sample along with test samples and was asked to assign scores on a nine-point hedonic scale for crumb color, texture, flavor, and overall acceptability. A sensory score of 5 or above was rated as acceptable, and a score below 5, was considered unacceptable. These samples were also tested for consumer acceptability among 120 of KISR's employees selected at random. These KISR employees had educational qualifications of graduation and/or above. They were provided with the control and test samples to categorize either as acceptable or unacceptable, based on their color, flavor, and textural attributes in the given templates (Supplementary Tables S-1A and S-B). Prior written consent from all the panelists and participants in sensory evaluation and consumer acceptability was obtained.

2.5. Statistical analysis

All the chemical analyses were reported on a moisture-free basis. All the experimental data obtained were analyzed statistically for analysis of variance, and the mean values were evaluated for statistical significance using Duncan's New Multiple Range Test (SAS Program, Windows Version 6.08). Inferences were reported at the appropriate places. Significance (α) was accepted at the $P \leq 0.05$ level.

3. Results

3.1. Chemical analyses

The shelf life of extruded snacks and digestive biscuits under Kuwait's food laws is limited to six months, whereas for pan bread, it is only five days. The chemical composition and sensory quality of the extruded snacks as well as the digestive biscuits were evaluated at time zero and at the end of six months of storage at room temperature (22 ± 1 °C).

3.1.1. Extruded snacks

The β -carotene, FFAs and PVs of the extruded snacks, initially as well as at the end of six months of storage at room temperature are presented in Table 1. In case of Plant No.1, the β-carotene content in control snacks decreased from 39.5 to 26.8 mg/kg fat compared with 440.4 to 430.9 mg/kg fat in test snacks made with RPOL during six months of storage. In case of Plant No.2, the average β carotene contents of the control snacks decreased from 61.4 to 56.1 mg/kg fat compared with 468.5 to 462.9 mg/kg fat for test snacks at the end of six months of storage. Overall, the loss of β carotene in control extruded test snacks ranged from 8.6 to 32.2%, whereas for the test snacks, it ranged from 1.2 to 2.2%, thus showing that in the test snacks made with RPOL, this decrease was relatively insignificant during six months of storage at room temperature. In absolute terms, one serving (a 20-g package) of a control extruded snack would provide consumers with only 0.32 mg of β -carotene, whereas same serving size of the test snacks made with RPOL would provide about 3.38 mg of β -carotene. Thus, the extruded snacks made with RPOL could serve as an excellent source of this important antioxidant vitamin in one's daily diet. The FFA contents of the control as well as the test snacks were extremely low (0.19-0.30%). The PVs for the control and test snacks ranged from 5.2 to 9.7 and from 5.1 to 14.0 meq/kg, respectively. The data presented in Table 1 show that the PVs for the extruded snacks made in Plant No. 1 were significantly higher than those for the corresponding snacks made in Plant No. 2, which could be due their manufacturing technology variations. For the cooking oils, the Codex Alimentarius Commission (CAC, 1993) has suggested a limit for FFA at 1% and PV values at 10 meq/kg fat.

The total vitamin E content in control and test snacks made in Plant No. 1 decreased after six months of storage from 786.1 to 704.4 mg/kg fat, and from 765.1 to 695.4 mg/kg fat, respectively (Table 2). The loss of vitamin E contents in control extruded snacks was 10.4%, whereas for the test snacks, it was 9.1%, thus showing that the extruded snacks made with RPOL, the decrease in vitamin E content was reasonably low. Interestingly, the total tocotrienol contents in these snacks were almost five to six times the respective tocopherol contents. The total vitamin E contents in control and test snacks prepared in Plant No. 2 differed significantly at time zero as well as after six months of storage. During the six months of storage at room temperature, the total vitamin E content in the control and test snacks decreased from 766.5 to 625.7 mg/kg fat and from 863.0 to 708.7 mg/kg fat, respectively (Table 2). The overall loss of vitamin E in the control and test snacks during the six months of storage at room temperature was significantly higher (17.9–18.4%) from Plant No. 2 than from Plant No. 1 (9.1–10.4%).

3.1.2. Digestive biscuits

Three types of digestive biscuits were prepared (only Plant No. 2 had biscuit-making facilities) using control shortening, RPS, and equal proportions of both the RPS and RPOL. They were stored at room temperature for six months. The changes in β -carotene, FFAs, and PVs during this storage period are summarized in Table 3. No significant differences were found in the FFAs and PVs in these

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Table 1

ß-	Carotene contents,	free fat	tv acids	(FFAs)	and	peroxide	values	(PVs)) of	f extruded	snacks d	luring st	orage	at room te	emperature	e.

Sample	β-Carotene (m	g/kg fat)	FFA (%)		PV (meq/kg)		
	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo	
Plant No.1 Control Snack	39.5 ^a	26.8 ^a	$0.29^{\rm a}_{\rm b}$	$0.27_{\rm b}^{\rm a}$	9.7 ^a	9.6^{a}_{d}	
Plant No. 1 Test Snack with RPOL	440.4_{g}^{b}	430.9 ^b _g	0.24_{c}^{a}	0.26 ^a _c	11.8 ^a	14.0 ^b _e	
Plant No. 2 Control Snack	61.4 ^c	56.1 ^c	0.30^{a}_{d}	0.19 ^a	5.2 ^c	6.1 ^c	
Plant No. 2 Test Snack with RPOL	468.5 ^d	462.9 ^d	$0.27_{\rm f}^{\rm a}$	$0.26_{\rm f}^{\rm a}$	9.5 ^a g	5.1 ^c _h	

RPOL = Red palm olein, FFAs = Free Fatty Acids, PVs = Peroxide Values.

For comparison among the four snack samples, β -carotene, FFAs and PVs (at zero or after six months) with same superscript in a single column do not differ significantly (P \leq 0.05). Among any snack sample at zero and six months, β -carotene, FFAs and PVs with different subscripts differ significantly (P \leq 0.05), (n = 12 for each column).

Table 2

Vitamin E content of extruded snacks made in two different Plants (#1 and #2) during storage at room temperature.

Vitamin E forms	Control sna	acks	10.5 128.0 97.0 199.0 .5 ND 7.4 15.6 20.5 365.0 .5 3.0 8.0 54.5 11.5 131.0 92.9 634.1 04.4 ^b 765.1 ^c 07.5 160.5 82.5 250.0 ID 0.8 5.0 32.0 74.0 337.5 .7 0.4 4.0 81.8 10.2 161.7	s made
	0 mo	6 mo	0 mo	6 mo
Plant #1				
α-t	122.0	110.5	128.0	121.0
α-t ₃	228.0	197.0	199.0	181.5
β-t	ND	0.5	ND	ND
β-t ₃ &γ-t	18.5	17.4	15.6	15.8
γ-t ₃	354.0	320.5	365.0	307.5
δ-t	1.4	0.5	3.0	3.8
δ-t ₃	62.2	58.0	54.5	65.8
Total t	123.4	111.5	131.0	124.8
Total t ₃	662.7	592.9	634.1	570.6
Total Vitamin E	786.1 ^a	704.4 ^b	765.1 ^c	695.4^{d}
Plant # 2				
α-t	144.5	107.5	160.5	103.0
α-t ₃	211.5	182.5	250.0	190.0
β-t	ND	ND	0.8	ND
β-t ₃ & γ-t	18.8	15.0	32.0	16.3
γ-t ₃	331.5	274.0	337.5	331.5
δ-t	2.4	2.7	0.4	4.9
δ-t ₃	57.8	44.0	81.8	63.0
Total t	146.9	110.2	161.7	107.9
Total t ₃	619.6	515.5	701.3	600.8
Total Vitamin E	766.5 ^a	625.7 ^b	863.0g	708.7^{d}_{h}

NB: Amounts are given as mg/kg fat, RPOL = Red palm olein, ND = Not detected. t = tocopherols, t_3 = tocotrienols.

For comparison of the total vitamin E contents in control or test snacks between zero and six months, values with different superscripts differ significantly ($P \le 0.05$). For comparison of the total vitamin E contents between control and test snacks at zero or after six months, values with different subscripts differ significantly ($P \le 0.05$), (n = 12 for the last row).

three biscuit samples during six months of storage. The values for FFA and peroxide remained reasonably low and within permissible limits of less than 1% (CAC, 1993). The β -carotene contents at the beginning of the storage period for biscuits made with control shortening, RPS and RPS:RPOL, were 22.0, 311.9 and 335.1 mg/kg fat, respectively, but after six months of storage at room temperature, these values decreased significantly to 18.3, 281.8 and 310.3 mg/kg fat, respectively. The overall average losses of

 β -carotene in biscuits made with control shortening, RPS and RPS: RPOL, were 16.8, 9.7 and 7.4%, respectively.

The total vitamin E content of biscuits made with control shortening, RPS and RPS: RPOL decreased significantly in all three samples at the end of six months of storage at room temperature (Table 4). At the end of six months of storage, the total tocotrienol contents for control, RPS and RPS: RPOL biscuits were 419.0, 391.3 and 424.5 mg/kg fat, respectively. The corresponding total vitamin E contents at the end of the storage period of six months were 498.4, 447.8 and 509.2 mg/kg fat. The overall loss of total vitamin E in control, RPS and RPS: RPOL biscuits were 20.1, 25.9 and 29.7%, respectively.

3.1.3. Pan bread

Palm oil and palm shortening, being good sources of vitamin E, contributed significantly to the tocopherol and tocotrienol contents of bread samples (Table 5). The total vitamin E content among these nine bread samples with zero storage time ranged from 279.0 to 600.1 mg/kg fat and decreased at the end of five days of storage to between 159.9 and 440.0 mg/kg fat. Significant overall vitamin E losses of 36, 58 and 27% were observed at the end of five days of storage for wholegrain wheat flour bread, white wheat flour bread, and brown bread samples, respectively. In absolute terms, the average vitamin E contents of wholegrain wheat flour bread, white wheat flour bread and brown bread, which were initially 383.8, 481.9 and 392.3 mg/kg fat, were reduced significantly at the end of five days of storage at room temperature to 246.0, 195.3 and 294.8 mg/kg fat, respectively.

3.2. Sensory analysis and consumer acceptability

3.2.1. Extruded snacks

The extruded snacks prepared in both commercial plants were evaluated for sensory quality on a nine-point hedonic scale, and the results are summarized in Table 6. For the snacks from Plant No. 1, there were no significant differences in any of the sensory attributes between the control and test samples. Though the test samples had slightly lower salt contents than their controls, they were well accepted by consumers. In the case of Plant No. 2, both the control and test snack samples received similar sensory scores from the panelist, and no significant differences were observed in

Table 3

β-Carotene contents, free fatty acids (FFAs) and peroxide values (PVs) of digestive biscuits made in Plant no. 2 during storage at room temperature.

Sample	β-Carotene (m	g/kg fat)	FFA (%)		PV (meq/kg)	PV (meq/kg)		
	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo		
Control Digestive Biscuits Digestive Biscuits with RPS	22.0 ^a 311.9 ^b	18.3 ^a 281.8 ^b	0.06^{a}_{b} 0.06^{a}_{c}	0.07^{a}_{b} 0.08^{a}_{c}	1.7^{a}_{c} 0.4^{b}_{d}	1.1 ^a _c 0.3 ^b _d		
Digestive Biscuits with RPOL:RPS	335.1 ^c	310.3 ^c _k	$0.08^{\mathrm{a}}_{\mathrm{d}}$	$0.07^{\rm a}_{\rm d}$	0.1_e^b	0.1 ^b _e		

RPS = Red palm shortening; RPOL = Red palm olein, FFAs = Free Fatty Acids, PVs = Peroxide ValuesFor comparison among the three digestive biscuit samples, β -carotene, FFAs and PVs (at zero or after six months) with same superscript in a column do not differ significantly (P \leq 0.05). Among any digestive biscuit sample at zero and six months, β -carotene, FFAs and PVs with the same subscript do not differ significantly (P \leq 0.05), ((n = 9 for the column).

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Table 4

Vitamin E content of digestive biscuits made in Plant no. 2 during storage at room temperature.

Vitamin E Forms	Control digestiv	ve biscuits	Digestive biscu	its with RPS	Digestive biscuits with RPO RPS	
	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo
α-t	102.0	68.5	102.0	49.5	122.5	76.0
α-t ₃	166.5	141.0	150.5	92.8	175.5	136.0
β-t	10.7	6.7	10.2	4.7	10.9	5.5
β-t ₃ &γ-t	64.5	44.5	60.5	31.0	67.0	39.5
γ-t ₃	234.5	199.5	236.5	243.5	289.5	212.5
δ-t	5.4	4.2	4.4	2.3	5.0	3.2
δ-t ₃	39.8	34.0	40.0	24.0	53.8	36.5
Total t	118.1	79.4	116.6	56.5	138.4	84.7
Total t ₃	505.3	419.0	487.5	391.3	585.8	424.5
Total Vitamin E	623.4 ^a g	498.4 ^b _h	604.1 ^c _g	447.8 ^d _h	724.2 ^e g	$509.2^{\mathrm{f}}_{\mathrm{h}}$

NB: Amounts are given as mg/kg fat.

RPS = Red palm shortening; RPOL = Red palm olein; t = tocopherols; t₃ = tocotrienols.

For comparison of total vitamin E contents in control or test digestive biscuits between zero and after six months of storage, values with different superscripts differ significantly ($P \le 0.05$). For comparison of total vitamin E contents between control and test digestive biscuits at zero or after six months of storage, values with same subscripts do not differ significantly ($P \le 0.05$), (n = 18 for the last row).

Table 5

Vitamin E content of various optimized pan bread formulations made on a pilot scale in a commercial bakery at zero and five days of storage at room temperature.

Vitamin E Forms	Storage time (d)	Control Wholegrain flour wheat bread	Wholegrain wheat flour bread with RPS	Wholegrain wheat flour bread with RPS: RPOL	Control white wheat Flour Bread	White wheat Flour bread with RPS	White wheat flour bread with RPS: RPOL	Control brown bread	Brown bread* with RPS	Brown bread* with RPS: RPOL
α-t	0	53.5	56.0	70.5	51.0	99.5	88.0	56.5	67.5	92.5
	5	25.5	47.0	60.5	30.3	33.5	40.0	46.0	42.8	85.0
α-t ₃	0	51.0	62.0	67.0	63.5	120.5	93.5	56.5	59.5	78.0
	5	21.5	43.5	66.0	30.2	40.5	58.0	46.5	42.5	80.0
β-t	0	28.8	20.8	32.0	13.0	24.1	24.0	13.5	29.5	38.8
	5	13.5	19.0	18.5	6.9	6.9	10.8	9.5	18.0	32.5
β-t ₃ & γ-t	0	119.0	88.5	138.5	85.0	156.5	155.5	59.0	116.0	149.5
	5	58.1	81.0	70.0	46.9	46.0	69.5	45.0	69.0	118.5
γ -t ₃	0	91.7	101.8	117.0	85.0	166.5	131.0	80.5	92.0	119.5
	5	34.6	61.5	81.5	38.6	44.0	56.3	62.0	44.5	102.5
δ-t	0	ND	ND	ND	2.8	5.2	9.9	ND	ND	ND
	5	ND	4.5	1.4	1.4	ND	6.5	ND	ND	4.5
δ-t ₃	0	15.5	14.8	23.0	12.5	27.8	31.0	13.0	21.5	33.5
	5	6.7	11.3	12.5	5.8	5.0	9.1	8.7	10.0	17.0
Total Vitamin E	0	359.5 ^{de}	343.9 ^e	448.0 ^c	312.8 ^g	600.1 ^a	532.9 ^b	279.0_{d}^{f}	386.0 ^d	511.8 ^b
	5	159.9 ^e b	267.8 ^{bc} _b	310.4 ^b _a	160.1 ^b _d	175.9 ^{de}	250.0 ^c _b	217.7_{e}^{bc}	226.8 ^{cd} _b	440.0 ^a _c

NB: All amounts are given as mg/kg fat, RPOL = Red palm olein, RPS = Red palm shortening, t = tocopherols, t₃ = tocotrienols, ND = Not detected.

For comparison of total vitamin E contents in different bread samples at day zero or after five days of storage at room temperature, values in a row with different superscripts differ significantly ($P \le 0.05$). For comparison of total vitamin E contents in different bread samples between day zero and after five days of storage at room temperature, values in a column with different subscript differ significantly ($P \le 0.05$), (n = 9 for the last row).

^{*} Brown bread was made with white wheat flour and 20% fine wheat bran.

any of the sensory attributes of these two samples. There were no significant differences in the average sensory scores in any of the sensory attributes of the four snacks initially or after six months of storage at room temperature.

3.2.2. Digestive biscuits

Three types of digestive biscuits, i.e., the control with the regular shortening, a second with RPS, and a third with equal ratios of RPS and RPOL, prepared in Plant No. 2, were evaluated for sensory quality in terms of color, texture, flavor and overall acceptability on a nine-point hedonic scale, and the results are presented in Supplementary Table S-2. The panelists did not find any significant differences in any of the sensory attributes between the control biscuits and those made using either the RPS or RPOL: RPS in combination, indicating that all three biscuit samples were equally acceptable.

3.2.3. Pan bread

All of the bread samples prepared in commercial Plant No. 3 were evaluated for sensory quality and consumer acceptability at time zero only, because, in Kuwait, being a small country, most of the bread is consumed fresh on the very first day of purchase. The data presented here indicate that in terms of the sensory attributes, bread samples were found to be quite acceptable (Supplementary Table S-3). No significant differences were found in any of the sensory attributes between the control wholegrain wheat flour bread and the two other test bread samples made with RPS or with an RPS:RPOL combination. Similar sensory quality results were observed among the control and test white wheat flour bread and brown bread samples.

4. Discussion

4.1. Extruded snacks

Palm oil being rich in carotenoid pigments, vitamin E and many phenolic compounds has been suggested for consumption as it does not pose any health risks (Absalome et al., 2020). The loss of carotenoids and the vitamin E isomers during food processing

Table 6

Average sensory scores of extruded snacks made with red	palm olein (R	RPOL) a	pilot-scale in two commercial	plants during	storage at room temperature.

Type of snack	Color		Texture		Flavor		Overall acceptabi	lity	Consume acceptabi	
	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo	0 mo	6 mo
Plant No.1 Control*	7.2 ^a	6.8 ^a	7.1 ^b	6.9 ^b	7.3 ^c	6.7 ^c	7.3 ^d	6.9 ^d	92	98
Plant No.1 RPOL	7.1 ^a	6.9 ^a	6.3 ^b	6.8 ^b	6.6 ^c	6.7 ^c	6.6^{d}	6.5 ^d	87	91
Plant No. 2 Control*	7.4 ^a	6.7 ^a	7.1 ^b	6.5 ^b	7.1 ^c	7.1 ^c	7.5 ^d	6.9 ^d	97	98
Plant No. 2 RPOL	6.9 ^a	6.6 ^a	6.7 ^b	6.1 ^b	6.9 ^c	6.9 ^c	7.1 ^d	6.6 ^d	90	95

Values with the same superscripts for each sensory attribute do not differ significantly ($P \le 0.05$), (n = 40 for each column).

* Extruded snacks made with control palm oil.

** Extruded snacks made with RPOL.

have been reported to behave differently. During the deep fat frying of foods, Mba et al. (2017) have also shown the β -carotene to be more stable than the tocopherols and tocotrienols. Extrusion process also develops quite a higher temperature, which could result in the loss of these antioxidant compounds. The β -carotene have been shown to be less stable than the lutein during storage of various food products (Song et al., 2018). Tocotrienols are known to be more effective than tocopherols in quenching the harmful free radicals and protecting humans against cancer (Chandrasekharan, 1996). It could be assumed that the vitamin E isomers present in red palm oil might have given protection to the β -carotenes during extrusion, thus leading to lower loss of these carotenoids. The metalized BOPP film used in the packaging of these snacks has better barrier properties to light and oxygen, thus resulting in higher retention of vitamin E contents. The larger sized package $(13 \times 18 \text{ cm})$ used by Plant No. 2 as compared with Plant No. 1 $(13 \times 15 \text{ cm})$, might be the most probable reason for this higher loss, as the later package would have a larger void volume, thus leaving a higher amount of oxygen inside the package. The residual oxygen may be responsible for this higher loss of vitamin E in extruded snacks produced by Plant No. 2 (Lee et al., 2020; Mba et al., 2017).

4.2. Digestive biscuits

Red palm olein being rich in many bioactive phytochemicals has been utilized in making cookies which have been reported to rich in provitamin A carotenoids and vitamin E isomers (Perez-Santana et al., 2021). At the end of baking, the retention of carotenoids and α -tocopherol was reported to be in the range of 92–100% and 93-100%, respectively. These values were much lower in biscuit samples prepared using RPS and RPOL than in those prepared with the control shortening; evidently β -carotene and vitamin E might have acted as effective natural antioxidants. The loss of β carotene in test biscuits was, therefore, quite low (\sim 7–10%) and the β -carotene could be considered as quite stable under these processing, packaging, and storage conditions. Moreover, the vitamin E present in RPS and RPOL might be helpful in reducing the degradation of β-carotene in digestive biscuits (Trela and Szymanska, 2019; Lee et al., 2020). No significant differences were found in the total vitamin E contents among the control and test digestive biscuits either at time zero or after six months of storage at room temperature. Biscuits being a high-fat product, the normal shortening used in their formulations is also sufficiently rich in naturally occurring vitamin E. As expected, the total tocotrienol content was four to five times higher than the total tocopherols in these biscuits. Biscuits prepared with the RPS: RPOL combination were slightly higher in total vitamin E content than the other samples. In absolute terms, 50 g of control, RPS and RPS: RPOL biscuits would provide about 5.5, 5.0 and 5.5 mg of total vitamin E to consumers, which is quite significant in terms of the daily requirements for this vitamin, thus making digestive biscuits made with red palm oil, a reasonably healthy snack product.

4.3. Pan bread

Production of pan bread is a leaner formula when compared with extruded snacks or digestive biscuits (Al-Hooti et al., 2002). Compared with the total fat contents of extruded snacks (35–41%) and digestive biscuits (21–22%), pan bread has only about 3–4% fat. The possible contribution of vitamin E via pan bread to our diet, therefore, seems to be quite meager, but on the other hand, the extruded snacks and digestive biscuits made with RPOL and RPS could serve as significant sources of vitamin E in our diet. The red palm oil has been reported to reduce the toxicity produced by reactive oxygen species (ROS) generated by carbon tetrachloride in rats (Emmanuel et al., 2021). The ROS are implicated in the lipid peroxidation which leads to damage of many organelles membranes containing lipids. The consumption of such food products enriched with RPO or RPOL are definitely expected to provide health benefits to humans too.

4.4. Sensory analysis and consumer acceptability

Sensory quality and physicochemical properties of food products is known to affected by the source, processing and storage conditions. This has recently been studied by a number of workers corroborating our findings (Majid et al, 2021a,b; Al-Ghamdi et al., 2019; Kumar et al. 2018). For the consumer acceptability results, the extruded snacks prepared in both plants were highly acceptable to a large majority of consumers. The consumer were not able to distinguish the quality of snacks made either with RPOL:RPS (1:1) or with RPS alone. It can, therefore, be deduced from the sensory analysis and consumer acceptability data that nutritious extruded snacks rich in vitamin E and β -carotene, with a shelf life of six months, can be produced commercially using either RPOL: RPS (1:1) or RPS alone, in place of regular shortening, thus offering healthy eating alternatives to the consumers.

The consumer acceptability scores for the three types of digestive biscuits were also higher among general consumers. From this sensory analysis and the consumer acceptability data, it became clear that good-quality, nutritious, digestive biscuits rich in vitamin E and β -carotene can be prepared commercially using RPS alone or in a 1:1 combination of RPS:RPOL. It must be pointed out that biscuits are now becoming quite popular as convenient snack items among consumers. Obviously, biscuits, being high in fat content, could serve as a good source of these antioxidants in our diet.

Comparing all nine bread samples together, the panelists could not find any significant differences for any of the sensory attributes. It can, therefore, be accepted that by using RPS alone or a combination of RPS:RPOL (1:1), acceptable quality wholegrain wheat flour, white wheat flour bread, and brown bread can be produced commercially. However, these test breads do have significant nutritional advantages over the control bread, especially in terms of higher β -carotene and vitamin E contents, mainly because of very low fat content (3 to 4% only) in bread samples.

5. Conclusions and recommendations

RPOL and RPS are rich in vitamin E and B-carotene, both these antioxidants have been reported to be essential to protect the human body from the onslaught of damaging free radicals produced during normal metabolic activity. RPOL and RPS, being rich in these naturally occurring antioxidants, have been shown in this study to offer a promising future in the development of various functional foods for consumption by the general population. The results of this study bring out the possibility of producing extruded snacks, digestive biscuits and pan bread using RPOL and RPS on a pilot scale in commercial food plants. The β -carotene and the various vitamin E isomers were found to be quite stable, and their retention in finished products was good. Moreover, the addition of RPOL and RPS not only improved the shelf life but also the nutritional quality of these products as well. The digestive biscuits and extruded snacks made with RPS or RPOL were found to be rich in β-carotene and the various vitamin E isomers, and these antioxidant vitamins were very stable during the storage period of about six months.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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All authors contributed to the research. Dr. Sidhu conceived this research idea and guided the research team, Dr. Sharifa, Dr. Fatima, Dr. Ahmed developed and put together the manuscript, Ms. Hanan helped in the pilot scale production of these products. Mr. Nissar and Mr. Anwar carried out the chemical analyses work. Ms. Amani carried out the statistical analyses of research data. All the authors have read and approved the final manuscript for submission to this journal.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.06.032.

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