Potential Health Implication of in Vitro Human LDL-Vitamin E Oxidation Modulation by Polyphenols Extracted from Côte d'Ivoire's Red Sorghum

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Abstract

Previous studies have shown that sorghum phenolic compound levels would be significantly greater than those of rice and corn, with a variety of genetically dependent types and levels, including phenolic acids, flavonoids and condensed tannins in some varieties. Firstly, in this study, 3-deoxyanthocyanidins and other phenolic compound extracted from red sorghum were characterized by HPLC technique with spectro-photometric detection. Secondly, we tested whether red sorghum phenolic compound had the capacity to protect LDL (Low Density Lipoprotein) against in vitro oxidation. LDL, with or without red sorghum phenolic compound, was oxidized by 5 µM-Cu2+ and 5 mM-AAPH systems Moreover, the antioxidant capacity of red sorghum was analyzed in comparison to purified 3-deoxyanthocyanidins (i.e. luteolinidin and apigeninidin). A synergic effect was evaluated. We also demonstrated that red sorghum phenolic compound was effective at preventing LDL-vitamin E depletion in vitro. We hypothesized that red sorghum phenolic compounds could exert direct beneficial antioxidant effects on vitamin E and other antioxidants contained in food and beverages in vivo, within the gastrointestinal tract. These data could also be of particular importance for a healthier nutrition or for the management of chronic diseases by a polyphenol-rich diet.

Keywords— *Cu2+- and AAPH-mediated oxidations; dietary polyphenols; LDL-vitamin E; red sorghum.*

ABBREVIATIONS USED

AAPH: 2-2' azobis (2-amidinopropane) hydrochloride; **CD**: conjugated-dienes; **DAD**: diode array detector; **EWH+:** ethanol/acidified water extract; **EWH+-PC:** phenolic compounds of EWH+; **GAE:** Gallic acid equivalents; **HPLC:** high performance liquid chromatography; **Ox-LDL:** oxidized low density lipoprotein; **PCs:** phenolic compounds; **SAA:** specific antioxidant activity; **Tlag:** lag time.

I. INTRODUCTION

The purpose of this study is to promote the consumption of sorghum in West Africa, a grain that grows in harsh climatic conditions in regions with low rainfall. Compared to rice and corn, sorghum is cheaper and thus reachable for rich and poor people (FAO). Moreover, ecologically its production needs less water. Reasoned production of sorghum would allow diversification of cereal-based foods in those regions of West Africa and reduce money allocated for imports of wheat, corn and rice. Several reports have shown that sorghum [1] is inexpensive and nutritionally comparable or even superior to major cereals. Grain sorghum is also important food cereal in many parts of Africa, Asia and the semi-arid tropics worldwide. In Africa, India and China, grain sorghum comes third among cereals for human consumption, superseded only by rice and wheat [2]. Recent studies [3,4] have shown that cereal grains contain constituents that have demonstrated health benefits for humans, such as antioxidants and anti-disease factors. Other studies have also demonstrated that diets high in carbohydrate, rich in dietary fiber, and largely of cereal origin, allowed withdrawal of oral hypoglycemic agents or a reduction of insulin dose in diabetic subjects [5].

Sorghum contains various phytochemicals, phenolic compounds [6], flavonols [7] and thiols [8]. Further, the sorghum grain cuticle is reported to contain flavonoids, anthocyanins [9]. These phytochemicals have gained increased interest due to their antioxidant activity, cholesterol-lowering and vitamin E protection in the oxidation process. Yet many study in the United States of America and Europe shows the antioxidant potential of these grains. However, the consumption of this cereal is not very popularized by our competent authorities in Côte d'Ivoire.

Previous studies [10,11] demonstrated that polyphenol effects on LDL oxidation may vary, depending on the oxidant system used and on the polyphenol structure, origin or dose. The purpose of

this study was therefore firstly to identify the phenolic compounds (PCs) present in a red sorghum variety commonly used in Côte D'Ivoire 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 PCs on LDL-vitamin E protection, these data could also be used as fundamentals of food-based dietary guidelines for a healthier nutrition or for 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.

II. MATERIAL AND METHODS

A. 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, CuCl2, butylated hydroxytoluene and 2N-Folin Ciocalteu reagent. Gallic acid was obtained from Merck (Darmstadt, Germany). Methanol used for 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. AAPH was from Biovalley (Conches, France).

B. Sample preparation and quantization

Sorghum grains were powdered in a 'Disk Mill' (Glen Mills Inc., Cliffton, NJ, USA) and the whole flour was employed in this study. Extraction procedure involved the addition of 50 mL- ethanol and 50 mL-water acidified by acetic acid (pH 2.6) to 10g of whole flour to obtain an ethanol/water/H+ extract (EWH+). Sample was shacked during three hours at room temperature and centrifuged at 3000 g for 10 min. Supernatant was then concentrated on a Rotavapor at 40°C to a final volume of 25 mL. This EWH+ extract was used for HPLC and spectral characteristic analyses.

Then, 200 µL-EWH+ extract was added to 800 µL-chloroform and 1 mL of HPLC-water. Sample was then mild vortexed and centrifuged at 1000 g for 10 min to obtain 11 mL of the polyphenol aqueous extract named EWH+-PC. This stage of extraction is used to eliminate tocopherols, tocotrienols, carotenoids and other no polar compounds contained in EWH+. 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 [12], products of interest were measured at 765 nm. A Gallic acid stock solution was prepared in ethanol and used as a standard. Results were expressed in mmol of Gallic acid equivalents per liter (mmol-GAE.L-1).

C. Polyphenol analysis by HPLC/DAD

EWH+-PC separation was carried out as follows: HPLC analysis was performed using HPLC-LCQ Advantage from Thermofinnigan (USA) equipped with an electrospray source and an ion trap analyzer with a mass range up to m /z = 2000. The source voltage is 4.5 kV (positive mode) and 4 kV (negative mode). The capillary voltage was respectively 23.5 V and - 45 V for both positive and negative modes, and temperature of 250 ° C. The nitrogen flow is 50 mL /min and auxiliary nitrogen flow of 10mL/min. The separation achieved by a chain Alliance equipped with a separation module 2690 (including the pumping system, autosampler, column oven) and a diode array detector (DAD) 996. The computerized control of the chain is ensured by the Millennium software that handles the automation of injections and recordings UV-visible spectra (from 250 nm to 600 nm with a spectral resolution of 4.8 nm and chromatographic resolution of 1 spectrum/s). Separation of compounds is obtained on a reversed phase column Lichrospher 100 RP-18 (250 X 2 mm / 5µm) protected by a precolumn of the same phase. The oven is thermostated at 30°C for all tests. The injected volume is 5µL. The mobile phase consists of solvent A: water/formic acid (95:5, v / v) and solvent B: water/ formic acid/acetonitrile (15:5:80, v/v/v). The flow rate was at 0.250 mL/min. HPLC analyses showed four main peaks and many minor undefined peaks. Concentrations were calculated from the chromatogram peak areas. Identification was achieved by comparison of both retention times and absorption spectra obtained for each eluted peak with those obtained for the standards.

D. DL 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 nm and 245 nm for 5 µmol. L-1-Cu2+- and 5 mmol. L-1-AAPH-oxidation, respectively, as previously indicated [10,11]. Isolated LDL was diluted to 1 µmol-apoB.L-1, added with the various EWH+-PC concentrations to be tested and then 10-fold diluted in oxygenated PBS (phosphate-saline buffer), Ph. 7.4. For the rendering of the antioxidant abilities of the EWH+-PC, we used the notion of specific antioxidant activity (SAA) as previously described [10,13]. EWH+-PC influence on the time course of in vitro LDL tocopherol consumption was determined after vitamin E extraction by hexane/ethyl acetate (3/1; v/v) from oxidized LDL. In order to do this, we measured the extracted products by means of a HPLC method with a Lichrocart 125-4

(5 µm-particle size) column (Merck, France), with α-tocopherol as an internal standard [13].

E. Statistical analysis

Analyses were carried out in several independent times (n) which were specified within tables. Results were reported as mean values ± SD. Data were compared on the basis of the mean values. Spearman's correlation was used for some studies. For all comparison, significance was set at P< 0.05. Statistical analyses were performed using the Stata Software V10.0 (Stata Corp, 2007 edition).

III.RESULTS AND DISCUSSION

A. Total Phenolic Contents and Phenolic Compounds Identified by HPLC

Total phenolic content of our Côte d'Ivoire's red sorghum variety, evaluated in EWH+-PC extract as 31.0 ± 0.1 mmol-GAE.L-1 was also reported to whole flour as 0.39 ± 0.04 mmol-GAE/g on a dry matter basis. This value was higher than that previously reported by Ragaee et al. [14] or by Awika et al. [15] due to the difference in our extraction procedure using an ethanol/water acetic acid pH 2.6 (50/50; v/v) mixture and that used by these authors, 80% aqueous methanol or 70% aqueous acetone; respectively.

The most important anthocyanin compounds analyzed at 480 nm were the luteolinidin and its derivatives as shown in Figure 1, which give a cumulative total anthocyanin weight percentage of 64.1% while the apigeninidin and derivatives represents only 22 %. Despite this importance, 3-deoxyanthocyanins are only 5% of the total identified compounds in red sorghum. Results are shown in Table 1 only for anthocyanin derivatives. It should be remembered that sorghum does not contain only anthocyanins. It also contains phenolic acids such as ferulic, sinapic, para-coumaric acids, and tannins [15]. But our present study focused only on specific sorghum anthocyanins.

The antioxidative phytochemicals in grains, vegetables and fruit have received increased attention recently for their potential role in prevention of human diseases as well as in food quality improvement [16,17]. Phenolics are considered as a major group of compounds that contribute to the antioxidant activities of grains, particularly in sorghum and barley [18]. Despite this, sorghum grain and its products have not been explored for their phytochemical attributes. In this study, we investigated EWH+-PC from red sorghum and identified their PCs by HPLC/DAD. Its profile was similar to those reported by Hahn et al [7] and was constituted by phenolic acids, mainly ferulic and para-coumaric acids and also by flavonoids, mainly luteolinidin and apigeninidin and their methoxylated or glycosilated derivatives.

Table 1: Phenolic Compounds of Red Sorghum EWH$^+$-PC at 480 nm

TR (min)	Phenolic compounds	Conc. (mg.L^{-1})	Conc. (%)
44.4	luteolinidin	4.8	29
46.9	apigeninidin	1.6	9.9
48.5	5-methoxyluteolinidin	3.2	19.2
51.2	5-methoxyapigeninidin	1.0	5.9
61.3	7-methoxyluteolinidin	1.1	6.6
63.3	7-methoxyapigeninidin	1.0	6.2
65.1	5,7-dimethoxyluteolinidin	1.5	9.3
67.5	derivative anthocyanidin*	1.5	9.0
70.2	derivative anthocyanidin*	0.8	4.8

TR: retention time; * not actually determined.
Conc.: concentration

B. Effects Of EWH$^+$ - PC And The 3-Deoxyanthocyanidin from Sorghum On Cu2$^+$- and AAPH-Mediated LDL Oxidation

Specific antioxidant activities (SAAs) of EWH+-PC were evaluated under the Cu2+- and AAPH-mediated oxidation systems and results were expressed as µmol-GAE-1.L (Table 2). SAA of EWH+-PC (163.0) was higher under Cu2+-, than under AAPH-mediated oxidation (46.5). SAA values for the main purified anthocyanidins identified in this red sorghum variety, the 3-deoxyanthocyanidins (Luteolinidin and Apigeninidin) in Cu2+- and AAPH-mediated oxidation were also evaluated and shown in Table 2. When using the Cu2+- and AAPH-mediated oxidation assay, the mixture of luteolinidin and apigeninidin at equal concentration (3 + 3 µmol-GAE.L-1) exhibited comparable SAA to that of EWH+-PC (148,8; 39.4) and (163.0; 46.5) respectively.

Table 2. A Standing Comparison of Specific Antioxidant Activities (SAA) of Red Sorghum, luteolinidin, Apigeninidin, and the Two-Compound Mixture, Under 5 µmol.L^{-1} Cu^{2+}- or 5 mmol.L^{-1} AAPH-Oxidation Assays. SAAs were Expressed as µmol-GAE.L^{-1}.

Designation	Cu^{2+}		AAPH	
	SAA	n; r	SAA	n; r
Red sorghum	163	n = 13; r = 0.95**	46.5	n = 10; r = 0.97**
Luteolinidin	67.0	n = 12; r = 0.96**	19.3	n = 10; r = 0.95**
Apigeninidin	19.7	n = 12; r = 0.67*	-2.7	n = 10; r = 0.64*
Luteolinidin+ Apigeninidin	148.8	n = 12; r = 0.99**	39.4	n = 10; r = 0.91**

* P<0.05; ** P<0.01

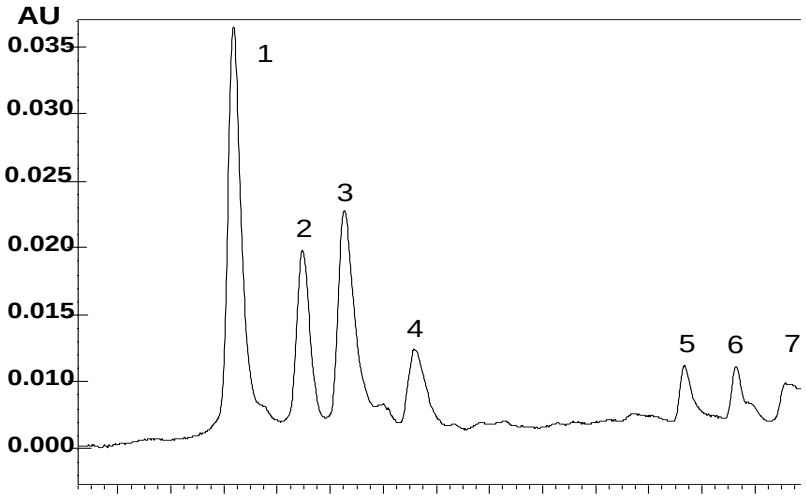

Figure 1: HPLC profile of red sorghum EWH+-PC detected at 480 nm.
Peaks were identified on the basis of matching retention times and spectral characteristics with standard, as follows: 1) Luteolinidin; 2) Apigeninidin; 3) 5-methoxyluteolinidin; 4) 5-methoxy-apigeninidin; 5) 7-methoxy-luteolinidin; 6) 7-methoxy-apigeninidin; 7) 5,7-methoxy-luteolinidin; 8 and 9) non identified derivative anthocyanins.

Moreover, luteolinidin and apigeninidin had individually lower SAA values than the EWH+-PC under the Cu2+-mediated oxidation. Surprisingly, apigeninidin even has a prooxidant activity under AAPH-mediated oxidation as shown by a negative SAA value **(Table 2).**

Results concerning SAA values allowed us to say that there is a complementarity between these two specific compounds (luteolinidin and apigeninidin) and, may be, other PC of the red sorghum whole flour, showing that PC mixture had a broader biological activity compared with individual phenolics [19]. Finally, SAA of EWH+-PC was about threefold higher in the copper- than in the AAPH-assays, as previously shown for PC obtained from other vegetable sources [11,13]. Moreover, anthocyanins with a 3-hydroxyl group, common in foods, are highly conjugated with sugars; but 3-deoxyanthocyanidins can occur naturally in the 3-aglycone or in 5- or 7-glycosylated forms. However, Awika et al. have previously shown that antioxidant properties of the 3-deoxyanthocyanidins were similar to those of the corresponding anthocyanins [19].

We noticed that EWH+-PC prolonged Tlag (time corresponding to the end of the first kinetic phase during which the conjugated-dienes do not or only slowly increase) with a dose-dependent effect in both oxidation assays. Moreover, under Cu2+-mediated oxidation, during the propagation phase of conjugated-diene production, oxidation rate named Rp(CD) decreased when TLag lenghtening was obtained by increasing EWH+-PC concentrations (**Table 3**). We established a significant linear correlation by plotting the logarithm of Rp(CD)+/Rp(CD)- versus the logarithm of Tlag+/Tlag- ($y = -3.4 x + 0.07$; $R2 = 0.93$, $P<0.01$); (+) and (-) denoting LDL with and without EWH+-PC.

Table 3. Effects of EWH$^+$-PC of Red Sorghum and Luteolinidin, Apigeninidin and the Mixture of Luteolinidin and Apigeninidin on LDL Cu^{2+}-Mediated Peroxidation

Compounds	**Vitamin E degradation Ri (Vit E)** (µmol.L^{-1} × min^{-1})	**Conjugated diene production R_p (CD)** (µmol.L^{-1} × min^{-1})
LDL without EWH$^+$-PC; n=5	0.46±0.02	8.3±0.8
LDL with EWH$^+$-PC (2 µmol-GAE.L^{-1}); n=2	0.11±0.001*	6.4±0.03
LDL with EWH$^+$-PC (4 µmol-GAE.L^{-1}); n=2	0.08±0.01*	6.0±0.7
LDL with Luteolinidin (3 µmol-GAE.L^{-1}) ; n=2	0.28±0.005	8.4±1.1
LDL with Apigeninidin (3 µmol-GAE.L^{-1}) ; n=2	0.47±0.06	8.5±0.5
Apigeninidin +Luteolinidin (3+3 µmol-GAE.L^{-1}) ; n=3	0.22±0.008*	7.4±0.9

* Significant values ($P<0.05$) compared with the value obtained for LDL without EWH+-PC; n denotes the number of independent experiments.

Table 4. Effects of EWH$^+$-PC of Red Sorghum and Luteolinidin, Apigeninidin and the Mixture of Luteolinidin and Apigeninidin on LDL AAPH-Mediated Peroxidation.

	Vitamin E degradation R_i (Vit E) ($\mu mol.L^{-1} \times min^{-1}$)	**Conjugated diene production R_i (CD)** **R_p(CD)** ($\mu mol.L^{-1} \times min^{-1}$)	
LDL without EWH$^+$-PC; n=6	0.40±0.03	0.32±0.1	3.6±0.8
LDL with EWH$^+$-PC (2 µmol-GAE.L^{-1}); n=2	0.16±0.01*	0.30±0.05	3.2±0.8
LDL with EWH$^+$-PC (4 µmol-GAE.L^{-1}); n=2	0.16±0.01*	0.24±0.01	3.3±0.8
LDL with EWH$^+$-PC (6 µmol-GAE.L^{-1}); n=2	0.15±0.003*	0.07±0.06	2.80±0.3
LDL with Luteolinidin (3 µmol-GAE.L^{-1}); n=2	0.34±0.001*	0.30±0.001	3.6±0.8
LDL with Apigeninidin (3 µmol-GAE.L^{-1}); n=2	0.54±0.07*	0.45±0.01*	4.5±0.2
LDL with Luteolinidin+Apigeninidin (3+3 µmol-GAE.L^{-1}); n=1	0.36	0.29	4.5

* Significant values (P<0.05) compared with the value obtained for LDL without EWH$^+$-PC; n denotes the number of independent experiments.

We obtained different results by using AAPH-mediated oxidation with EWH+-PC, with luteolinidin or apigeninidin alone or with the mixture of the two later compounds because they induced a slight increase in the Vitamin E degradation rate and a significant increase (P<0.05) in the initial rate of conjugated diene (CD) production (Table 4).

EWH+-PC influence on the time course of LDL-vitamin E consumption under Cu2+-mediated oxidation was shown in Figure 1 in parallel with CD production. Comparable results were obtained for mixture of luteolinidin and apigeninidin and were shown in Table 3. We found that Vitamin E consumption, as well as CD production were delayed in a dose-dependent manner by the EWH+-PC. Firstly, Vitamin E degradation rate named Ri(VitE) was about twice lower when PC concentration was twice higher, pointing out a direct protection of vitamin E by EWH+-PC. However, the very beginning of the propagation phase (i.e. the end of the inhibition period corresponding to TLag) of CD production did not coincide with the time of total consumption of vitamin E. Unlike other phenolic compounds previously studied by us [10,13], red sorghum PC and the 3-deoxyanthocyanidin (luteolinidin and apigeninidin) protected LDL from oxidation, despite the absence of vitamin E. We think, in this case, there could be a greater affinity of EWH+-PC for LDL-lipid core than for LDL-Vitamin E surface. The most efficient antioxidant in our study was EWH+-PC extract which was able to reduce significantly (P<0.05) Vitamin E consumption from Ri(VitE) = 0.46±0.02 to 0.06±0.08 µmol.L-1 min-1 for LDL without and with 4 µmol-GAE.L-1 EWH+-PC respectively. The LDL-Vitamin E protection afford by EWH+-PC was significantly higher than that of the luteolinidin and apigeninidin mixture [Ri(vitE) = 0.22±0.008 µmol.L-1 min-1] (Table 3). We can hypothesize that while the mixture (luteolinidin and apigeninidin) is as complementary as to have a SAA similar to that of red sorghum, other phenolic compounds of red sorghum intervene actively in the protection of vitamin E.

Concerning AAPH-mediated oxidation and under our experimental conditions, radical generation rate (Rg) was constant and taken to be Rg = 0.39 µmol.L-1 □ min-1 according to Bowry et al. [20]. The time-dependent absorbance increase could be characterized by a "three phase kinetic profile" as shown in Figure 3 (A and B). During the first part of this kinetic (initiation phase), accumulation of oxidation products - CD initiation rate named Ri (CD) - was very slow and slightly lower than Rg, for LDL oxidized with or without EWH+-PC (Table 4). This contrasted with copper-induced peroxidation where no peroxidation product was formed until TLag. Moreover, for LDL without EWH+-PC and during this first phase, vitamin E was progressively consumed with a rate Ri (vitE) = 0.40±0.03 µmol.L-1 □ min-1, equal to Rg. During the second phase of this kinetic (propagation phase), auto-acceleration occurs with an increased and practically constant peroxidation rate named Rp(CD), for LDL without or with EWH+-PC and 3-deoxyanthocyanidins.

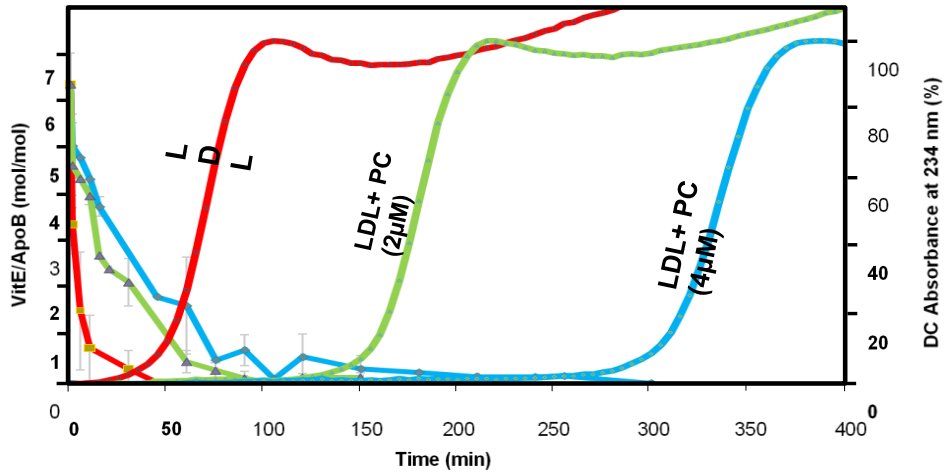

Figure 2: *In vitro* Cu^{2+}-degradation of LDL-vitamin E, in parallel with CD production, with or without red sorghum EWH^{+}-PC.
LDL oxidation was induced by 5 µmol.L^{-1}-copper ions at 37°C. Vitamin E was analyzed by an HPLC method (left y-axis) and CD production was detected at 234 nm (right y-axis). 2 and 4 µmol-GAE.L^{-1} of red sorghum EWH^{+}-PC (2 and 4 µM) were added to LDL before oxidation, in comparison to LDL alone.

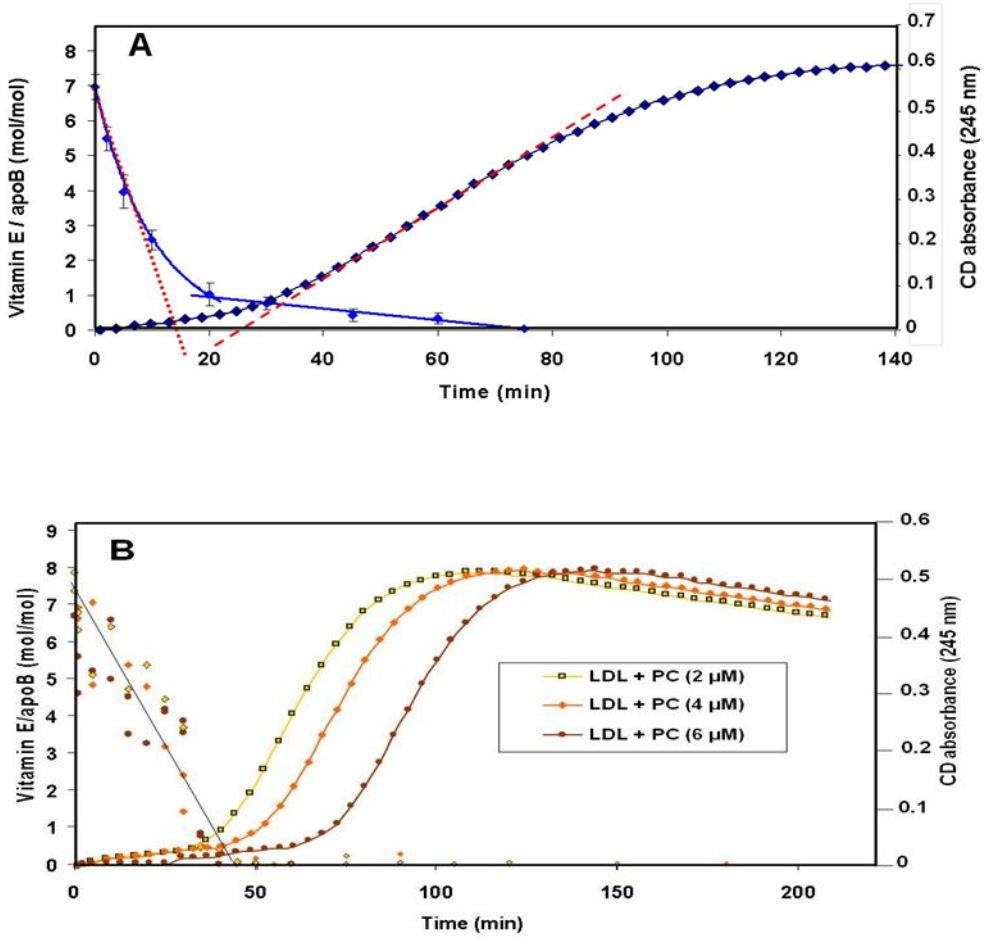

Figure 3 (A and B): *In vitro* AAPH-Degradation of LDL-Vitamin E, in Parallel with Conjugated-Diene Production, without (A) or with (B) EWH^{+}-PC of Red Sorghum.
LDL Oxidation was Induced by 5 Mmol L^{-1}-Water Soluble Radical Initiator AAPH At 37°C (Rg = 0.39 µmol L^{-1} × Min^{-1}). Vitamin E was Analyzed by an HPLC Method (Left Y-Axis) and CD Production was Detected at 245 Nm (Right Y-Axis). 0 (A) Or 2, 4 and 6 µmol GAE.L^{-1} Of Red Sorghum EWH^{+}-PC (2, 4 And 6 µm) was Added To LDL Before Oxidation (B).

This propagation phase could begin before complete degradation of vitamin E for LDL without EWH+-PC (Figure 2), unlike under copper-induced peroxidation. However, with EWH+-PC, the propagation phase started after complete Vitamin E depletion. We also found that the rate of degradation of Vitamin E is not EWH+-PC dose-dependent in the presence of AAPH, as shown in Table 4. The mixture of luteolinidin and apigeninidin (3 + 3 µmol-GAE.L-1) was unable to reduce the consumption rate of Vitamin E, as noted by the same R i (VitE) value compared with that of LDL without EWH+-PC.

The peroxyl radical (ROO•) formed by thermal decomposition of AAPH is responsible for the hydrogen abstraction from peroxidizable lipid species [20-22] and/or from vitamin E [10]. Protecting effect of EWH+-PC 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.

Our data demonstrate that EWH+-PC exhibit LDL-Vitamin E antioxidant activities against both inducers used. These results shown that vitamin E and polyphenols may be very significant bioactive compounds [13,23]. Conflicting results were shown concerning anthocyanin human absorption compared to other flavonoids [24,25]. Most of these data were obtained from fruit anthocyanins which are thought to contribute significantly to the health benefits of fruit consumption. The high antioxidant capacity of black sorghum and their brans were correlated with their anthocyanins contents [15]. Hence, anthocyanins may contribute significantly to any potential health benefits of these sorghums. Boveris et al. demonstrated that a 3-deoxyanyhocyanin (apigeninidin) isolated from soybean had strong dose-dependent quenching ability against lipid radicals [26]. Anthocyanins from fruits have been shown to possess several therapeutic benefits, including vasoprotective and anti-inflammatory properties [27], anti-cancer and chemo protective properties [28] as well as anti-neoplastic properties [29].

IV. CONCLUSION

The results presented here is our contribution to the study of PCs in sorghum to better advice its use in developing countries where problems of malnutrition and even starvation are recurrent. We could hypothesize that red sorghum PCs could exert direct beneficial antioxidant effects on vitamin E and other antioxidants contained in food and beverages in vivo, within the gastrointestinal tract. Further studies will be focused on the chemical properties and biological activities of EWH+-PC and their effect pattern in experimental animal models and humans. The sorghum anthocyanins should be later investigated for any other health properties.

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