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Developing functional foods using red palm olein. IV. Tocopherols and tocotrienols

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Abstract

Two functional foods (pan bread and sugar-snap cookies), were prepared using red palm olein (RPOL) and red palm shortening (RPS) with the objective of providing higher amounts of antioxidant vitamin E in our diet. The total vitamin E contents in the palm oils used in this study were significantly higher (ranging from 717.8 to 817.5 mg/kg fat) than those in the palm shortenings (ranging from 451.0 to 479.9 mg/kg fat). Tocotrienols were found to be the predominant compounds (about 81–84% of the total vitamin E) in all of the palm oil and palm shortening samples. The total tocopherol and total tocotrienol contents were significantly higher in bread made with control shortening (325.0 and 468.0 mg/kg fat) than in that made with a 75% replacement level of RPOL (192 and 407 mg/kg fat) or RPS (101.4 and 300 mg/kg fat). Similar trends were observed in the white bread and the brown bread made with RPOL and RPS. The tocotrienol contents in cookies made with varying levels of replacement RPS ranged from 229.1 to 317.8 mg/kg fat, compared with 379.4 to 672.3 mg/kg fat in cookies made with RPOL. The tocotrienols were found to be the predominant fraction in cookies made either with RPS (58 to 68%) or with RPOL (about 69 to 83% of the total vitamin E). Cookies, being higher in fat contents, would be better providers of these desirable phytochemicals and antioxidant vitamins than breads.

Keywords: Functional foods; Red palm olein; Vitamin E; Tocopherols; Tocotrienols; Bread; Cookies; Nutritive value

1. Introduction

A recent survey in the United States (US) showed that majority of people are becoming more aware of the relationship between diet and disease, and are believing that foods can be designed to help prevent various diseases (Hollingsworth, 1997). The concept that phytochemicals can play a role in the prevention of cancer can as well be extended to other chronic diseases like arthritis, coronary heart disease, osteoporosis, and possibly many other diseases (Wrick, Friedman, Brewda, & Caroll, 1993). Free-radical-mediated lipid peroxidation has been implicated in a variety of pathological processes, especially in both the initiation and promotion of atherosclerosis (Kehrer, 1993). Vitamin E, ascorbic acid and β-carotenes are the major dietary factors that are known to affect the antioxidative ability of the organism (Steinbrecher, Parthasarathy, Leake, Witztum, & Steinberg, 1984).

Palm oil is the largest potential source of vitamin E, which has been advocated for the prevention of heart attacks (Pantazaris & Elias, 1996). The vitamin E content in 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, especially α-tocopherol, is a superior radical chain-breaking antioxidant compared with synthetic ones. The presence of this natural vitamin E in palm oil ensures a longer shelf-life for palm-based food products. As the vitamin E acts as an antioxidant, it plays an important role in the stabilization of oils and

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fats. Vitamin E is major fat-soluble antioxidant acting in the cellular membrane. The potential role of vitamin E in providing protection against free-radical-mediated diseases has been the subject of many studies. Recently, Jiang, Christen, Shigenaga, and Ames (2001) reviewed the role of γ-tocopherol in the US diet. They indicated that γ-tocopherol might be more important to human health because it is more effective in trapping lipophilic electrophiles than α-tocopherol. Park and Choi (2002) investigated that α-tocopherol supplementation was beneficial in decreasing blood-lipid peroxide concentrations without affecting antioxidant activities in patients with type-2 diabetes treated with continuous subcutaneous insulin infusion.

There are a number of natural antioxidants in crude palm oil. The unsaponifiable fraction of crude palm oil, which account for 1% of the oil, are rich in carotenes, vitamin E (tocopherols and tocotrienols), sterols, phospholipids, glycolipids and squalenes (triterpenoids), ubiquinone-9 (UQ9), ubiquinone-10 (UQ10), ergosterol, fucosterol and tocopherolquine (Berger, 2000). Recently, UQ9 was detected at 5 ppm and UQ10 at 80 ppm in crude palm oil, but in palm oil products, more UQ10 was found in the olein (liquid) than in the stearin (solid) fractions. Apart from their role as electron transporters in oxidative phosphorylation, ubiquinones have also been reported to show antioxidant activity (Bonnie & Choo, 2000).

Studies have shown that dietary supplementation with palm tocotrienols for 6 weeks lowered lipid-associated coronary heart disease risk factors significantly, i.e., total serum cholesterol by 20%, LDL—by cholesterol 28% and apo-lipoprotein by 18% (Leong & Thiagarajan, 1999). The current, rising scientific interest in these minor components of palm oil is due to their potent antioxidant properties and their influence in reducing oxidative stress (Kamal-Eldin & Appelqvist, 1996). Edible red palm olein (RPOL) is a new product developed by the Malaysian Palm Oil Board, which is rich in important phytochemicals like vitamin E, carotenoids, ubiquinones and sterols (Choo, 1996; Ping & May, 2000).

A few studies have reported in the literature about the use of synthetic forms of vitamin E in bread and cookies. According to Wennermark and Jaegerstad (1992), dough making resulted in the highest losses of vitamin E in French bread and wheat/rye bread (20–40%). Park, Seib, and Chung (1997) who had fortified bread with fat-coated ascorbic acid, protein-encased β-carotene, and cold-water-dispersible all-*rac*-α-tocopheryl acetate, have reported a loss of less than 10% for vitamin E and about 25% for β-carotene at the end of seven days of storage at room temperature. Bread being a dietary staple, Ranhotra, Gelroth, and Okot-Kotber (2000) have recommended fortification of bread with vitamin E as a better choice. Apart from good retention (67.2%) of vitamin E, no adverse effect on bread quality was observed by them. Hix, Klopfenstein, and Walker (1997) have investigated the use of certain natural antioxidant compounds such as ascorbyl-2-phosphate, α-tocopherol, sodium phytate and ferulic acid as replacements for butylated hydroxyanisole (BHA) in preserving cookies. From the instrumental texture analysis and consumer acceptance data, they recommended the use of these natural antioxidants for effectively substituting BHA in sugar-snap cookies. Because of the importance of antioxidant vitamins (tocopherols and tocotrienols) in our diet, red palm olein (RPOL) and red palm shortening (RPS) were used in the production of two important functional foods (bread and cookies).

2. Materials and methods

2.1. Raw materials

RPS and RPOL were provided by the Carotino Company of Malaysia. White flour, whole-wheat flour, and fine bran were obtained from the Kuwait Flour Mills and Bakeries Co., Kuwait. Fine granulated sugar, common salt, bakery shortening (Wesson brand, US), instant dry yeast and nonfat dry milk were procured from the local market. Diacetyl tartaric acid esters of monoglycerides (DATEM) and sodium stearoyl-2-lactylate (SSL) emulsifiers were procured from the American Ingredients Co., Kansas City, US. All other chemicals were of analytical grade.

2.2. Pan bread formulation

Whole-wheat bread, white bread, and brown bread (80 parts white flour, 20 parts fine bran) were prepared by the optimized straight-dough bread-making method (Method 10-10B) of the American Association of Cereal Chemists (AACC, 1990). These bread-making details were reported earlier (Sidhu, Al-Hooti, Al-Saqer, & Al-Othman, 2001). Initial trials were conducted for replacement of bakery shortening (Wesson, US) with RPOL or RPS at 0, 25, 50, 75, and 100% levels. The control breads in all trials contained only Wesson shortening and no RPOL or RPS. The different combinations of RPS and RPOL i.e., 0:100, 25:75, 50:50 and 100:0% were also used.

2.3. Sugar-snap cookie formulations

The cookies were made as described by Standard Method 10-50D of the American Association of Cereal Chemists (AACC, 1990). The cookies were baked at 205 °C for 10 min. The basic cookie dough formulation contained 225 g of white flour, 135 g of sucrose, 2.25 g of salt, 70 g of shortening, 1 g of sodium bicarbonate, 2.75 g of ammonium bicarbonate, 7 g of nonfat dry milk, 2.25 g of dextrose and 1.25 g of SSL. Initial trials were conducted by replacing bakery shortening (Wesson, US) with RPOL or RPS at 0, 25, 50, 75, and 100% levels.

2.4. 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 containers in a refrigerator for further chemical analysis. The cookies were directly ground, as they had very low moisture contents, and stored in airtight containers for further chemical analysis.

The RPOL, RPS and oil extracted from prepared samples were analyzed for eight forms of vitamin E using the American Oil Chemists Society Method Ce 8-89 (AOCS, 1997). Internal standards for all eight isomers of vitamin E were used for the standardization of high-pressure liquid chromatography (HPLC) conditions HPLC (Shimadzu, Japan, model LC6A) 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; tetra-hydrofuran (60 ml) BDH; and isopropanol (3 ml) BDH.
- Standards: 10 ppm.
- Sample size: Approximately 0.1 g/ml of hexane.

Vitamin E lipids were extracted by the method of Bligh and Dyer (1959) and were analyzed, in duplicate, using the above-mentioned methods. The average values based on extracted fat are presented.

3. Results and discussion

3.1. Characteristics of oils and shortenings

The RPS and RPOL used in this study and commercial samples of palm oil and palm shortening were analyzed for all eight forms of vitamin E and the results are presented in Table 1. The total vitamin E content in the palm oils was significantly higher (ranging from 717.8 to 817.5 mg/kg fat) than that in the palm shortenings (ranging from 451.0 to 479.9 mg/kg fat). Tocotrienols were the predominant isomers (about 81–84% of the total vitamin E isomers) in all of the palm oil and palm shortening samples. In general, all of the palm oil, palm shortening and control (Wesson brand) shortening were as good sources of vitamin E as the RPOL and RPS. The crude palm oil, commercial RPOL, and refined bleached deodorized palm oil have been reported to be rich in vitamin E, with ranges from 600 to 1000, 717 to 863 and 515 to 800 ppm, respectively (Kamal-Eldin & Appelqvist, 1996; Ping & May, 2000). A part of the vitamin E is lost during the refining and hydrogenation processes. Because of the milder processing conditions used, the RPOL was found to be very high in vitamin E (717.8 ppm) and could, thus, serve as a good source of this important antioxidant vitamin in formulated food products.

3.2. Vitamin E contents in baked products

The vitamin E isomer contents of the fat extracted from whole-wheat bread using varying levels of RPS and RPOL are shown in Table 2. The total tocopherol and total tocotrienol contents were significantly higher in bread made with control shortening (325.0 and 468.0 mg/kg fat) than in that made with 75% replacement with RPOL (192 and 407 mg/kg fat) or RPS (101.4 and 300 mg/kg fat). Similar trends were observed in the white bread and the brown bread (Table 3) made with RPS or RPOL. The bread samples prepared with control shortening were richer in vitamin E than those prepared with RPS and RPOL. In all of these bread samples, the tocotrienol content far exceeded the tocopherol content at all levels of RPS or RPOL replacement used in this study. Similar results have earlier been reported on the stability of synthetic vitamin E in bread (Ranhotra, Gelroth, & Okot-Kotber, 2000).

Tocotrienol contents were also higher than tocopherols in sugar-snap cookies (Table 4). The tocotrienol contents in cookies made with varying levels of replacement with RPS ranged from 229.1 to 317.8 mg/kg fat compared with 379.4 to 672.3 mg/kg fat in cookies

Table 1
Vitamin E content of various oils and shortenings

Type of oil	α-t	α-t_3	β-t	β -t_3&γ-t	γ-t_3	δ-t	δ-t_3	Total t	Total t_3	Total Vitamin E
RPOL	131.3	190.0	1.2	17.0	316.0	0.5	61.8	133.0	584.8	717.8
RPS	57.8	123.0	ND	13.9	226.4	ND	42.5	57.8	405.8	463.6
Wesson brand Shortening	35.5	ND	ND	299.1	ND	ND	116.4	35.5	415.5	451.0
Plant No. 1 Palm oil	140.5	211.0	0.5	22.2	371.5	4.8	67.0	145.8	671.7	817.5
Plant No. 2 Palm oil	118.0	211.5	ND	20.8	352.5	ND	56.0	118.0	640.8	758.8
Plant No. 2 Palm shortening	70.5	138.0	ND	13.5	204.5	ND	30.0	70.5	386.0	456.5
Plant No. 3 Palm shortening	82.2	121.0	0.9	12.3	222.0	1.7	39.8	84.8	395.1	479.9

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

Table 2
Vitamin E content of prepared whole-wheat bread using red palm olein (RPOL) and red palm shortening (RPS) at varying replacement levels

Vitamin E	Control	Replacement level of RPOL			Replacement level of RPS		
		25%	50%	75%	25%	50%	75%
α-t	108.0	94.0	85.0	95.0	18.0	17.0	33.4
α-t_3	46.0	53.0	55.0	68.0	11.0	13.0	35.0
β-t	44.0	45.0	35.0	44.0	25.0	28.5	30.0
β-t_3& γ-t	391.0	319.0	263.0	245.0	160.0	185.0	180.0
γ-t_3	27.0	37.0	52.0	78.0	28.0	34.0	63.0
δ-t	173.0	114.0	85.0	53.0	93.0	70.0	38.0
δ-t_3	4.0	7.8	9.0	16.0	20.0	16.5	22.0
Total t	325.0	253.0	205.0	192.0	136.0	115.5	101.4
Total t_3	468.0	416.8	379.0	407.0	219.0	248.5	300.0
Total vitamin E	793.0	669.8	584.0	599.0	355.0	364.0	401.4

NB: Amounts are given as mg/kg fat, t = tocopherols, t_3 = tocotrienols.

Table 3
Vitamin E contents of prepared white bread and brown bread using red palm shortening (RPS) and red palm olein (RPOL) at varying replacement levels

Vitamin E	Control	White bread with RPS				Control	Brown bread[a]	
		25%	50%	75%	100%		100% RPS[a]	100% RPOL[a]
α-t	84.0	64.3	40.4	57.2	91.4	7.8	6.5	17.0
α-t_3	63.9	24.4	37.9	60.3	35.0	5.3	24.2	24.7
β-t	27.5	22.0	18.6	19.0	41.8	22.5	28.5	29.2
β-t_3& γ-t	458.8	169.4	251.0	236.2	349.2	153.7	145.0	126.5
δ-t_3	3.7	12.0	58.5	64.3	42.1	ND	54.5	65.0
δ-t	197.5	103.0	75.2	47.2	135.4	87.0	9.0	11.80
δ-t_3	ND	33.0	11.0	16.6	20.1	ND	20.2	28.5
Total t	309.0	189.3	134.2	123.4	268.6	117.3	44.0	58.0
Total t_3	526.4	238.8	358.4	377.4	446.4	159.0	243.9	244.7
Total vitamin E	835.4	428.1	492.6	500.8	715.0	276.3	287.9	302.7

NB: Amounts are given as mg/kg fat, ND = Not detected, t = tocopherols, t_3 = tocotrienols.
[a] Brown bread was made from white flour (80 parts) and fine bran (20 parts).

Table 4
Vitamin E content of prepared sugar-snap cookies made with varying levels of red palm shortening (RPS) and red palm olein (RPOL)

Vitamin E	Control	Replacement level of RPS				Replacement level of RPOL			
		25%	50%	75%	100%	25%	50%	75%	100%
α-t	66.2	52.8	41.3	40.2	60.1	76.2	72.3	57.9	123.0
α-t_3	7.2	29.9	48.8	46.8	7.9	109.9	99.1	92.8	208.8
β-t	14.8	9.2	7.5	8.1	38.4	8.2	7.3	4.3	9.1
β-t_3 & γ-t	380.0	229.7	139.9	132.4	214.6	178.0	157.4	708.0	47.8
γ-t_3	3.4	48.1	86.3	83.9	6.6	204.0	175.4	181.0	352.0
δ-t	307.0	166.0	106.5	110.7	39.7	146.0	121.4	48.3	5.3
δ-t_3	3.0	10.1	17.0	16.9	ND	35.8	33.5	34.8	63.7
Total t	388.0	228.0	155.3	159.0	138.2	230.4	201.0	110.5	137.4
Total t_3	393.6	317.8	292.0	280.0	229.1	527.7	465.4	379.4	672.3
Total vitamin E	781.6	545.8	447.3	439.0	367.3	758.1	666.4	489.9	809.7

NB: Amounts are given as mg/kg fat, ND = Not detected, t = tocopherol; t_3 = tocotrienol.

made with RPOL. The various tocotrienol forms were found to be the predominant fraction in cookies made either with RPS (58 to 68% of the total vitamin E) or with RPOL (about 69 to 83% of the total vitamin E). The cookies made with 100% replacement with RPOL were higher in total tocotrienol content (672.3 mg/kg fat) than the cookies made with 100% replacement with RPS (229.1 mg/kg fat) and all other prepared cookies.

Synthetic antioxidants such as BHA, butylated hydroxytoluene (BHT), propyl gallate (PG) and t-butylhydroquinone (TBHQ) are being widely used by the baking industry to retard rancidity and preserve freshness in cookies (Hix et al., 1997). RPOL and RPL are not only rich in natural antioxidant vitamin E compounds, but have also been reported to produce sugar-snap cookies and bread with good sensory characteristics and consumer acceptability (Al-Hooti et al., 2002).

As in bread, the total vitamin E content was significantly higher (781.6 mg/kg fat) in cookies made with control shortening than in those made with 100% RPS (367.3 mg/kg fat) or 100% RPOL (489.9 mg/kg fat), but the use of RPOL or RPS in bakery products has another added advantage of contributing higher contents of other valuable phytonutrients like β-carotenes, ubiquinones and sterols to these functional foods (Ooi, Choo, Yap, & Ma, 1996). Cookies being higher in fat contents would be better carriers than bread for these desirable phytochemicals and antioxidant vitamins in our diet. It can, therefore, be concluded that RPOL as well as RPS could be used as a good source of these valuable phytonutrients in pan bread and sugar-snap cookies.

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