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Potential of dried bread enriched with red palm oil to improve lipid profile, and control oxidative stress: An experimental study in adult men with dyslipidaemia

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Abstract

Dyslipidaemia is an abnormality of lipid metabolism, characterised by the increased levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and decreased levels of high-density lipoprotein cholesterol (HDL-C), which cause antioxidant enzymes to decrease, and trigger oxidative stress, characterised by an increase in lipid peroxidation. The present work examined the effect of consumption of dried breads enriched with red palm oil (RPO) to improve serum lipid profiles, and control oxidative stress in adult men with dyslipidaemia. This randomised double-blind controlled trial involved adult men with an abnormal lipid profile, and had a body mass index between 18.5 and 26.9 kg/m^2. Each subject consumed two pieces of dried bread per packet as a snack for eight weeks. Subjects were randomly assigned into two groups: those who consumed dried breads with RPO (experiment), and those who consumed dried breads without RPO (control). Serum levels of lipid profile parameters (total cholesterol, triglyceride, LDL-C, HDL-C) and control oxidative stress markers [superoxide dismutase (SOD) enzyme, malondialdehyde (MDA), oxidised LDL (oxLDL), and β-carotene serum] were measured before and after the intervention. In subjects who consumed dried breads with RPO, there was a significant decrease in total cholesterol and LDL-C levels (p = 0.020 and 0.041, respectively); whereas serum triglyceride and HDL-C levels were not significantly affected (p = 0.083 and 0.233, respectively). Further, the consumption of dried breads with RPO significantly (p = 0.018) increased SOD activity which led to a significant decrease in MDA and oxLDL levels (p = 0.036 and 0.047, respectively), but non-significant on the increase in β-carotene serum levels (p = 0.166). Therefore, daily consumption of 60 g dried breads with RPO has the potential to be a functional food that can be used as a snack that efficiently lowers total and LDL cholesterol levels in adult men with dyslipidaemia, but without deleterious effects on biomarkers of oxidative stress.

Introduction

Coronary heart disease (CHD) is one of the cardiovascular diseases causing death worldwide (Piepoli *et al*., 2016), especially in Indonesia. Basic health research data (RISKESDAS, 2013; KEMENKES RI, 2012) reported that the highest prevalence of cardiovascular disease in Indonesia is CHD (1.5%). The prevalence and susceptibility of CHD are not only restricted to the elderly, but also to the young people. CHD occurs due to the disruption of blood supply to the heart muscle, which is caused by the narrowing or blockage of the arteries due to dyslipidaemia. Dyslipidaemia is a condition in which lipid level abnormalities occur in the blood; increase in the levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglyceride, and decrease in the levels of high-density lipoprotein cholesterol (HDL-C) (Singh *et al*., 2010), which later causes a decrease in antioxidant enzymes, and an increase in lipid peroxidation. The prevalence of dyslipidaemia in Indonesia is still quite high. The 2007 Biomedical RISKESDAS report showed that the national prevalence of total cholesterol was 44.9%, LDL was high at 73.1%, and HDL was low at 35% (KEMENKES RI, 2012).

Another factor for CHD is oxidative stress which is a condition of imbalance between the free radical production or reactive oxygen species (ROS) and antioxidants; free radical levels are higher than antioxidant levels (Kurkcu, 2010). Free radicals are molecules that have a deficiency or excess of

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unpaired electrons in their orbits (Dasgupta and Klein, 2014). Free radical compounds can damage lipids, especially LDL-C. Free radical attacks on LDL-C result from LDL-oxidation, and cause the formation of lipid peroxidation which produces malondialdehyde (MDA); an indicator of lipid peroxidation is high number of MDA. There is a relationship between MDA in the blood and atherosclerosis in the arterial wall; therefore, high levels of MDA is categorised as one of the risk factors for CHD. The first defence mechanism for excessive oxidation in the body will be carried out by the activity of superoxide dismutase (SOD) enzyme, which is one of the antioxidant enzymes (Gropper and Smith, 2013). High free radicals in the body result from low SOD activity.

Free radicals cause damage to several organs such as heart, liver, and kidneys. This organ damage can end in cell death, thus resulting in accelerated emergence of degenerative diseases such as CHD. However, oxidative stress can be prevented by antioxidant intake which accelerates the degradation of free radicals. Controlling CHD is possible with some prevention measures such as decreasing the negative impact of free radicals with the consumption of foods rich in antioxidants. Red palm oil (RPO) contains β-carotene which is a type of antioxidant (Small and Gobe, 2013). Therefore, RPO can be a functional food which prevents the occurrence of CHD, and decreases the probability of lipid peroxidation (Atawodi *et al*., 2011).

Antioxidants protect cells by capturing the free radicals (radical scavenger), and neutralising the action of the free radicals to prevent chain reactions so that lipid peroxidation does not occur. In addition, antioxidants damage the chain reaction of lipid peroxidation (chain-breaking antioxidant), and protect the cell membranes via lipid repair and replacement (Colombo, 2010). The interaction of β-carotene and vitamin E also leads to a variety of biological effects ranging from the scavenging of the free radicals to the modulation of signals that are associated with a decreased risk of CHD (Vrolijk *et al*., 2015). According to Yuliasari *et al*. (2014), RPO has the potential to be a functional food ingredient owing to its content of β-carotene and other functional components. Indonesia is one of the countries with the largest palm oil production in the world, and the production increases annually. However, its utilisation in processed food, particularly in the form of RPO, remains limited. The main objective of the present work was therefore to examine the consumption effect of dried breads enriched with RPO to improve serum lipid profiles, and control oxidative stress in adult men with dyslipidaemia.

Materials and methods

Subject

Subjects were recruited using a simple random sampling among the educational staff of Bogor Agricultural University, Indonesia. The study protocol was carefully explained to all subjects before they were provided with a written informed consent. The present work received the ethics approval from the Human Research Ethics Committee of Bogor Agricultural University, Indonesia (approval no.: 09/IT3.KEPMSM-IPB/SK/2017, dated October 2nd, 2017). The present work was an experimental trial with a randomised control trial pre-post study design. Subjects were eligible if they (1) were an adult man aged 35 - 45, with a body mass index (BMI) ranging from 18.5 - 26.9 kg/m^2, (2) had an abnormality in the lipid profile (total cholesterol, triglycerides, LDL-C, and HDL-C of ≥ 200, ≥ 150, ≥ 130, and < 40 mg/dL, respectively), (3) did not use statins, and (4) were willing to sign the informed consent. Those who had participated in other researches, congenital abnormalities, food allergies, or did not complete the research procedures were excluded. The size of the subjects was determined using a large formula of two different hypothesis test samples on average. The minimum number of required subjects was determined using Eq. 1:

$$n = 2\,(\sigma/\delta)^2\,(z_\alpha + z_\beta)^2 \qquad \text{(Eq. 1)}$$

where, n = number of subjects; Z_α = random variable value, $Z_{0.025}$ = 1.96; Z_β = random variable value, $Z_{0.178}$ = 0.84; σ = standard deviation from SOD level (9.6 U/mL); and δ = difference or expected effect (-9.2 U/mL; Utari, 2011). Based on the calculations, the minimum number of subjects required for each treatment group was 17 adult men, and we added 10% to this number in anticipation of dropouts. Thus, the final number of subjects in each treatment group was 19 adult men, making a total of 38 adult men. Based on the screening result, 36 subjects were enrolled; however, three subjects dropped out because they (1) were unable to participate in the intervention, (2) had a low level of adherence, and (3) indicated a clinical symptom of CHD. Therefore, a total of 34 adult men, 17 in each group, completed the study. The baseline for clinical and biological characteristics of all subjects (n = 34) are shown in Table 1.

Table 1. Clinical and biological characteristics of the subjects at baseline.

Variable	Dried bread treatment		*p*-value
	With RPO	Without RPO	
Age (y)[1]	40 ± 2.74	38 ± 2.37	
35-39	8 (47.06)	12 (70.59)	0.30
40-45	9 (52.94)	5 (29.41)	
Education			
Elementary-Junior high school	0 (0)	4 (23.53)	
Senior high school	10 (58.82)	12 (70.59)	0.10
College	7 (41.18)	1 (5.88)	
Smoking			
No	9 (52.94)	7 (41.18)	0.73
Yes	8 (47.06)	10 (58.82)	
Exercise			
No	3 (17.65)	7 (41.18)	
Yes			0.29
Not regularly	10 (58.82)	8 (47.06)	
Regularly	4 (23.53)	2 (11.76)	
NCD genetic history[2]			
No	17 (100)	15 (88.24)	0.49
Yes	0 (0)	2 (11.76)	
Abdominal circumference (cm)			
Normal	10 (58.82)	13 (76.47)	0.47
Not normal	7 (41.18)	4 (23.53)	
Blood pressure (mmHg)			
Normal	12 (70.59)	10 (58.82)	0.72
Hypertension	5 (29.41)	7 (41.18)	
Nutritional status (kg/m^2)			
Skinny (< 18.5)	0 (0)	0 (0)	
Normal (≥ 18.5 - 25)	11 (64.71)	10 (58.82)	1.00
Overweight (> 25 - 27)	6 (35.29)	7 (41.17)	
Obesity (> 27)	0 (0)	0 (0)	

[1]percentage (%); [2]NCD = non communicable diseases. Comparison between dried bread with or without RPO group were performed by using independent sample t-test for continuous data or chi-square test for qualitative data.

Study design

The present work employed a double-blind randomised control trial pre-post study design, where dried breads [dried bread without RPO (control) and with RPO (treatment)] had the same shape and colour, so that each subject and researcher would not know the type of treatment received. Thirty-four subjects were randomised into each group using a computer programs. Research subjects were randomly divided into the following two treatment groups: (1) those who consumed dried breads without RPO, and (2) those who consume dried breads with RPO. The duration of the intervention period was eight weeks.

Instruction and collection of the subjects' blood samples were conducted at the Nutrition Clinic, Department of Community Nutrition, Bogor Agricultural University, Indonesia. Analyses of the serum lipid profiles (total cholesterol, triglycerides, HDL-C, and LDL-C) were performed at the Regional Health Laboratory in Bogor City, while serum levels of SOD, MDA, oxidized LDL, and β-carotene were analysed at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, Indonesia.

Administration of dried bread with RPO

Dried breads with and without RPO were prepared at the Bread Unit, SEAFAST Centre, Bogor Agricultural University, Indonesia. These dried breads were made of flour, instant yeast, sugar, milk, eggs, margarine, RPO (in the case of treatment), and salt. The products were yellow in colour, had a slight aroma of RPO (in the case of treatment), slightly sweet taste, and slightly crunchy texture. A single portion (60 g) of dried bread with RPO contained about 291 kcal of energy. The daily energy requirement in the general group based on the nutritional label reference was 2,150 kcal. The proportion of the snack was 10 - 15% for one time snacking of the total daily energy needs. Therefore, this dried bread contributed to 14% of the total daily energy needs. This product also contained β-carotene (4,074 µg/100 g of the food product). The regulation prescribed by The National Agency of Drug and Food Control of Republic of Indonesia or NADFC (Indonesian: Badan Pengawas Obat dan Makanan) (No. 13/2016) controls the monitoring of claims on labels and processed food advertisements. Food is considered a source of vitamins or minerals if it meets 15% of the Nutrient Reference Values per 100 g (in solid form), and is considered high in vitamins or minerals if it meets 30% of the Nutrient Reference Values (BPOM RI, 2016).

Research subjects were men aged 35 - 45 years who had vitamin A level of 600 µg/day. However, this regulation does not monitor the claims of bioactive compounds such as β-carotene. We then applied the regulations to establish β-carotene standards using the equations of vitamin A and β-carotene (1 µg RE = 6 µg β-carotene). We established a cut-off of 1,080 µg/day of β-carotene to express high β-carotene food. The amount of β-carotene required to be present in this dried bread to qualify it as high in vitamin A was 30% of the Nutrient Reference Values, that is, 180 µg/day; therefore, the level of β-carotene adequacy in the general population is 3,600 µg/day. The β-carotene content of RPO dried bread was 40.74 ppm, equivalent to 4,074 µg/100 g of the food product. The level of β-carotene in RPO dried breads met the high claim of β-carotene because it exceeded 1,080 µg. The antioxidant activity of RPO dried breads was 470.44 mg/100 g AEAC (ascorbic acid equivalent antioxidant capacity). This figure shows that the antioxidant activity of 100 grams of these products was equivalent to that of 470.44 mg ascorbic acid (vitamin C).

Blood sampling

Venous blood samples were obtained after the subjects had fasted overnight. A 5-mL sample of venous blood was collected from the median cubital vein (antecubital fossa) in vacutainers containing EDTA anticoagulants using a syringe. Blood collection was performed by a health analyst who has a competency certificate. The samples were immediately put into a sterile Falcon tube, and centrifuged at 3,000 rpm for 10 min.

Analyses of the lipid profile levels included total cholesterol (colorimetric enzymatic CHOD-PAP method with a spectrophotometer at λ of 500 nm wavelength), triglyceride (colorimetric GPO-PAP method with a spectrophotometer at λ of 500 nm), and HDL-C (precipitation method with a spectrophotometer at λ of 546 nm). LDL-C levels were calculated using Eq. 2:

$$\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - [\text{triglycerides}/5] \quad \text{(Eq. 2)}$$

Analysis of serum total cholestrol, LDL-C, triglyceride, and HDL-C was done using a ‘‘Roche Modular P’’ system and the associated kits (Roche Diagnostics, Indianapolis, United States).

SOD activities were determined by measuring the inhibition levels of the reaction of xanthine and xanthine oxidase that produced superoxide with 2-(4-iodophenyl)-3-(4-nitrophenol)-5 phenyltetrazolium chloride (INT) radical, which produced a red colour, and was measured using a spectrophotometer at λ of 480 nm (Ukeda *et al*., 1999). MDA levels were determined using the thiobarbituric acid reactive substances assay method; the absorbance of the pink colour was measured using a spectrophotometer at λ of 532 nm (Capeyron *et al*., 2002). OxLDL and β-carotene levels were determined using the commercially available Mercodia ELISA kits as per the manufacturer's instruction using an ELISA reader (Mercodia AB, Uppsala, Sweden).

Compliance

Subject compliance for dried bread consumption was assessed and evaluated by interviewing the subjects, monitoring, and collecting product packages during the intervention by counting the unopened and unconsumed product packages returned. Monitoring the subject's compliance was done by reminding them in the morning via a short message (SMS) to eat the dried bread following the predetermined schedule. The subjects were expected to finish the dried breads each day at a specified time, namely, the first pack (30 g) between 09.00 - 10.00 WIB and the second pack (30 g) between 15.00 - 16.00 WIB. At the beginning of the intervention, the researchers requested the subjects to fill out the compliance questionnaire honestly, and then, it was validated by interviewers. Compliance was recorded as the percentage of the scheduled servings consumed. Non-compliance was defined as the consumption of < 80% of the scheduled servings during the study period.

Statistical analysis

Data analysis were performed using SPSS program version 23. Data regarding the subject characteristics were analysed descriptively for each variable, and were presented as mean ± standard error. The significance level was 0.05. The chi-square test was used to examine the effect of two nominal variables. ANCOVA test (covariate analysis) was used to determine the treatment effect on the response variables by controlling the covariate variables.

Results

Study participants

We enrolled only male subjects in the present work considering that the effect of oestrogen in pre-menopausal women is associated with an increase in the HDL-C levels, and a decrease in the LDL-C levels. We selected subjects in the age group of 35 - 45 years to avoid a large effect of external factors because this was a preventive study. There were no significant differences between dried breads with and without RPO groups at baseline (Table 1), where smoking was 58.82% as compared to 47.06%, exercise was 41.18% as compared to 17.65%, abdominal circumference was 23.53% as compared to 41.18%, and blood pressure was 41.18% as compared to 29.41%, respectively.

Subjects were selected on the basis of screening values for the mean fasting abnormalities lipid profile. The mean serum levels of total cholesterol, tryglicerides, and LDL-C were higher in dried breads with RPO group than without RPO group (204.32 vs 197.47 mg/dL, 154.82 vs 131.12, and 128.41 vs 116.82, respectively) ($p > 0.05$). Neither biological criteria nor dietary intake at baseline showed that there were significant changes in either of the groups.

Compliance and side effects during study

Compliance for the consumption of dried breads was determined using the proportion of the actual consumption of the product in relation to the provided supplies, and defined as consumption of > 80% of the scheduled servings during the study period. Subjects had highly good compliance, given that on an average of > 99% of the provided dried breads were consumed every day. The average consumption of dried breads without and with RPO for eight weeks was 99.42 ± 2.75% and 99.32 ± 3.56%, respectively.

The consumption of dried breads without RPO was higher than that with RPO; however, there were no significant differences between the two groups ($p = 0.421$). All subjects followed the dietary recommendations. No adverse events related to the consumption of the product occurred during the experimental trial.

Serum lipid profile

The mean serum lipid levels at baseline and endline consumption of the dried breads with and without RPO groups are shown in Table 2. The decrease in total and LDL cholesterol levels during the intervention was 3.09 and 4.21%, respectively, in the dried breads with RPO group, whereas in the dried breads without RPO group, it increased by 1.78 and 6.49%, respectively. This corresponded with 6.53 and 5.41 mg/dL decrease in total and LDL cholesterol levels, respectively, in the dried breads group with RPO, and 3.53 and 7.59 mg/dL increase in total and LDL cholesterol levels in the dried breads group without RPO ($p = 0.020$ and 0.041, respectively). HDL cholesterol and serum triglyceride levels were not significantly affected by the consumption of dried breads with RPO ($p = 0.233$ and 0.083, respectively).

Oxidative stress

The increase in serum SOD levels was 7.03% greater in the dried breads with RPO group than without RPO group ($p = 0.018$). However, serum levels of MDA were decreased significantly in both groups (33.92 vs 68.43 ng/mL, $p = 0.036$). Serum levels of oxLDL were decreased more significantly in

Table 2. Serum lipid levels at baseline and endline for consumption of dried bread with and without RPO, and the absolute change between baseline and endline of the trial.

	Dried bread treatment group		
	with RPO (n = 17)	without RPO (n = 17)	p-value
Total cholesterol (mg/dL)			
Baseline	211.18 ± 4.50	197.47 ± 5.42	
Endline	204.65 ± 6.41	201 ± 5.43	0.020
Absolute change[1,2]	6.53[3]	-3.53	
LDL cholesterol (mg/dL)			
Baseline	128.41 ± 5.29	116.82 ± 6.67	
Endline	123 ± 7.23	124.41 ± 5.45	0.041
Absolute change[1,2]	5.41[3]	-7.59	
HDL cholesterol (mg/dL)			
Baseline	51.94 ± 2.19	54.41 ± 2.38	
Endline	46.76 ± 1.35	50 ± 1.93	0.233
Absolute change[1,2]	5.18	4.41	
Triglyceride (mg/dL)			
Baseline	154.82 ± 16.20	131.12 ± 16.43	
Endline	169.65 ± 17.91	132.82 ± 14.59	0.083
Absolute change[1,2]	-14.82	-1.71	

Values are mean ± SE; [1]absolute change was calculated for each subject as baseline value minus endline value; [2]comparison between dried bread with and without RPO groups was performed using ANCOVA on raw data; [3]significantly different from dried bread without RPO = $p < 0.05$.

the dried breads with RPO group (0.01 vs -0.03 ng/mL; p = 0.047). The increase in β-carotene serum levels was 1.68% smaller in the dried breads with RPO group than without RPO group (3.59%). Although serum β-carotene levels in both groups increased, variation in β-carotene levels during the study did not differ significantly between both groups (p = 0.166) (Table 3).

Discussion

Compliance is obviously a key aspect in the success of the intervention, especially if the purpose was for disease prevention. In the present work, the compliance was excellent (reaching > 99% by the end of the study), and consumption of dried breads with or without RPO did not give rise to any clinical adverse events.

Double-blind randomised controlled trial (dried breads with RPO and without RPO) pre-post study and daily consumption of 60 g dried breads with RPO as snack led to a decrease in total cholesterol levels of 3.09 and 4.21%, respectively in subjects of dyslipidaemia after the trial study. Experimental and epidemiological researches have demonstrated that dyslipidaemia plays an important role in the incidence of atherosclerosis. The present work observed a significant decrease in the total cholesterol levels following the consumption of dried breads with RPO, and this agrees with the results of meta-analysis by Odia *et al*. (2015) which stated that foods containing palm oil were more effective in improving the serum lipid profile. Similar results have been reported in other researches from China (Zhang *et al*., 2003) and Malaysia (Alias *et al*., 2002), based on which, palm oil significantly decreased the total cholesterol levels of the subjects.

The mechanism of decrease may be attributable to the synergistic action of several micronutrients in RPO that inhibit the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme in the mechanism of cholesterol biosynthesis. Saturated fatty acids in MSM have a unique structure, the sn-2 position, which is mostly absorbed in the intestine, and serves to maintain stability and protection against oxidation (Rooyen, 2013). The main saturated fatty acid in MSM is palmitic acid (16:0); but, palmitic acid is neutral to cholesterol. In addition, unsaturated fatty

Table 3. Serum levels of superoxide dismutase (SOD), malondialdehyde (MDA), oxidised LDL (oxLDL), and β-carotene at baseline and endline of trial and study product effect.

	Dried bread treatment group		
	with RPO (n = 17)	without RPO (n = 17)	p-value
SOD (U/mL)			
Baseline	22.61 ± 2.71	23.28 ± 3.32	
Endline	24.20 ± 2.72	20.88 ± 1.59	0.018
Absolute change[1,3]	-1.59[4]	2.40	
MDA (ng/mL)			
Baseline	326.13 ± 28	324.85 ± 26.92	
Endline	292.21 ± 26.23	256.42 ± 14.43	0.036
Absolute change[1,3]	33.92[4]	68.43	
OxLDL (ng/L)			
Baseline	1967.43 ± 224.95	2203.31 ± 320.98	
Endline	1945.67 ± 196.84	2278.41 ± 303.07	0.047
Absolute change[2,3]	0.01[4]	-0.03	
β-carotene (nmol/mL)			
Baseline	1.79 ± 0.14	1.67 ± 0.11	
Endline	1.82 ± 1.14	1.73 ± 0.10	0.166
Absolute change[2,3]	-0.02	-0.04	

Values are mean ± SE; [1]absolute change was calculated for each subject as baseline value minus endline value; [2]absolute change was calculated for each subject as ratio (baseline minus endline, divide by baseline) value; [3]comparison between dried bread with and without RPO groups was performed by using ANCOVA on raw data; [4]significantly different from dried bread without RPO = $p < 0.05$.

acids (oleic and linoleic), which predominate in MSM, also have a positive effect on decreasing blood cholesterol so that they can neutralise the negative effects of sterols present in the body, and inhibit the occurrence of atherosclerosis (Halim and Rahmayuni, 2015), and also have a role in transport and fat metabolism, improve body immune function, and prevent CHD (Sartika, 2008).

There was a significant decrease in the LDL-C levels in subjects who consumed dried breads with RPO, in line with the report by Alias *et al*. (2002), wherein palm oil consumption decreased the LDL-C levels of the subjects. Similar findings have also been reported in China (Sartika, 2008). The ability of RPO to decrease LDL-C is related to its fatty acid composition. MSM contains about 50% unsaturated fatty acids in the form of oleic acid (18:1) and linoleic acid (18:2). Unsaturated fatty acids increase the amount and activity of LDL receptors so that they can increase the catabolic LDL-C and will decrease LDL-C (Fernandez-Sanchez *et al*., 2011). This mechanism can be attributable to the type of fat in RPO that affects LDL-C levels, explained through the LDL receptor pathway. RPO contains unsaturated fatty acids in the form of oleic, linoleic, and linolenic acids, which can be esterified by acyl CoA cholesterol acyl transferase enzyme; this enzyme acts on unsaturated fatty acids (MUFA and PUFA) as substrates for the esterification, during which, the free cholesterol in intracellular is used, thus causing a decrease in free cholesterol. The esterification stimulates gene transcription and produces new LDL receptors through the sterol regulatory element binding the protein mechanism, which eventually decreases LDL-C levels.

HDL-C levels in both the treatment groups decreased but without significant differences. These are in line with Alexandre *et al*. (2017) who recruited 120 healthy young volunteers (18 - 30 years) with similar eating habits, regardless of the amount of palm oil consumed in Ivory Coast, Abidjan, and West Africa, and showed that there was no significant difference in HDL-C levels between subjects. Decreased HDL-C levels are also associated with the risk of heart disease and development of oxidative stress. Several studies have also shown that a decrease in HDL-C levels are associated with a higher risk of CHD, and an increase in HDL-C levels is associated with a lower risk of CHD. However, how effective a good serum HDL-C levels in decreasing the risk of CHD is not very clear (Rajagopal *et al.*, 2012). High HDL-C levels are associated with low cardiovascular risk; however, the role of HDL-C in heart disease is very complex, and includes increased reverse cholesterol transport and macrophage activation, anti-inflammation, inhibition of LDL oxidation, and endothelial cell apoptosis.

Triglyceride levels in both treatment groups showed a non-significant increase. This is in agreement with Alexandre *et al*. (2017) who showed that there were no significant differences in the triglyceride levels between the two treatments, with the subjects who were given palm oil had higher triglyceride levels than the controls. Triglycerides store excess energy from the diet. Excessive energy is converted to triglycerides, transported to fat cells, and stored as a source of energy for the body. High energy intake is due to high intake of sugar, fat, and low physical activity. Fat stored are the result of a synthesis of fatty acids and glycerol known as lipogenesis. If the cell needs energy or energy input is lower than the energy output, the lipase enzyme in fat cells will break down triglycerides into glycerol and fatty acids, and release them into blood vessels, known as the lipolysis process. Triglycerides are not only derived from dietary fat (saturated and unsaturated fatty acids) but also from foods containing carbohydrates (simple and complex). Excessive carbohydrate consumption can increase the glucose levels in the blood, and decrease HDL-C levels. Increased carbohydrate intake will increase the triglyceride levels by increasing the formation of pyruvate and acetyl-CoA, thus leading to an increase in *de novo* fatty acid formation from acetyl-CoA. The fatty acids will experience esterification with the triphosphate that is produced from glycolysis to produce triglycerides, thus resulting in an increase in the triglyceride levels.

Oxidative stress, defined as an imbalance between anti- and pro-oxidant factors, is implicated in the development of numerous chronic pathologies including atherosclerosis (Hansel *et al.*, 2007). SOD is a type of endogenous antioxidant enzyme that plays a specific role in protecting cells from oxidation (oxidative damage). High levels of free radicals in the body results from the low activity of SOD in the cells that prohibit the cells from stopping free radical production in the body; this in turn leads to lipid peroxidation and an increase in MDA levels. MDA is a low molecular weight aldehyde compound produced by lipid peroxidation in the body from the free radical reactions (Savini *et al.*, 2013). High MDA levels are categorised as CHD risk factor (Gropper and Smith, 2013). Several studies have shown eating foods that have antioxidants, such as RPO that contains β-carotene, may inhibit lipid peroxidation, and increase the levels of enzyme antioxidants.

SOD acts as the first line of defence against free radicals by converting superoxide anion radicals to hydrogen peroxide and oxygen molecules (Djordjevic *et al*., 2010). Superoxide is an initiator of an acute free radical chain reaction. The SOD activity increases with the induction of enzymes by chemicals or by a condition that increases the SOD production (Pham-Huy, 2008). A significant increase in the SOD levels of subjects who consumed dried breads with RPO was believed to be due to the antioxidant content that protected the protein from oxidative damage, and the interaction of several vitamins and minerals that led to more efficient production and activity of SOD. Valko *et al*. (2007) stated that under the conditions of oxidative stress, there is excessive production of free radicals in the body, and this decreases the antioxidant activity. Therefore, the intake of exogenous antioxidants is crucial for efficient functioning of enzymatic antioxidants. In humans and animals, carotenoids such as β-carotene are important components and play an important role in providing protection against photo-oxidative processes by acting as an oxygen and peroxyl radical scavenger. The synergistic action of carotenoids such as β-carotene with other antioxidants makes it a stronger compound.

Lipids are susceptible oxidation targets owing to their abundant molecular structures that have reactive double bonds. One of the best markers of lipid peroxidation is MDA (Ho *et al*., 2013). In the body, MDA is formed because of oxidative stress conditions that cause an imbalance between the formation of reactive oxygen species (ROS) and the presence of antioxidants (more free radicals than antioxidants are formed). Excess hydroxyl radicals and peroxinitrite may attack the cell membranes and lipoproteins which form lipid peroxides and produce MDA (Savini *et al*., 2013). With age, the formation of free radicals increases, thus resulting in cellular oxidative damage.

The MDA levels in each treatment group decreased significantly. Such a decrease in the MDA levels was also reported by Alias *et al*. (2002) who stated that there was a significant decrease in the MDA levels of subjects who consumed palm oil. This showed that the intervention with dried cakes enriched with RPO significantly decreased the serum MDA levels in the subjects (Alias *et al*., 2002). The decrease in the MDA levels could be attributable to the action of the antioxidants, in particular β-carotene, which might have counteracted the free radicals by cutting off the chain oxidation reaction of free radicals, and provided protection against lipid peroxidation. Antioxidants are molecules that possess the ability to prevent the negative effects of oxidation, and prevent cell and tissue damage caused by free radicals. Free radicals are unstable molecules produced by the body during normal metabolism; they play an important role in disease development (Peng *et al*, 2016). Free radicals oxidise other molecules and produce oxidised products that may cause biochemical changes and damage various living cell components such as proteins, lipids, carbohydrates, and nucleates. Free radical reactions with lipoprotein LDL produce oxLDL that interferes with the LDL-C function, and may play an important role in the aetiology of CHD and atherosclerotic plaque formation (Mitra *et al*., 2011).

It has been suggested that even a minor decrease in β-carotene levels may be proatherogenic. However, antioxidants including numerous substances or enzymes and liposoluble vitamins may interact synergistically. Therefore, analysis of one component independently of the others may not accurately reflect their combined action, and therefore, does not properly estimate systemic oxidative stress. Hence, we measured the effect of dried breads with RPO on the levels of oxLDL, an integrative marker of systemic oxidative stress. In all likelihood, serum oxLDL levels may depend on the capacity of *in vivo* antioxidants to protect LDL particles against oxidation in the arterial wall. OxLDL has been shown to be a strong predictor of acute coronary heart disease events. Interestingly in the present work, the consumption of dried breads with RPO was associated with a significant decrease in the serum levels of oxLDL as compared to the consumption of dried breads without RPO.

OxLDL plays a crucial role in the development of atherosclerosis via several acceleration initiations and functions, including induction attachment between endothelial and monocyte cells, recruitment of monocyte macrophages attached to the vascular wall, and the formation of foam cells from macrophages. OxLDL that circulates in the body is a marker for identifying the incidence of heart disease. A high oxLDL level is an independent predictor of the incidence of heart disease (Hiki *et al*., 2009). A significant decrease in the oxLDL levels in the subjects who consumed dried breads with RPO reported in the present study has also been reported by Rodella and Favero (2013) who stated that antioxidant compounds might prevent atherosclerosis at some point, thus preventing monocytes from entering the intima, oxLDL and ROS formation, transcription factors, and pro-inflammation by cytokines in macrophage foam cells.

The serum β-carotene levels of subjects in both treatment groups showed a non-significant increase. β-carotene, either alone or with other carotenoid compounds, may act as an antioxidant by fighting free radicals to suppress lipid peroxidation, thus resulting in decreased levels of MDA and oxLDL, and an increase in SOD. The antioxidant properties of β-carotene are involved in the molecular mechanisms responsible for disease prevention, particularly diseases that are fully initiated by oxidative stress at their developmental stage. β-carotene may modulate the free radical processes by acting as a chain-breaking antioxidant in lipid peroxidation (Mueller and Boehm, 2011). An antioxidant-rich diet helps decrease oxidative stress.

The consumption of dried breads with RPO by subjects improved their oxidative status via increased SOD activity and significantly decreased MDA and oxLDL levels; however, the increase in the serum β-carotene levels was insignificant. This might have been due to the fact that β-carotene, as a component with antioxidant activity, worked against free radicals to suppress lipid peroxidation, thus resulting in decreased MDA levels. In contrast, β-carotene as a free radical scavenger may help the SOD work as antioxidant.

The main weakness of the present work was its short duration due to concerns about the large dropout rate because the intervention given was in the form of food with a risk of boredom. Decrease in cholesterol and the effect of oxidative stress might sustain over the long term, especially when compliance is maintained. In addition, no adverse effects arising from consuming the breads indicated that their consumption could be safe for a relatively long period of time.

Conclusion

The present work demonstrated that the daily consumption of 60 g dried breads with RPO as a snack, at two times of consumption, resulted in total- and LDL-cholesterol-lowering effects in subjects as compared to the control group. Subjects did not report any adverse effects of dried breads with RPO consumption. The levels of oxidative stress as assessed by SOD, MDA, and adjusted serum levels of oxLDL were significant. Therefore, the consumption of dried breads with RPO may represent a useful preventive measure to people with dyslipidaemia and oxidative stress conditions which lead to cardiovascular disease risk.

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