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ORIGINAL ARTICLE

Effect of added *Theobroma cacao* Leaves Extract on the Oxidative Stability of Refined Palm Olein during Accelerated Storage

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Abstract

This study was conducted to evaluate the antioxidant potential of *Theobroma cacao* leaves extract on the oxidative stability of palm olein (PO). The plant material was extracted with methanol and its total phenolic, flavonoid contents and radical scavenging activity were evaluated, followed by the identification of some phenolic antioxidants by high-performance liquid chromatography (HPLC). The cocoa leaves extract was added in palm olein at concentrations of 500–2000 ppm. Oil containing butylhydroxytoluene (BHT) and oil without antioxidants served as positive and negative controls respectively. The oxidative stability of these oil samples was evaluated by determining their induction times on Rancimat (at 110°C) and measuring their oxidative state using the Schaal oven for a period of 46 days at 70°C (8 hours of heating per day) for measurement of their primary and secondary oxidation products. Results showed *Theobroma cacao* leaves extract to be rich in phenolic antioxidants and to be a good radical scavenger. Caffeic acid and vanillic acid were the phenolic antioxidants detected by HPLC. The induction times of palm olein supplemented with the extract were found to be ranged between 26.0 –27.99 hours, values significantly ($p < 0.01$) higher than that of the negative control. The extract, at all concentrations, were also found to be efficient in delaying oxidation of palm olein during 46 days storage at 70°C. It can be concluded that *Theobroma cacao* leaves extract can be a natural source of antioxidants for the stabilization of palm olein.

Practical Application

The research findings of this study can be useful to valorize waste products as cocoa leaves in the production of natural antioxidants who are supposed to be safer than synthetic antioxidants and their utilization as ingredient to limit palm olein oxidation.

Keywords: Accelerated storage, oxidation, palm olein, *Theobroma cacao* leaves, antioxidants

1. Introduction

In the early 1990s, palm oil and palm olein entered the world market. They are increasingly used in frying and cooking due to their techno-economic advantages compared to other oils (Ismail, 2005). It is estimated that several million tonnes of palm olein are used annually for domestic and commercial frying. This oil is rich in monounsaturated fatty acids and more stable than other refined oils (Sambanthamurthi *et al.*, 2000). However, the presence of unsaturated fatty acids in these oils also gives them a chemical reactivity (oxidation) which can take

place during storage, culinary and technological treatments. However, their quality and oxidative stability are the main factors that influence their market value and acceptability (Ofosu *et al.,* 2012). Lipids oxidation is considered as one of the main reactions responsible for the quality deterioration of fats, vegetable oils and other food systems (Che Man & Tan, 1999). The adverse effects of lipid oxidation are the reduction of the sensorial and nutritional properties of foods as well as the formation of undesirable compounds (organic acids, aldehydes, ketones, …) which are dangerous for health (Saad *et al.,* 2007).

To solve this problem of oxidation, the agro-food industries have practiced for many years the addition of synthetic antioxidants in oils. These include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-Butylhydroquinone (TBHQ). These food additives significantly delay the oxidation of unsaturated fatty acids during storage and culinary processes (Pimpa *et al.,* 2009). However, reports show that despite their ability to slow down oxidation, these additives are implicated in the occurrence of several pathologies, including cancer (Iqbal *et al.,* 2008; Krishnaiah *et al.,* 2010). Also, they are easily volatilized at high temperature (Pitchaon *et al.,* 2007). As a result, significant works have been done in recent decades to find compounds from natural sources, supposed to be safer (Houlian & Ho, 1985) as an alternative to these chemicals. In this approach, stabilization of vegetable oils has been the subject of numerous researches having opted for the use of extracts, essential oils and powders of some spices, herbs and agrowastes rich in phenolic compounds with antioxidant properties (John & Grohmann, 2001; Anwar *et al.,* 2006; Nyam *et al.,* 2013; Mousavi *et al.,* 2013; Mei *et al.,* 2014; Womeni *et al.,* 2016; Teboukeu *et al.,* 2018). Nyam *et al.* (2013) showed the good thermal stability of kenaf seed and roselle seed extracts and their performance in protecting fatty acids double bonds and limiting the appearance of primary oxidation products under accelerated storage. Similar results have been found with others natural sources. Beyond all these previous investigations, only few natural sources have been authorized for industrial purpose (case of rosemary) (Cuvelier & Maillard, 2012). Therefore, there is a strong rational to continue the search of new natural sources of antioxidants by screening the numerous plant sources and investigate the possibilities of their application to overcome the lipids oxidation of oils.

Theobroma cacao is the name given to the cocoa tree, belonging to the family *Malvaceae.* Cocoa beans and derived products contain bioactive compounds, especially polyphenols that have potential health benefits against chronic disorders such as inflammation, neurodegenerative diseases, cardiovascular diseases and cancer (Jalil & Ismail, 2008; Rimbach *et al.,* 2009). Cocoa leaves are rarely used and contain a large amount of polyphenols (Osman *et al.,* 2004). For an economic and environmental reasons, plant by-products such as *Theobroma cacao* leaves, produced in the field should be valorized as natural sources of antioxidants to replace synthetic antioxidant. Thus, the objective of this study was to assess the antioxidative potential of phenolic extracts from cocoa leaves on the oxidative stability of palm olein during an accelerated oxidation.

2. Materials and Methods

2.1. Materials

Refined, bleached, and deodorized palm olein, free from additives was obtained from SCS/RAFCA Palm Oil Industry Company Ltd, Bafoussam, West-Cameroon. Fresh cocoa leaves were collected at Bafang, Haut Nkam Department, West-Cameroon. All the chemicals and reagents used were of analytical reagent grade.

2.2. Methods

2.2.1 Preparation of methanolic extract

Fresh *Theobroma cacao* leaves were dried in an electric oven at 45°C for 48 hours. The dried leaves were ground to pass through a 1 mm sieve. About 25 g of powder was extracted into 500 mL of methanol. The extract was regularly shaked at room temperature. The mixture was filtered with a Wathman N°1 filter paper and residue was again extracted to ensure the maximum extraction of bioactive compounds. The filtrate was subjected to rotary evaporation at 45°C under reduced pressure for the removal of the solvent. The extract was stored at 4°C before for further analysis.

2.2.2 Determination of total phenolic content

Total phenolic content was evaluated using the Folin-Ciocalteu colorimetric method as described by Gao *et al.* (2000). Plant extract (20 µL) was mixed in a test tube with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of distilled water and incubated at room temperature for 3 min. After this, 1 mL of 20% sodium carbonate was added and the mixture re-incubated for 2 hours at room temperature. The absorbance of the resulting solution was measured at 765 nm. Gallic acid was used as standard and total phenolic content expressed as gallic acid equivalents (GAE) milligrams per gram of extract.

2.2.3 Estimation of total flavonoid content

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to the method described by Ordonez *et al.* (2006) using catechin as standard. The extract (0.1 mL) was added to 0.3 mL distilled water followed by 5% $NaNO_2$ (0.03 mL). After 5 min incubation at 25°C, 0.03 mL of 10 % $AlCl_3$ was added. After 5 min of incubation, the reaction mixture was treated with 0.2 mL of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm. The results were expressed as catechin equivalents (CAE) milligrams per gram of extract.

2.2.4 High-performance liquid chromatography analysis

Reverse-phase HPLC was used to analyze the phenolic composition of the extract (1 mg/mL in methanol). The HPLC Agilent system 1200 series used was equipped with a quaternary pump G11311A and Diode Array Detector (DAD) G11315B in combination with Chemstation software. The column type was an RP-C18 Lichrospher column, 5 µm, 4.0 mm internal diameter × 250 mm. Separations were done in the isocratic mode, using acetonitrile - 1% orthophosphoric acid in water (70:30 v/v) at a flow rate of 1 mL/min; with an injection volume of 20 µL. DAD detection was at 280 nm. Identification of the phenolic compounds was achieved by comparing their retention time to those of standards (Gallic acid, Vanillic acid, Caffeic acid, Ferulic acid, Ellagic acid, Quercetine).

2.2.5 Preliminary antioxidant tests

The DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging activity was assessed according to the method of Mensor *et al.* (2001).

1 mL of 0.3 mM alcoholic solution of DPPH was added to 2.5 mL of the samples with different concentrations of the extract. The samples were kept at room temperature in the dark and after 30 min of incubation, the optical density was measured at 517 nm. The optical densities (Abs) of the samples, the control and the blank, was measured in comparison with methanol. BHT (synthetic antioxidant) were used as positive control. The antiradical activity (AA) was calculated according equation (1):

$$AA\% = [(Abs_{control} - Abs_{sample}) \times 100/Abs_{control}] \quad (1)$$

2.2.6 Effect of cocoa leaves extract on palm olein (PO) oxidation

2.2.6.1 Samples preparation

The samples were prepared according to the method of Iqbal & Bhanger (2007). The crude concentrated methanolic extract was dissolved in a small volume of methanol and separately added to 100 g of preheated palm olein (at 50°C) at concentrations of 500, 720, 1250, 1780 and 2000 ppm. Synthetic antioxidant (BHT) was employed at it legal limit of 200 ppm (Duh & Yen, 1997) to compare the efficacy of natural antioxidants. A palm olein free from additives and prepared under similar conditions served as negative control. After removing the methanol, each sample was separated in two portions, 10 and 90 g, for Rancimat and Schaal oven tests respectively.

2.2.6.2 Rancimat test

Induction periods of stabilized and control palm olein samples were evaluated using an automated Metrohm Rancimat model 892 as described by Womeni *et al.* (2016). About 5 g of each oil sample was weighed in individual reaction vessels of the instrument and vessels were placed in a heating block for 10 min for preheating of the sample. After that, the air was supplied by a built-in-pump at a flow rate of 20 L/hr. Temperature was adjusted to 110°C and absorption vessels filled with 60 mL deionized water were connected with reaction vessels via Teflon tubing. Induction period, the time elapsed from the beginning until the oil starts to become rancid, was automatically recorded, and the protection factor (equation (2)) was calculated using the induction time of oil with antioxidant (I) and the induction time of oil without antioxidant (I_0) (Yanishlieva & Marinova, 2001):

$$\text{Protection factor} = I / I_0 \quad (2)$$

2.2.6.3 Schaal oven test

Enriched and control oil samples (90g) were placed in dark brown airtight glass bottles with narrow necks and subjected to accelerated storage in an electric hot air oven at 70°C (8 hours heating cycle per day) for 46 days. Samples were collected every 7, 23, 40 and 46 days and stored for further analysis. The oxidative deterioration level was evaluated by measuring oxidation parameters.

2.2.6.4 Measurement of oxidation parameters

Determination of the peroxide value (PV) of stabilized and control palm olein samples were made following the spectrophotometric IDF standard method, 74A: 1991 (IDF, 1991). *p*-anisidine (AV) was determined according to the procedure in AOCS Official Method CD 18–90 (AOCS, 1999). Total oxidation (TOTOX) values of oil samples were determined using the equation (3) (Shahidi & Wanasundara, 2008):

$$TOTOX = 2PV + AV \quad (3)$$

2.3 Statistical Analysis

The tests were performed in triplicate and the results are representative of the mean ± standard deviations (SD). The obtained data were submitted to the statistical analysis of variance at 5% probability level. The Student-Newmann-Keuls and Dunnett tests were used to compare means using the software Graphpad-InStat, version 3.05 for windows.

3. Results and Discussion

3.1. Extraction yield, total phenolic and flavonoid contents, HPLC-DAD analysis

The yield of methanolic extract obtained from cocoa leaves was 13.60 %. Its total phenolic and flavonoid contents were found to be 61.21 ± 1.118 mg GAE/g and 36.21 ± 0.99 mg CAE/g respectively. Results of the HPLC analysis of the extract (Figure 1) showed that vanillic and cafeic acids were the phenolic antioxidants detected; caffeic acid was the most abundant compound.

Phenolic compounds are considered as a major group of chemicals that contribute to the antioxidant potential of plants (Liu & Yao, 2007). The result from spectrophotometric determination of total phenolic content of *Theobroma cacao* leaves extracts (61.21 ± 1.118 mg GAE/g) was lower than that obtained by Osman *et al.* (2004) with steam-blanched cocoa leaves extract (28.4 mg/100mg). However, this value was higher than that found by Karim *et al.* (2014) in cocoa pods extract (49.54 + 3.69 mg GAE/g). Flavonoids were the most abundant polyphenols in our extract (36.21 ± 0.99 mg CAE/g) and their rate was higher than that reported by Karim *et al.* (2014) in cocoa pods extract (22.42+ 0.99 mg RE/g). Differences between these results might be attributed to environmental factors, choice of parts tested,

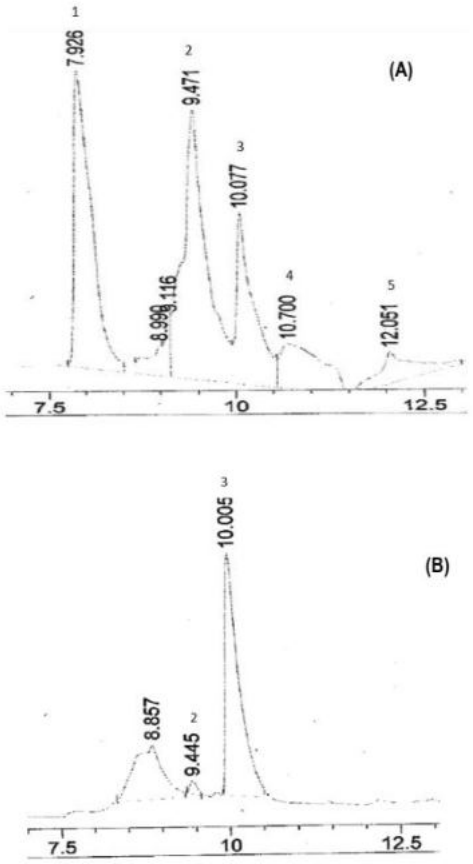

Figure 1. HPLC-DAD Chromatogram of standards (A) (*1 = Gallic acid, 2 = Vanillic acid, 3 = Caffeic acid, 4 = Ferulic acid, 5 = Ellagic acid, pic 6 = Quercetine)* and *Theobroma cacao* leaves extract (B) *(2 =Vanillic acid, 4 = Caffeic acid*).

harvesting time and method used (Shan *et al.,* 2005). The presence of caffeic acid revealed by HPLC-DAD in the cocoa leaves extract has been previously reported by Osman *et al.* (2004). This author also showed that steam-blanched cocoa leaves contain other phenolic compounds like gallate, epicatechin, epigallocatechin, gallic acid and epicatechin gallate. However, the presence of vanillic acid in the cocoa leaves extract has not been reported previously. This difference could be due to many factors such as genetic characteristics of the plant, extraction procedure, environmental differences, and its state of maturity (Shan *et al.,* 2005; Kim & Choe, 2004).

3.2 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of cocoa leaves extract in comparison with butylated hydroxytoluene is presented in Figure 2. It can be seen that below 50 µg/mL, the radical scavenging activity of the extract is significantly lower ($p < 0.001$) than that of BHT. However, from 50 to 200 µg/mL, no significant difference ($p > 0.05$) was registered between the activity of both BHT and the extract respectively.

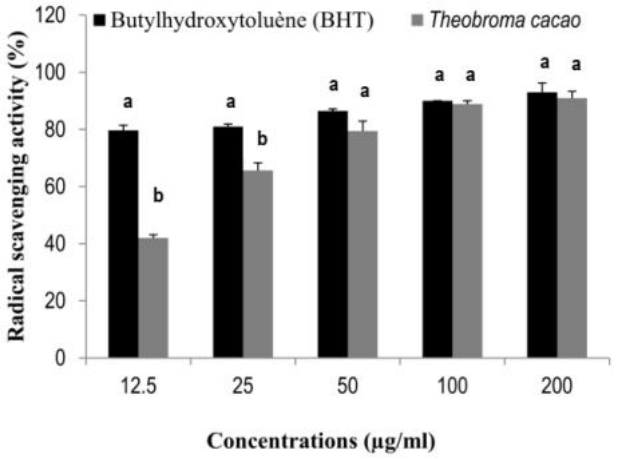

Figure 2. DPPH radical scavenging activity of *Theobroma cacao* leaves extract. *Bars represent mean ± SD. Bars with the same lowercase letter are not significantly different (p > 0.05).*

Theobroma cacao leaves extract which previously showed a high content of phenolic compounds, exhibited high antiradical activity toward the DPPH. It has been proven that the radical scavenging activity of a plant extract is mainly attributed to its concentration of phenolic compounds. Several studies showed a good correlation between the total phenolic content and antioxidant activity of plant extracts (Huang *et al.,* 2005; Moure *et al.,* 2001). Indeed, the degree of hydroxylation of phenols gives them more abilities to stabilize free radicals (Roginsky & Lissi, 2005). Results obtained in this study are in agreement with those of Baharum *et al.* (2014) who showed that cocoa leaves extract is a powerful antioxidant.

3.3 Rancimat test

The effects of different concentrations of extract on the induction period and protection factors of palm olein in comparison with the control and oil enriched with BHT are presented in Table 1. It was observed that the added extract, at all concentrations, significantly ($p< 0.01$) prolonged the induction period (IP) of oxidation and protection factors (PF) of palm olein (IP ≥ 26.07 hours; PF ≥ 1.12), compared to the control (IP = 23.19 hours; PF =1.00) and palm olein containing BHT as antioxidant at concentration of 200 ppm ($PO+BHT_{200ppm}$) (IP = 24.41 hours; PF =1.05). Additionally, the activity of the extract increased with its concentration.

The Rancimat test is a technique which is currently used for the evaluation of the oxidative stability of fats and oils as well as the study of antioxidant potential of new molecules (Jain *et al.,* 2005). Longer induction period indicates good efficiency of the added antioxidants or higher resistance of oil towards oxidation. Results of the analysis showed that oil samples containing the extracts as antioxidants presented the highest induction period. This might be attributed to their high amount of phenolic compounds. Phenols can give their hydrogen atoms for the stabilization of free radicals. By this way, they reduce the formation of peroxide and their decomposition into volatiles organic acid, and then prolong the oil induction time (Womeni *et al.,* 2016; Teboukeu *et al.,* 2018). The fact that the activity of the extract was higher than that of BHT might be due to the rapid decomposition of BHT at high temperatures as reported by Hamama & Nawar

(1991). Similar results have been reported by Abd el ghany *et al.* (2010) in sunflower oil supplemented with olive cake extract at concentrations ranging between 100 and 600 ppm.

Table 1. Induction time and protective factor of oil samples during storage at 110°C

Samples	Induction time (hours)	Protection factor
Control	23.19 ± 0.12^{a}	1.00 ± 0.00^{a}
PO + $BHT_{200\ ppm}$	24.41 ± 0.23^{b}	1.05 ± 0.00^{b}
PO + $Thca_{2000\ ppm}$	27.99 ± 0.56^{e}	1.20 ± 0.01^{f}
PO + $Thca_{1780\ ppm}$	27.11 ± 0.54^{d}	1.16 ± 0.00^{e}
PO + $Thca_{1250\ ppm}$	27.04 ± 0.43^{d}	1.17 ± 0.00^{e}
PO + $Thca_{720\ ppm}$	26.77 ± 0.16^{d}	1.15 ± 0.00^{d}
PO + $Thca_{500\ ppm}$	26.07 ± 0.19^{c}	1.12 ± 0.07^{c}

Values of columns with different letters differ significantly ($p < 0.05$). Control = palm olein without antioxidant, PO + $BHT_{200\ ppm}$ = palm olein containing BHT as antioxidant at concentration of 200 ppm, PO + $Thca_{xxxppm}$ = palm olein supplemented with Theobroma cacao leaves extract at concentration xxx ppm

3.4 Accelerated storage at 70 °C

3.4.1. Peroxide value

The degree of primary oxidation of enriched and control palm olein samples were assessed by measuring their peroxide values and the results are recapitulated in Table 2. A continuous increase in peroxide values was observed in all the samples during the storage. Control exhibited the highest peroxide value ($p < 0.05$) during the storage. However, oil samples containing the extract and BHT were less primarily oxidized.

Peroxide value is an indicator of the extent of primary oxidation products in oil (Chatha *et al.*, 2006). The general increase in peroxide value observed in all samples might be attributed to the formation of hydroperoxides which are the principal markers of primary oxidation of lipids. A low concentration of peroxide obtained in palm olein enriched with the extract and BHT in comparison to the control indicates the preservative effect of these additives. The phenolic antioxidants present in this extract might be responsible for the registered activity. They can provide a hydrogen atom to stabilize the free radicals present in oil and consequently increase its oxidative stability (Gordon, 1990). The lowest peroxide value observed in the sample containing *Theobroma cacao* at 2000 ppm (PO+$Thca_{2000ppm}$) clearly indicated that the antioxidant effect of the extract was concentration-dependent. Similar results were reported by Womeni *et al.* (2016) with palm olein supplemented with tea leaves extracts.

3.4.2. p-Anisidine value

Table 2 also shows the relative increase in *p*-anisidine value of oil samples supplemented with the extract and control. *p*-Anisidine value increased in all the samples with storage time. This augmentation was significantly pronounced in the control ($p < 0.01$) compared to oil samples enriched with antioxidants. The activity of the extract was not far from that of the synthetic antioxidant.

The second stage of oil oxidation is the decomposition of hydroperoxides into secondary oxidation products (expressed as *p*-anisidine value), mainly carbonyls (2, 4-alkadienal and 2-alkenal). The highest *p*-anisidine value of control shows a higher degree of secondary oxidation due to the absence of antioxidants. Cocoa leaves extract and BHT inhibited the formation of secondary oxidation products and their effectiveness could be related to the additives present, which, by inhibiting peroxide formation have reduced by the same way the amount of secondary oxidation products in oil. Hydroperoxides are a thermolabile species and their breakdown causes secondary products. Consequently, the limitation of peroxide formation by cocoa leaves extract leads to the reduction of 2, 4-alkadienal and 2-alkenal formation. These results were found to agree with the studies of Asma *et al.* (2005) and Womeni *et al.* (2016) who respectively found that *Moringa oleifera* and tea leaves extract delay the formation of secondary oxidation products in oils.

3.4.3. TOTOX Value

The changes in total oxidation value of stabilized and control palm olein samples during storage are illustrated in Table 2. As previously observed with the peroxide and *p*-anisidine values, there was a significant increase in total oxidation in all samples. The oxidation rate was significantly higher ($p < 0.05$) in control, while it was very low in palm olein samples supplemented with antioxidants. Palm olein supplemented with *Theobroma cacao* leaves extract at concentration 2000 ppm (PO+Thca$_{2000\ ppm}$) was the lowest oxidized sample, and its TOTOX value was not different ($p > 0.05$) from that of palm olein supplemented with BHT.

Table 2. Changes in peroxide, *p*-anisidine and total oxidation (TOTOX) values during storage of palm olein

Characteristic	Days	Control	PO + BHT$_{200ppm}$	PO+Thca$_{500ppm}$	PO+Thca$_{750ppm}$	PO+Thca$_{1250ppm}$	PO+Thca$_{1750ppm}$	Po+Thca$_{2000ppm}$
Peroxide value (meq O_2/Kg)	0	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$	$4.23 \pm 0.16^{a}_{A}$
	7	$14.45 \pm 0.1^{a}_{B}$	$5.65 \pm 0.00^{e}_{A}$	$10.44 \pm 0.00^{b}_{B}$	$9.35 \pm 0.10^{c}_{B}$	$8.00 \pm 0.00^{d}_{B}$	$7.65 \pm 0.15^{e}_{B}$	$6.19 \pm 0.01^{f}_{B}$
	23	$27.15 \pm 0.06^{a}_{C}$	$16.04 \pm 0.07^{e}_{B}$	$25.45 \pm 0.16^{b}_{C}$	$24.23 \pm 0.23^{c}_{C}$	$22.65 \pm 0.08^{d}_{C}$	$22.83 \pm 0.04^{d}_{C}$	$15.45 \pm 0.00^{f}_{C}$
	40	$30.23 \pm 0.12^{a}_{D}$	$24.34 \pm 0.00^{g}_{C}$	$25.34 \pm 0.08^{b}_{D}$	$27.87 \pm 0.15^{d}_{D}$	$28.23 \pm 0.15^{c}_{D}$	$26.32 \pm 0.00^{e}_{D}$	$25.61 \pm 0.19^{f}_{D}$
	46	$32.45 \pm 0.11^{a}_{E}$	$28.45 \pm 0.15^{b}_{D}$	$29.50 \pm 0.10^{d}_{C}$	$27.39 \pm 0.23^{e}_{D}$	$28.20 \pm 0.00^{b}_{D}$	$28.69 \pm 0.12^{b}_{E}$	$27.16 \pm 0.23^{c}_{E}$
p-Anisidine value	0	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$	$2.76 \pm 0.13^{a}_{A}$
	7	$4.45 \pm 0.10^{a}_{B}$	$3.15 \pm 0.00^{e}_{B}$	$3.31 \pm 0.00^{d}_{B}$	$3.60 \pm 0.10^{c}_{B}$	$3.49 \pm 0.00^{c}_{B}$	$4.16 \pm 0.15^{b}_{B}$	$4.36 \pm 0.01^{a}_{B}$
	23	$6.78 \pm 0.06^{a}_{C}$	$3.04 \pm 0.07^{d}_{B}$	$4.76 \pm 0.16^{b}_{C}$	$4.20 \pm 0.23^{c}_{C}$	$4.62 \pm 0.08^{b}_{C}$	$4.71 \pm 0.04^{b}_{C}$	$4.72 \pm 0.00^{b}_{C}$
	40	$10.23 \pm 0.12^{a}_{D}$	$5.02 \pm 0.00^{c}_{C}$	$5.75 \pm 0.08^{b}_{D}$	$5.55 \pm 0.15^{b}_{D}$	$4.06 \pm 0.15^{e}_{D}$	$4.18 \pm 0.00^{e}_{B}$	$4.50 \pm 0.19^{d}_{BC}$
	46	$13.23 \pm 0.11^{a}_{E}$	$7.45 \pm 0.15^{e}_{D}$	$8.34 \pm 0.10^{b}_{E}$	$8.23 \pm 0.23^{b}_{E}$	$5.43 \pm 0.00^{d}_{E}$	$5.23 \pm 0.12^{d}_{D}$	$4.99 \pm 0.23^{e}_{C}$
TOTOX value	0	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$	$11.23 \pm 0.48^{a}_{A}$
	7	$33.36 \pm 0.3^{a}_{B}$	$14.46 \pm 0.01^{f}_{B}$	$24.20 \pm 0.01^{b}_{B}$	$22.32 \pm 0.31^{c}_{B}$	$19.50 \pm 0.02^{d}_{B}$	$19.48 \pm 0.45^{d}_{B}$	$16.76 \pm 0.05^{e}_{B}$
	23	$61.09 \pm 0.19^{a}_{C}$	$35.13 \pm 0.23^{e}_{C}$	$55.67 \pm 0.49^{b}_{C}$	$52.66 \pm 0.69^{c}_{C}$	$49.93 \pm 0.25^{d}_{C}$	$50.37 \pm 0.13^{d}_{C}$	$35.62 \pm 0.02^{e}_{C}$
	40	$70.70 \pm 0.37^{a}_{D}$	$53.70 \pm 0.01^{f}_{D}$	$64.44 \pm 0.26^{b}_{E}$	$61.31 \pm 0.47^{c}_{D}$	$60.53 \pm 0.46^{c}_{D}$	$56.83 \pm 0.00^{e}_{D}$	$55.73 \pm 0.59^{d}_{D}$
	46	$78.13 \pm 0.33^{a}_{E}$	$64.36 \pm 0.46^{b}_{E}$	$59.35 \pm 0.31^{e}_{D}$	$63.03 \pm 0.70^{c}_{E}$	$61.84 \pm 0.01^{d}_{D}$	$62.62 \pm 0.38^{cd}_{E}$	$59.33 \pm 0.70^{e}_{E}$

(a- e) Means ± SD within each row with different superscripts are significantly different ($p < 0.05$). (A–D) Means ± SD within each column with different superscripts are significantly different ($p < 0.05$). Control = Palm olein without antioxidant, PO + BHT 200ppm = palm olein containing BHT as antioxidant at concentration of 200 ppm, PO + Thca$_{xxxppm}$ = palm olein supplemented with the extract at concentration of xxxx ppm

TOTOX value provides a better estimation of the progressive oxidative deterioration of fats and oils measuring both hydroperoxides and their breakdown products (Womeni *et al.*, 2016). The results obtained during this investigation shows

once again that cocoa leaves extract and BHT has a good antioxidant activity toward palm olein oxidation. These results are in accordance with those reported in previous work (Nyam *et al.,* 2013; Womeni *et al.,* 2016) have proven that natural plant extract can limit total oxidation of vegetable oils.

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4.0 Conclusion

The objective of this work was to assess the ability of *Theobroma cacao* leaves extract to delay palm olein oxidation during accelerated storage at 70 °C. Based on the obtained results, the addition of the extract at concentrations 500, 720, 1250, 1720 and 2000 ppm in palm olein is effective to ensure its oxidative stability. This protective effect depends proportionally of the concentration of the extract and might be attributed to the presence of phenolic antioxidants (particularly vanillic and cafeic acids). For economic and environmental perspective, the "waste" leaves from cocoa plantations can be used as a new source of natural bioactive extract for the protection of vegetable oils from oxidation.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Ethics

This Study does not involve Human or Animal testing.