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Potential Health Implication of *in Vitro* Human Low-Density Lipoprotein−Vitamin E Oxidation Modulation by Polyphenols Derived from Côte d'Ivoire's Oil Palm Species

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ABSTRACT: Antioxidant activities of polyphenolic compounds extracted (PPEs) from ripe fruits of oil palms are investigated by studying their *in vitro* effects on human low-density lipoprotein (LDL) oxidation. Four oil palm species (*Elaeis guineensis*) are issued from the National Centre of Agronomic Research of Côte d'Ivoire, of which two are parental varieties (HP1 and HP2), while the other two are crossing varieties (HP3 and HP4). The main identified compounds were rutin (HP3 and HP4) and caffeic and chlorogenic (5-caffeoyl quinic) acids (HP1, HP3, and HP4). The highest total phenolic content was found for HP4, while it was significantly lower for HP2. Antioxidative effects were monitored by Cu^{2+}- or 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH)-induced generation of conjugated dienes (lag time and oxidation rate). The highest PPE specific antioxidant activity (SAA) values were obtained with crossing varieties (HP3 and HP4) in the copper-oxidation assay. In the AAPH-oxidation assay, SAA values were comparable for all four varieties. PPEs were effective at preventing LDL−vitamin E depletion *in vitro*. They could exert direct beneficial antioxidant effects on vitamin E and other antioxidants contained in food and beverages *in vivo*, within the gastrointestinal (GI) tract. These data could also be of particular importance for a healthier nutrition or the management of chronic diseases by a polyphenol-rich diet.

KEYWORDS: LDL−Vitamin E, Cu^{2+}- and AAPH-mediated oxidations, dietary polyphenols, crude palm oil

INTRODUCTION

Numerous epidemiological and intervention studies suggested associations between consumption of polyphenol-rich foods or beverages and prevention of certain chronic diseases, such as cancers, cardiovascular diseases, and type-2 diabetes, or side effects of aging.[1] Particularly, a recent epidemiological study[2] revealed an increasing incidence of cardiovascular diseases in west Africa, related to a decreased consumption of such compounds, which is the drawback of modified dietary habits. Given the large number of potential mechanisms of polyphenolic action in preventing these degenerative diseases, there is a great interest in studying the role of human low-density lipoprotein (LDL)−vitamin E in the oxidation process. In African and Asian diets, the main source of fat is palm oil, which is characterized by its richness in antioxidants.[3] Among these, data regarding fatty acids, carotenoids, and vitamin E of palm oils are now available,[4,5] while few reports concerned palm oil polyphenols. However, some authors previously showed that palm oil mill effluents are a rich source of water-soluble biologically active polyphenolic compounds.[6]

In Côte d'Ivoire, many varieties of oil palms have been selected by the National Centre of Agronomic Research to improve oil quality, particularly by increasing the content of polyunsaturated fatty acids, tocopherols, tocotrienols, or β-carotene, and have been previously described.[5] Considering the wealth of bioactive components in these crude palm oils, we investigated the antioxidant potential effects. Previous studies[7−9] demonstrated that polyphenol effects on LDL oxidation may vary, depending upon the oxidant system used and the polyphenol structure, origin, or dose. The purpose of this study was therefore at first to identify the phenolic compounds present in crude palm oils of Côte d'Ivoire varieties and then to assess their antioxidative effects through their ability to protect LDL−vitamin E against *in vitro* oxidation. Should we show beneficial effects of biologically active secondary plant phenolic compounds on LDL−vitamin E protection, these data could also be used as fundamentals of food-based dietary guidelines for healthier nutrition or the management of chronic diseases by a polyphenol-rich diet. This study was also undertaken to gain further insight into a new biomarker that could be used to evaluate supposed protection against oxidative damage.

MATERIALS AND METHODS

Chemicals and Standards. All chemicals used were analytical-grade. The following reagents were purchased from Sigma Aldrich Chemical Co. (Saint Quentin Fallavier, France): chlorogenic acid, $CuCl_2$, butylated hydroxytoluene, and 2N Folin−Ciocalteu reagent. Gallic acid was obtained from Merck (Darmstadt, Germany). Methanol used for

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chromatography was high-performance liquid chromatography (HPLC)-grade supplied by Merck. Ethanol was from Prolabo (Paris, France) and was used for preparing standard solutions. Membrane filters with 0.45 μm pores from Millipore were used for filtration of the mobile phase and the samples. Chloroform was from Prolabo. 2,2′-Azobis(2-amidinopropane) hydrochloride (AAPH) was from Biovalley (Conches, France).

Sample Preparation and Quantization. Palm fruit extracts (PFEs) were prepared as previously described,[5] from ripe fruits of oil palm trees grown from a genetic improvement program through breeding. Briefly, four different varieties of oil palm were selected and analyzed, with two samples being from base collections and two others coming from crossing of the base collections. Both samples from the base collections "Lamé" (Côte d'Ivoire) and "Deli" (Malaysia, variety cultivated in Côte d'Ivoire) were named HP1 and HP2, respectively. Samples from the first and second cycles of crossing selection were named HP3 and HP4, respectively. PFE preparation involved the following steps: ripe fruits of oil palm trees were selected and washed, and the pulp was weighed up to 50 g. An electrical mixer was used to crush it, and 100 mL of an ethanol/water mixture (70:30, v/v) was added. This mixture was boiled under vacuum for 30 min. After cooling, the alcoholic mixture obtained was filtered and dried under vacuum with a rotary evaporator at 40 °C. Then, the concentrated residue was dissolved in 10 mL of an ethanol/water mixture (70:30, v/v), and the final extract was kept at 4 °C before analysis.

Polyphenolic aqueous extracts, named PPEs, were prepared to eliminate triacyglycerols, tocopherols, tocotrienols, and carotenoids contained in PFEs. In line with this consideration, 200 μL was treated with 800 μL of chloroform and 1 mL of HPLC water. After mild vortex shaking and centrifugation for 10 min at 1000*g*, the upper phase was separated and used for determination of total hydrophilic phenolic contents according to a modification of Folin−Ciocalteu's colorimetric method. Using this variant, products of interest were measured at 765 nm.[10] A gallic acid stock solution was prepared in ethanol and used as a standard. Results were expressed in millimoles of gallic acid equivalents per liter (mmol of GAE L^{-1}). We also verified that these PPEs do not contain a lipophilic component, neither tocopherol and tocotrienol nor carotenoid (data not shown).

Polyphenol Analysis by HPLC/DAD. PPE separation was carried out as follows: HPLC analysis was performed using a HPLC Agilent 1200 apparatus, with a Waters C18 analytical column (5 μm particle size, 250 × 4.6 mm, AIT/ACE), and was equipped with a diode array detector (DAD, G-1315, Agilent), a quaternary pump (G-1311A), and an eluent mixing chamber. A manual injector with a 20 μL loop was used, and chromatographic data processing software (model D-6500, Agilent) was used. We operated at room temperature. PPE samples, standard solutions, and mobile phases were filtered through membrane filters with 0.45 μm pores and degassed before their use. Extracts were separated using a gradient elution comprising two solutions: a mobile phase A (acetonitrile/0.8% water at pH 3.8, v/v) and a mobile phase B (acetonitrile). After the initial condition [elution mixture = 90:10 (v/v) A/B], a linear gradient was run up to 35% B until 45 min, then adjusted to 100% B for 3 min, and maintained for 2 min, at which point the initial condition was restored. The flow rate was maintained at 1 mL min^{-1}. Detection was routinely carried out by monitoring absorbance signals at 280, 306, and 360 nm. Gallic and chlorogenic acid and rutin stock solutions were prepared as standards. HPLC analyses showed five main peaks and many minor undefined peaks. Concentrations were calculated from the chromatogram peak areas. Identification was achieved by a comparison of both retention times and absorption spectra obtained for each eluted peak to those obtained for the standards.

LDL Isolation and Oxidation Studies. LDL was isolated from fresh human plasma, obtained from the "French Blood Establishment", in accordance with the ethical rules of that establishment, and oxidizability was monitored at 234 and 245 nm for 5 μmol L^{-1} Cu^{2+} and 5 mmol L^{-1} AAPH oxidation, respectively, as previously indicated.[7−9,11] These absorbances are related to conjugated dienes (CDs) produced during oxidation. Isolated LDL was diluted to 1 μmol of apoB L^{-1}, added with the various PPE concentrations to be tested, and then 10-fold diluted in oxygenated phosphate-buffered saline (PBS) at pH 7.4. For the rendering of the antioxidant abilities of the PPE, we used the notion of specific antioxidant activity (SAA) as previously described.[7] Briefly, lag time (Tlag) was defined as the time corresponding to the end of the first kinetic phase during which CDs do not or only slowly increase. SAA was calculated as the slope of the linear relationship obtained between relative lag times and concentrations of tested antioxidants. Relative Tlag was defined as $[Tlag^{+}/Tlag^{-}] \times 100$, with + and − denoting LDL with and without PPE. Finaly, CD_{max} was the accumulation of oxidized products corresponding to the plateau of the kinetic curve. The PPE influence on the time course of *in vitro* LDL α-tocopherol consumption was determined after vitamin E extraction by hexane/ethyl acetate (3:1, v/v) from oxidized LDL. To do this, we measured the extracted products by means of a spectrophotometric detector after HPLC separation, with δ-tocopherol as an internal standard. A Lichrocart 125-4 (5 μm particle size) column (Merck, France) was employed for separation using a mobile phase consisting of a water/methanol mixture (3:97, v/v) at a 0.8 mL min^{-1} flow rate for 12 min.

Statistical Analysis. Analyses were carried out 3 times or more, and results were reported as mean values ± standard deviation (SD). Statistical significance was assayed using one-way analysis of variance, following by Student, Mann−Whitney, or Kruskal−Wallis tests, according to different results, with the Stata Software V10.0 (StataCorp, 2007 edition). Correlation analysis significances were also studied by Fischer test. The level of significance was set at $p < 0.05$.

RESULTS

Total Phenolic Contents and Phenolic Compounds Identified by HPLC. The highest total phenolic content was found for HP4 (1.23 ± 0.05 mmol of GAE L^{-1}) without significant difference with HP1 (1.11 ± 0.05) and HP3 (1.13 ± 0.02), while HP2 (0.5 ± 0.02) was significantly lower ($p < 0.05$). The major phenolic components found in PPE of these four varieties were mainly hydrophilic compounds and were identified on the one hand as caffeic and chlorogenic acids, which were predominant in HP1 (23.5 and 19.7%, respectively), and on the other hand as rutin and a quercetine derivative (not specifically identified), which prevailed in HP2 (54.9 and 31.4%, respectively) (Figure 1). Results were expressed as weight percentages of identified compounds. It must be noted that the two crossing varieties HP3 and HP4 have a similar polyphenolic pattern to the parental HP1 variety.

Effects of PPE on Cu^{2+}- and AAPH-Mediated LDL Oxidation. SAAs of PPE were evaluated under the Cu^{2+}-mediated oxidation system, and results were expressed as μmol of GAE L^{-1} (Table 1). SAAs were higher for the two crossing varieties HP3 and HP4 (without significant difference between them; 69.4 and 69.6, respectively) than those of parental varieties HP1 and HP2 (52.7 and 51.1, respectively). However, when SAA was evaluated using a AAPH-mediated oxidation assay (Table 1), the level for the HP4 variety was found to be slightly lower (26.6) compared to the three other varieties (29.9, 31.2, and 33.6 μmol of GAE L^{-1} for HP1, HP2, and HP3, respectively) but without significant difference. SAA values for the main purified phenolic acids identified in these palm oil varieties (caffeic and chlorogenic acids) and rutin in Cu^{2+}- and AAPH-mediated oxidation are also evaluated and shown in Table 1. When using the Cu^{2+}-mediated oxidation assay, rutin and chlorogenic acid, the most hydrophilic

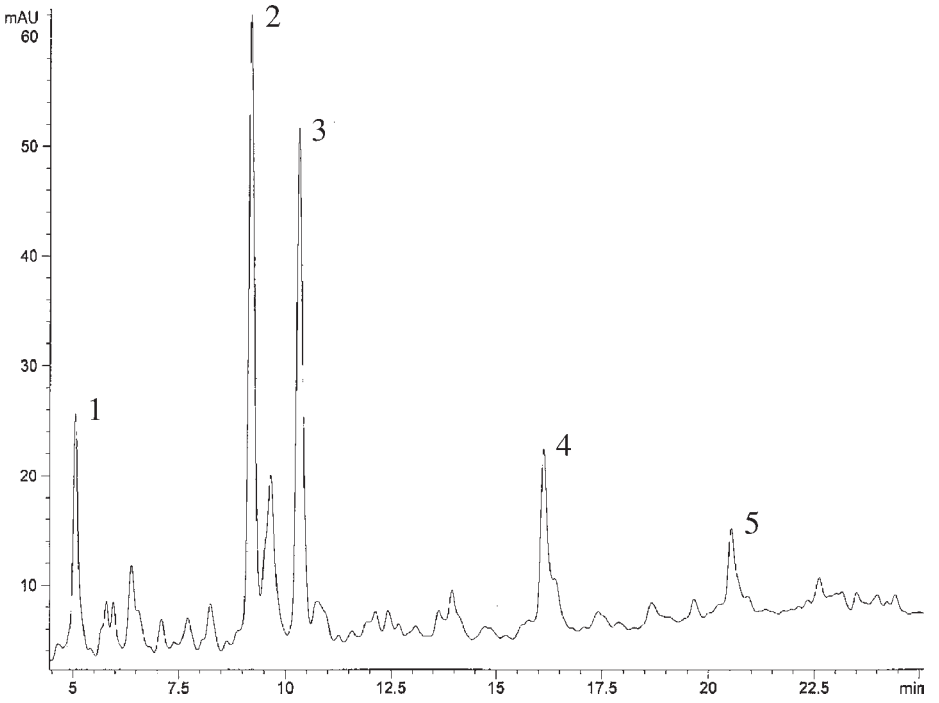

Figure 1. PPE representative HPLC/DAD chromatogram of the HP1 variety given as an example of the four varieties. Peaks were detected at 360 nm and identified as follows: 1, coumaric acid and derivative [retention time (RT) = 5 min]; 2, caffeic acid (RT = 9.2 min); 3, 5-caffeoyl quinic acid (or chlorogenic acid) (RT = 10.3 min); 4, rutin (RT = 16.1 min); and 5, quercetin derivative (RT = 20.5 min).

Table 1. Standing Comparison of SAAs of PPEs (HP1, HP2, HP3, and HP4) and Some Purified Phenolic Compounds, under 5 μmol L^{-1} Cu^{2+}- or 5 mmol L^{-1} AAPH-Oxidation Assays[a]

	Cu^{2+}		AAPH	
	SAA	*n*; *r*	SAA	*n*; *r*
HP1 (μmol of GAE L^{-1})	52.7 a	$n = 20$; $r = 0.99$	29.9 c	$n = 12$; $r = 0.93$
HP2 (μmol of GAE L^{-1})	51.1 a	$n = 20$; $r = 0.97$	31.2 c	$n = 12$; $r = 0.99$
HP3 (μmol of GAE L^{-1})	69.4 b	$n = 20$; $r = 0.97$	33.6 c	$n = 12$; $r = 0.98$
HP4 (μmol of GAE L^{-1})	69.6 b	$n = 20$; $r = 0.99$	26.6 c	$n = 12$; $r = 0.99$
caffeic acid (μmol L^{-1})[b]	54.0	$n = 24$; $r = 0.99$	73.6	$n = 8$; $r = 0.99$
chlorogenic acid (μmol L^{-1})[b]	155.5	$n = 8$; $r = 0.99$	55.3	$n = 12$; $r = 0.98$
rutin (μmol L^{-1})	187.8	$n = 10$; $r = 0.94$	82.6	$n = 10$; $r = 1$

[a] For PPE SAA, significant differences are shown by different letters. *n* denotes the number of independent experiments, and *r* denotes the regression coefficient of the linear relationship between Tlag and phenolic concentrations. [b] Previously shown by Cartron et al.[6]

among evaluated molecules, had stronger SAAs (187.8 and 155.5 μmol L^{-1}, respectively) than caffeic acid (54 μmol L^{-1}). Moreover, rutin and chlorogenic acid had stronger SAA values than the four PPE mixtures, even though caffeic acid SAA was similar to those of PPE mixtures. When using the AAPH-mediated oxidation assay, purified compounds exhibited comparable activities, whose values were stronger than those of the four PPE mixtures.

We noticed that PPE prolonged Tlag with a dose-dependent effect in both oxidation assays. Moreover, under Cu^{2+}-mediated oxidation, during the propagation phase of CD production, the oxidation rate R_p(CD) decreased significantly ($p < 0.01$) when Tlag lenghtening was obtained by increasing phenolic compound concentrations. We established linear correlations by plotting the logarithm of $R_p(CD)^+/R_p(CD)^-$ versus the logarithm of $Tlag^+/Tlag^-$ for the four varieties (data not shown). We obtained different results using AAPH-mediated oxidation, because the four varieties did not induce a significant decrease for the oxidative rate during the propagation phase.

The HP1 PPE influence on the time course of LDL−vitamin E consumption under Cu^{2+}-mediated oxidation is shown in Figure 2 in parallel with CD production. Comparable results were obtained for HP2, HP3, and HP4 and were not shown. We found that vitamin E consumption as well as CD production were delayed in a dose-dependent manner by the four PPE varieties. The very beginning of the propagation phase, corresponding to Tlag, coincided with the time of total consumption of vitamin E. Moreover, for all four varieties, the vitamin E degradation rate was twice as low when the phenolic concentration was twice as high, pointing out a direct protection of vitamin E by phenolic compounds.

Concerning AAPH-mediated oxidation and under our experimental conditions, the radical generation rate (R_g) was constant and taken to be $R_g = 0.39$ μmol L^{-1} min^{-1} according to Bowry et al.[12] A time-dependent absorbance increase could be characterized by a "three-phase kinetic profile", as shown in Figure 3, for HP3 PPE taken as an example. During the first part of this kinetics (initiation phase), accumulation of oxidation products [CD initiation rate = R_i(CD)] was very slow, with practically the same magnitude as R_g, for LDL oxidized with or without phenolic

compounds (Table 2). This contrasted with copper-induced peroxidation, where no peroxidation product was formed until Tlag (for this comment, see Figure 2). Moreover, for LDL without PPE, during this first phase, vitamin E is progressively consumed with a rate R_i(vitE) = 0.44 μmol L^{-1} min^{-1}, practically equal to R_g. During the second phase of this kinetics (propagation phase), auto-acceleration occurs with an increased and constant peroxidation rate named R_p(CD). This propagation phase could begin before complete degradation of vitamin E, unlike under copper-induced peroxidation. After the third phase revealed by an absorbance plateau level corresponding to CD_{max} production, hydroperoxides obtained from all unsaturated lipids decomposed, forming dienals and other aldehydes, while cholesterol became completely oxidized. This final phase was not explored here. As shown in Table 2, on one hand, 10 μmol of GAE L^{-1} PPE for HP1, HP3, and HP4 varieties was able to reduce the consumption rate of vitamin E, as noted by decreasing R_i(vitE) values compared to that of LDL without PPE. On the other hand, PPEs were without significant effect on both R_i(CD) and R_p(CD). It must also be noticed that both the crossing varieties HP3 and HP4 exhibited a more efficient protective effect against vitamin E consumption (R_i(vitE) = 0.07 and 0.08 μmol L^{-1} min^{-1}, respectively) than the parental variety HP1 (R_i(vitE) = 0.14 μmol L^{-1} min^{-1}). Concerning the protective effect of 10 μmol of GAE L^{-1} PPE for the HP2 variety, the initiation phase could be divided into two parts corresponding to first a very low rate of vitamin E degradation (R_{i-1}(vitE) = 0.03 μmol L^{-1} min^{-1}) and second a faster rate of vitamin E degradation (R_{i-2}(vitE) = 0.13 μmol L^{-1} min^{-1}), comparable to the rate obtained for the HP1 variety (Table 2).

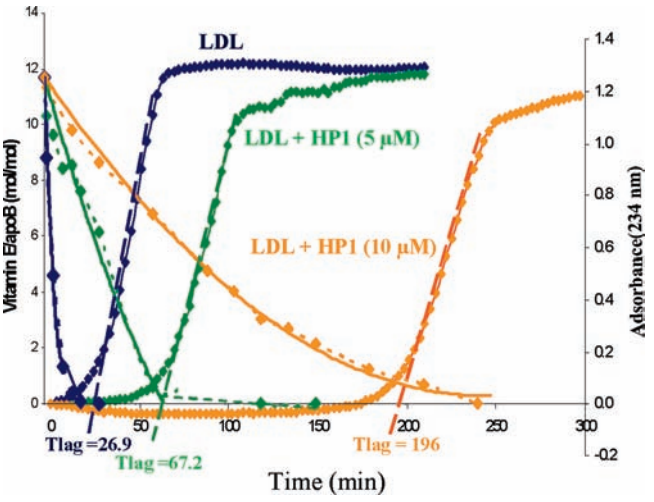

Figure 2. *In vitro* Cu^{2+} degradation of LDL−vitamin E, in parallel with CD production, with or without HP1 PPE. LDL oxidation was induced by 5 μmol L^{-1} copper ions at 37 °C. Vitamin E was analyzed by a HPLC method (left *y* axis), and CD production (absorbance) was detected at 234 nm (right *y* axis). PPE from the HP1 variety [5 and 10 μmol of GAE L^{-1} (5 and 10 μM)] was added to LDL before oxidation, in comparison to LDL alone. HP1 was chosen as an example of the four varieties.

DISCUSSION

In this study, we investigated PPEs from ripe fruits of four oil palm varieties and identified their phenolic compounds by HPLC/DAD. We also evaluated their antioxidative effects on *in vitro* LDL oxidation by two catalysts, namely, copper and AAPH. Total phenolic contents were higher in the crossing varieties HP3 and HP4 and in the basal collection variety HP1 than in the other basal one HP2. Phenolic compound profiles recovered in PPE were similar to those reported by Tan et al.[13] in the palm oil mill effluents and were constituted by phenolic acids, mainly caffeic and chlorogenic acids, and also flavonoids, mainly rutin. One peculiarity of this study was the presence of substantial levels of phenolic compounds in PFE, whereas most other studies demonstrated that these compounds were predominantly found in the wastewater mill effluents and, consequently, were discarded during the milling process.[6,13] Moreover, PPE profiles appeared

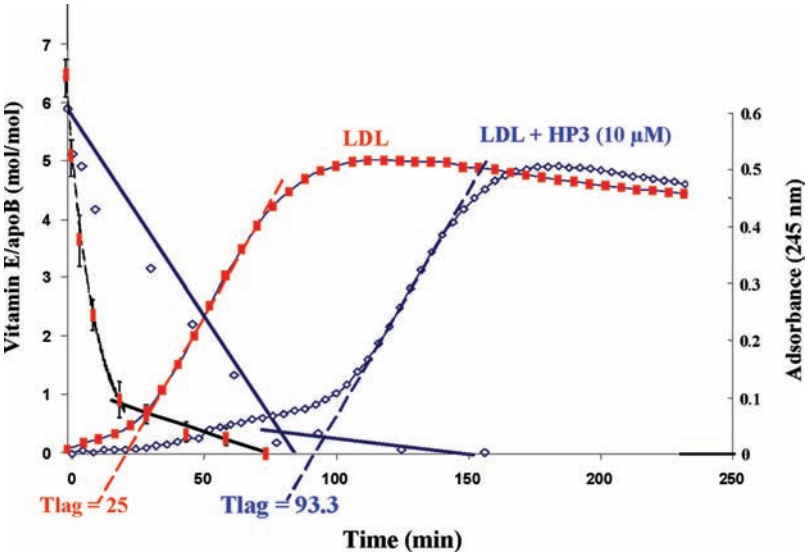

Figure 3. *In vitro* AAPH degradation of LDL−vitamin E, in parallel with CD production, with or without HP3 PPE. LDL oxidation was induced by 5 mmol L^{-1} water-soluble radical initiator AAPH at 37 °C (R_g = 0.39 μmol L^{-1} min^{-1}). Vitamin E was analyzed by a HPLC method (left *y* axis), and CD production (absorbance) was detected at 245 nm (right *y* axis). PPE from HP3 variety [10 μmol of GAE L^{-1} (10 μM)] was added to LDL before oxidation, in comparison to LDL alone. The HP3 variety was chosen because of its greatest protective effects on vitamin E degradation, as shown in Table 1.

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Table 2. Effects of PPEs (HP1, HP2, HP3, and HP4) on LDL AAPH-Mediated Peroxidation[a]

	Vitamin E degradation	Conjugated dienes production	
	R_i(Vit E) (μmol L^{-1} min^{-1})	R_i(CD) (μmol L^{-1} min^{-1})	R_p(CD) (μmol L^{-1} min^{-1})
LDL without PPE; n = 5	0.44 a	0.54	2.6
LDL with HP1 (10 μmol−GAE.L^{-1}); n = 2	0.14 b	0.24	2.6
LDL with HP2 (10 μmol−GAE.L^{-1}); n = 2	$R_{i\text{-}1}$(Vit E) = 0.03 c $R_{i\text{-}2}$(Vit E) = 0.13 b	0.38	2.3
LDL with HP3 (10 μmol−GAE.L^{-1}); n = 3	0.07 c	0.41	2.6
LDL with HP4 (10 μmol−GAE.L^{-1}); n = 3	0.08 c	0.47	3.0

[a] The radical generation rate is taken to be $R_g = 1.3 \times 10^{-6}$ [AAPH] s^{-1} = 0.39 μmol L^{-1} min^{-1}.[20] n denotes the number of independent experiments. Significant differences are shown by different letters.

to be widely different from those of olive oil, where oleuropein and derivatives were predominant.[14,15]

The second part of our study aimed to investigate the polyphenol antioxidant activity through inhibition of *in vitro* LDL oxidation, which is a very usual assay. Results evaluated as SAA showed that PPE from the two crossing varieties HP3 and HP4 had a higher efficiency in inhibiting copper-mediated oxidation than the parental varieties HP1 and HP2. However, in AAPH-mediated oxidation, all four varieties had similar protective effects. SAAs of polyphenol extracts were 2-fold higher in the copper assay than in the AAPH assay. Moreover, under copper-induced oxidation, PPE first prolonged the Tlag and delayed the vitamin E consumption in a dose-dependent manner and second significantly decreased the maximal rate of oxidation R_p(CD), whereas the formation CD_{max} remained constant. Lipid peroxidation started only when LDL−vitamin E was completely depleted, as previously shown for red wine polyphenols or caffeic acid.[7] In our study, the highest antioxidant PPE activities were from HP3 and HP4, followed by HP1 and HP2. Differences in structural features of PPE can be considered to explain this result.[16] The polyphenolic Cu^{2+} antioxidant activity is mainly defined by the presence of the *ortho*-dihydroxy substituents, giving maximum radical stabilization or allowing for metal chelation, as found in caffeic acid, quercetin, or their derivatives.[17] On the other hand, rutin, one of the most common flavonolic glycosides in the human diet, is able to chelate iron ions, to scavenge reactive oxygen species,[18] and to inhibit cellular enzymes.[19] It is worthy to notice that rutin was predominantly found in PPE, especially higher in the HP2 variety.

Unlike copper-induced peroxidation, in the AAPH-induced oxidation assay, PPE may induce increasing Tlag without significantly affecting either R_p(CD) or CD_{max}. The peroxyl radical (ROO•) formed by thermal decomposition of AAPH is responsible for the hydrogen abstraction from peroxidizable lipid species[16,17] and/or vitamin E.[12] The protecting effect of PPE may act by mechanisms that involve quenching and stabilization of free radicals, delaying the vitamin E consumption and reducing the CD production in the initiation phase. The most efficient antioxidant PPEs in our study were HP3 and HP4 varieties, which were able to reduce vitamin E consumption [R_i(vitE) = 0.07 and 0.08 μmol L^{-1} min^{-1}, respectively) more significantly than the parental HP1 variety [R_i(vitE) = 0.14 μmol L^{-1} min^{-1}]. Therefore, it may be supposed that cinnamic acid derivatives, found more predominantly in HP1, HP3, and HP4 varieties than in HP2, react with the peroxyl radical generated by AAPH, allowing for impairment of vitamin E oxidation. This effect could be afforded by the capacity of an α,β-unsaturated carboxyl moiety, with an aromatic hydroxyl group in the *para* position, to promote electron delocalization.[17] Surprisingly, concerning the parental HP2 variety, we observed a biphasic effect during the initiation phase, showing at first a very slow rate of vitamin E consumption [$R_{i\text{-}1}$(vitE) = 0.03 μmol L^{-1} min^{-1}] and then a faster rate of vitamin E consumption [$R_{i\text{-}2}$(vitE) = 0.13 μmol L^{-1} min^{-1}] comparable to that observed for the HP1 variety. It must be noted that this variety contains more quercetine derivatives (86.3%) than the other varieties.

Our data demonstrate that PPEs exhibit LDL−vitamin E antioxidant activities against both inducers used. Therefore, PPEs probably act through several mechanisms, first by scavenging free radicals at the LDL surface or reducing the oxidized vitamin E, yielding confirmation of our previous results[7] obtained with other hydrophilic polyphenolic compounds, and second by reducing the hydroperoxide transfer rate between lipoprotein particles.[16,17] This study obtained insight into a new biomarker, the LDL−vitamin E oxidation, that could be used to evaluate protection against oxidative damage.

This study added to our previous results[5] showed that vitamin E and polyphenols may be very significant bioactive compounds. Moreover, it is now well-known that a flavonoid-rich diet (onion and black tea) led to a significant increase of the plasma quercetine concentration.[20] With phenolic-acid-rich beverages (green coffee), chlorogenic acid derivatives were also significantly absorbed,[21] confirming their bioavailability, although they appeared to be rapidly metabolized in humans.[22] However, these phenolic compounds, such as PPE, could still exhibit some valuable effects on the long-term prevention of chronic diseases by their presence in the organism, even though at low but constant doses provided by a regular dietary intake.[23] Particularly, PPEs, which were able to delay the *in vitro* LDL−vitamin E oxidation, could exert *in vivo* direct beneficial antioxidant effects within the gastrointestinal (GI) tract on vitamin E and other antioxidants of food and beverages, because of their high GI concentrations.[24]

The data described here contribute to a better understanding of PPE effects. It could be anticipated that the intake of polyphenol-rich food, such as tea, wine, fruits, or vegetable oils, like crude palm oil, could lead to a decrease in the incidence of chronic diseases, particularly cardiovascular diseases. Further studies will be focused on the chemical properties and biological activities of PPE and their effect pattern on experimental animal models and humans. The potential benefits of crude palm oil for nutritional and health beneficial effects for economic benefits in west Africa must be more closely recognized. Finally, these results shoud be considered in future plam species breeding efforts.

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ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; DAD, diode array detector; GAE, gallic acid equivalent; HPLC, high-performance liquid chromatography; Ox-LDL, oxidized low-density lipoprotein; PFE, palm fruit extract; PPE, polyphenolic extract of PFE; SAA, specific antioxidant activity; CD, conjugated diene; Tlag, lag time

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