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Original article

# Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract

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ABSTRACT

*Background:* Lipid oxidation is responsible for the deterioration of the nutritional and organoleptic qualities of foods. It also leads to the formation in these products of compounds which are harmful for the consumer's health. The synthetic antioxidants used by industries in order to solve this problem are also prone to be toxic and quite volatile at high temperatures. On the other hand, the trend among researchers to replace the synthetic antioxidants with natural ones is increasing because of their potential health benefits. In this study, old Cameroonian green tea leaf methanolic extracts were added in palm olein and its efficiency in delaying the oxidation of oil under forced storage conditions was assessed.

*Methods:* The plant material was extracted with methanol and its total phenolics content evaluated by colorimetry, followed by the qualitative identification of some phenolic antioxidants by HPLC-DAD and ESI-MS. After preliminary antioxidant studies, the extract was added in oil at concentrations that ranged from 200 to 1800 ppm. Oil containing butylated hydroxytoluene (BHT) and oil without antioxidant served as positive and negative controls, respectively. The oxidative stability of these oil samples was evaluated by determining their oxidation induction times on Rancimat (at 110 °C) and measuring their oxidative state by the Schaal oven test method during 30 days of storage at 70 °C. Here, oil samples were collected every 10 days and their peroxide, *p*-anisidine, total oxidation, thiobarbituric acid, iodine values as well as changes in their linoleic acid profile gas chromatography coupled to flame ionization detector (GC/FID) were evaluated.

*Results:* The outcomes showed the total phenolics content of the extract to be 53.5 mg GAE/g. Gallic acid, epicatechin gallate, gallocatechin and epigallocatechin gallate were the phenolic antioxidants detected. The induction times of palm olein supplemented with the extract (200–1800 ppm) were found to be in the range of 24.8–28.9 h, while those of control and oil containing BHT were 20.1 and 22.7 h, respectively. The extracts, at all concentrations, were also found to be efficient like butylated hydroxytoluene in inhibiting the oxidation of palm olein during 30 days of storage at 70 °C.

*Conclusion:* The investigations showed that old Cameroonian green tea leaves are a viable source of natural antioxidants for delaying palm olein oxidation.



## 1. Introduction

Lipid oxidation that results from the reaction between unsaturated fatty acids and molecular oxygen is one of the culprits of deterioration in fats and oils. It decreases the nutritional properties of foods since it involves the loss of essential fatty acids, essential amino acids, destruction of vitamins, and reduction of protein digestibility [1]. It also alters the organoleptic properties of foods as well as changes in colour, texture, appearance of rancid odours and undesirable flavours [2]. Besides affecting the nutritional and organoleptic qualities of the products, during this process, it may generate potential toxic compounds through the action of free radicals and reactive oxygen species that are harmful to human health and are implicated in degenerative diseases such as cancer, cardiovascular diseases, and early ageing [3]. Due to these changes, consumers do not accept oxidized products and industries suffer from economic losses [4].

*Abbreviations:* DAD, diode array detector; ESI-MS, electrospray ionization-mass spectrometry; GAE, gallic acid equivalent; PO, palm olein; Ca.S, *Camellia sinensis*.

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In order to delay lipid oxidation, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butyl hydroquinone (TBHQ) have been used to maintain the quality and extend the shelf-life of oils. However, their use is increasingly contested or even banned in certain countries, due to their potential health risks [3]. As a consequence, they are negatively perceived by consumers. Besides, according to Chang et al. [5] and Thorat et al. [6], BHA and BHT are quite volatile and easily decompose at high temperatures. In order to overcome this challenge, food industries have started to search for alternative antioxidants that are more stable and that originate from natural sources, which in general are supposed to be safer.

Many studies have been previously conducted in view of finding natural sources of antioxidants which can limit vegetable oil oxidation [7,8,9,10,11]. However, recently, only a few natural sources have been authorized for industrial purposes (case of rosemary) [1]. It is necessary to find other natural sources of antioxidants. According to the literature, the antioxidant potential of natural plant extracts is mainly contributed by their phenolic compounds. Tea leaves are well-known for their high antioxidant activity and phenolic content. Up to now, only few studies have described the efficiency of old green tea leaf extracts in stabilizing vegetable oils. In one study, Navas et al. [12] employed black tea extract to stabilize corn oil under accelerated storage conditions. The results of their study suggest that black tea leaf extracts are an effective source of antioxidant for stabilization of corn oil. However, it has also been proven that green tea contains more antioxidant compounds than black tea leaves [13].

Green tea tree (*Camellia sinensis* L.) belongs to the family of Theaceae. Its leaves contain a variable amount of natural antioxidants, mainly phenolics, among which (+)-catechin, (+)-gallocatechin, (−)-epicatechin gallate, (−)-epicatechin, (+)-gallocatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate represent up to 90%. The strongest antioxidant among these molecules has been reported to be epigallocatechin gallate [14]. In Cameroon, old tea leaves (matures, found at the base of the tree) are generally considered as waste, and are always eliminated from the tree during cleaning to improve the development of young leaves, which are used for tea manufacturing. These old leaves, instead of being thrown away, could be exploited for other purposes. Hence, in this study, old Cameroonian green tea leaf methanolic extracts were added to palm olein and its effectiveness in delaying oil rancidity under forced storage conditions was investigated.

Palm olein has been used in this study as an oxidation substrate because it is consumed in most the countries all over the world, and is used for food formulations, cooking, and fast food manufacturing. It also contains non-negligible amounts of unsaturated fatty acids, among which linoleic acid, an essential fatty acid, is the most represented. However, relatively fewer studies have been reported on the effect of natural antioxidants in stabilizing palm olein under accelerated storage, probably because it belongs to the same oils as palm stearin, which is highly stable toward oxidation, so there is less interest to stabilize them with antioxidants. Only the effectiveness of synthetic antioxidants in retarding palm olein deterioration during accelerated storage and the effectiveness of some natural antioxidants on the palm olein stability during deep-fat frying have been reported in some papers [15,16].

## 2. Material and methods

### 2.1. Material

Refined, bleached, and deodorized palm olein, free from additives, was obtained from SCS/RAFCA Palm Oil Industry Company Ltd., Bafoussam, West Cameroon. Old green tea leaves (matures, found at the base of the tree) were freshly collected from Cameroon Tea Estate (CTE) industry's farm based in Djuttitsa, West Cameroon, on April 2013. All the chemicals and reagents used were of analytical reagent grade.

## 3. Methods

#### 3.1.1. Extraction of tea leaves antioxidants

The old tea leaves freshly collected from the farm were cleaned and dried in an electric air-dried oven at 50 °C for 48 h. The dried leaves were ground to pass through a 1 mm sieve. About 100 g of the obtained powder was extracted into 800 ml of methanol for 48 h at room temperature. The mixture was regularly subjected to shaking during the extraction. The extract was filtered with a Whatman No. 1 filter paper, and residue was again extracted with 400 ml of methanol to ensure maximum extraction of phenolic compounds. Methanol was used for extraction because of its good antioxidant extraction power compared to other solvents [4]. The combined filtrates were subjected to rotary evaporation at 40 °C under reduced pressure for the removal of the solvent, and the solvent residue was removed by drying the extract in the oven at 45 °C until the extract became solid and the weight became constant. The dried extract was stored at 4 °C prior to further analysis.

#### 3.1.2. Phytochemical characterization of the extract

##### 3.1.2.1. Colorimetric determination of the total phenolic content

The total phenolic content of the extract have been evaluated using the Folin–Ciocalteu colorimetric method, as described by Gao et al. [17]. Briefly, in a test tube of 5 ml volume, 20 μl of a 2 mg/ml extract solution was added, followed by the Folin–Ciocalteu reagent (0.2 ml) and distilled water (2 ml). After 3 min incubation of the solution mixture at room temperature, 1 ml of 20% sodium carbonate solution was added and the mixture re-incubated for 2 h under the same conditions. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract.

##### 3.1.2.2. High-performance liquid chromatography analysis

Reverse-phase HPLC was used to analyse the composition of phenolics in the extract (1 mg/ml in methanol). The HPLC Agilent system 1200 series used was equipped with a quaternary pump model G11311A and diode array detector (DAD) model G11315B in combination with Chemstation software. The column type was an RP-C18 Lichrospher column, 5 μm, 4.0 mm internal diameter × 250 mm length. 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 antioxidants was achieved qualitatively by comparing their retention time to those of standards.

##### 3.1.2.3. Electrospray ionization-mass spectrometry analysis (ESI-MS)

A qualitative ESI-MS analysis of the extract (1 mg/ml in methanol) was performed on a QUATTROMICRO instrument, equipped with an ESCI multimode ionizator, operating in negative ionization mode, in 100% methanol. The injection volume in the system was 50 μl. ESI-MS parameters were optimized by direct infusion with tuning mix. The other optimum values of the ESI-MS parameters were: temperature, 120 °C; desolvation temperature, 300 °C; desolvation gas flow, 500 l/h; RF lens, 0.2 V; extractor, 3 V; cone, 25 V; and capillary, 3.41 KV (setting); and temperature, 119 °C; desolvation temperature, 317 °C; desolvation gas flow, 423 l/h; RF lens, 0.2 V; extractor, 3.34 V; cone, 28.45 V; and capillary, 3.41 KV (readbacks). The accurate mass data for molecular ion were processed using the software Masslynx V4.1. The identification of compounds was made qualitatively by comparing $[M-1]^-$ fragment ions in samples with those of standards.

*3.1.3. Preliminary antioxidant tests*

*3.1.3.1. DPPH radical scavenging assay*

The radical scavenging ability of the extract was determined according to the method of Braca et al. [18]. A total of 4.5 ml of 0.002% alcoholic solution of DPPH was added to 0.5 ml of different concentrations (125, 250, 500, 1000, and 2000 μg/ml) of samples and standard solutions separately, in order to have final concentrations of products of 25–200 μg/ml. The samples were kept at room temperature in the dark and after 30 min and the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control, and blank was measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful hydrogen donor, was used as positive control. The antiradical activity (AA) was determined using the following formula:

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

*3.1.3.2. Ferric reducing antioxidant power*

The antioxidant potential of soursop flower extracts was also evaluated by their ability to reduce iron (III) to iron (II) following the method of Oyaizu [19]. An aliquot of 0.5 ml plant extract (125, 250, 500, 1000, and 2000 μg/ml) was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% aqueous $K_3Fe(CN)_6$ solution, well shaken, and incubated at 50 °C for 30 min. After incubation, 1 ml of 10% TCA solution was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 min. A total of 1.5 ml of supernatant, 1.5 ml of distilled water, and 0.1 ml of 0.1% $FeCl_3$ solution were mixed and incubated for 10 min and absorbance read at 700 nm on a spectrophotometer. A sample blank, containing all the reagents but no extract, was prepared in the same conditions. Catechin, a recognized powerful ferric reducer, was used as positive control to compare the reducing power of the extracts. A higher absorbance indicates a higher reducing power.

*3.1.3.3. Antioxidant activity in the linoleic acid system*

The antioxidant activity of the extract was also evaluated in a linoleic acid emulsion system as described by Iqbal and Bhanger [4]. A 0.02 M linoleic acid emulsion solution was prepared by adding 0.2804 g of linoleic acid and 0.2804 g of Tween 20 in 50 ml of a 0.05 M potassium phosphate buffer solution (pH 7.4). A total of 2.5 ml of the prepared emulsion was transferred in a 15 ml volume test tube containing 0.2 ml of 5 mg/ml extract solution and 2.3 ml of a 0.2 M potassium phosphate buffer (pH 7). The resulting solution was mixed with a homogenizer and incubated in the dark at 37 °C for 312 h, and the degree of oxidation measured at 500 nm using the thiocyanate method [20]. The percentage of inhibition of linoleic acid peroxidation was calculated according to the following formula:

$$(\%)inhibition = 100 - \left[\frac{\text{Absorbance increase of sample}}{\text{Absorbance increase of control}} \times 100\right]$$

*3.1.4. Effect of green tea leaves extract on palm olein oxidation*

*3.1.4.1. Sample preparation*

The samples were prepared according to the method of Iqbal et al. [21]. The crude concentrated methanolic extract was dissolved in 1 ml of methanol and separately added to 100 g of preheated palm olein (at 50 °C for 3 h) at concentrations of 200, 600, 1000, 1400, and 1800 ppm. Synthetic antioxidant, butylated hydroxytoluene, was employed at the legal limit of 200 ppm [22] to compare the efficacy of natural antioxidants. Palm olein free from additives and prepared under the same conditions, served as control. It is important to note that the concentration of added methanol (1 ml/100 g of oil) was similar or less than 10 mg/kg (in Europe) and 50 mg/kg (in Japan), which are the recommended concentrations of methanol to be added in oils and other foods as supplement and additive, respectively [23,24,25]. However, after shaking the oils for 30 min, the samples were stored in the oven at 45 °C for 48 h to reduce the amount of the added methanol to a value less than 10 mg/kg as recommended by the regulations. After this storage, each sample was separated into two portions, 10 and 90 g, for Rancimat and Schaal oven tests, respectively.

*3.1.4.2. Rancimat test*

Induction times of control and palm olein samples supplemented with antioxidants were determined using an automated Metrohm Rancimat instrument (Model 892). Each oil sample (≥5 g) was separately weighted in a Rancimat test tube. The instrument was switched on and the heating block temperature set at 110 °C. After reaching 110 °C, the measuring vessels filled with 60 ml deionized water were connected to the instrument via electrodes. The tubes containing the sample were sealed using the appropriate caps, and connected to both instrument and measuring vessels via the appropriate cables. After starting the gas flow (20 l/h), the reaction tubes were individually placed in their respective heating blocks and the reaction started. Induction time, the time elapsed from the beginning until the oil starts to become rancid, was automatically recorded by the instrument, and the protection factors calculated following the formula: protection factor = induction time of oil with antioxidant/induction time of oil without antioxidant [26].

*3.1.4.3. Schaal oven test*

The method used by Sultana et al. [35] has been used with slight modifications. Stabilized and control oil samples (90 g) 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 h heating cycle per day) for 30 days. Samples were collected every 10 days and stored in the refrigerator for further analysis. The oxidative deterioration level of oil samples was assessed by measuring peroxide, *p*-anisidine, TOTOX, thiobarbituric acid, and iodine values, as well as some changes in the fatty acid profile of oils.

*3.1.4.4. Measurement of oxidation parameters*

Determination of the peroxide value of stabilized and control palm olein samples were made following the spectrophotometrical IDF standard method, 74A: 1991 [27]. *p*-Anisidine and iodine values assays were carried out according to the procedure of AOCS Official Method CD 18–90 and CD 1–25, respectively [28]. Total oxidation (TOTOX) values were calculated using the equation TOTOX = 2PV + AV according to Shahidi and Wanasundara [29]. Thiobarbituric acid value was evaluated as described by Draper and Hadley [30].

*3.1.4.5. Changes in linoleic acid profile of oil during storage*

*3.1.4.5.1. Preparation of fatty acid methyl esters.* Fatty acid methyl esters (FAMEs) of stabilized and control palm olein samples were prepared by transesterification using 2% sulphuric acid in methanol [31]. The FAMEs were extracted into ethyl acetate and thoroughly washed with water to make them free of acid and dried over anhydrous sodium sulphate. The dried esters were analysed in gas chromatograph coupled to flame ionization detector GC/FID.

*3.1.4.5.2. Gas chromatography.* The GC/FID analyses were performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 7890A series gas chromatograph equipped with a FID detector using a DB-225 capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The column temperature was initially maintained at 160 °C for 2 min, increased to 220 °C at 5 °C/min and maintained for 10 min at 220 °C. The carrier gas was nitrogen at a flow rate of 1.5 ml/min. The injector and detector temperatures were maintained at 230 °C and 250 °C, respectively, with a split ratio of 50:1. Identification of linoleic acid was based on comparison of its retention time with that of standard reference fatty acid methyl esters ($C_{4-24}$) performed under the same conditions.

*3.1.5. Statistical analysis*

Results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Dunnet and Student–Newman–Keuls tests using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at $p < 0.05$ was considered statistically significant.

## 4. Results and discussion

*4.1. Extraction and phytochemical characterization of the extract*

*4.1.1. Extraction yield and total phenolics content*

The extraction yield and total phenolics content of the extract were found to be 11.1% and 53.5 mg GAE/g of extract, respectively. The yield obtained in this study was significantly higher compared to that found by Yen and Chen [32], who showed aqueous green tea leaves extract yield to be 3.9%. Concerning the total phenolics content, the registered value was not far from that reported by Izzreen and Fadzelly [33], who demonstrated that the total phenolics content of mature green tea leaves was 63.9 mg EQ/g of extract. The variations observed in these parameters compared to those of the literature might be attributed to environmental differences (climate, location, and harvest period), nature of the solvent used, extraction procedure, and the state of maturity of the plant [34,35].

*4.1.2. HPLC-DAD and ESI-MS analysis*

The HPLC analysis of old green tea leaves methanolic extract is shown in Fig. 1A. The peaks 1, 2, 3, and 4 were found to match well with gallic acid (RT: 7.765 min), gallocatechin (RT: 8.903 min), epicatechin gallate (RT: 9.225 min), and epigallocatechin gallate (9.995 min), respectively. The presence of gallic acid and catechin derivatives in

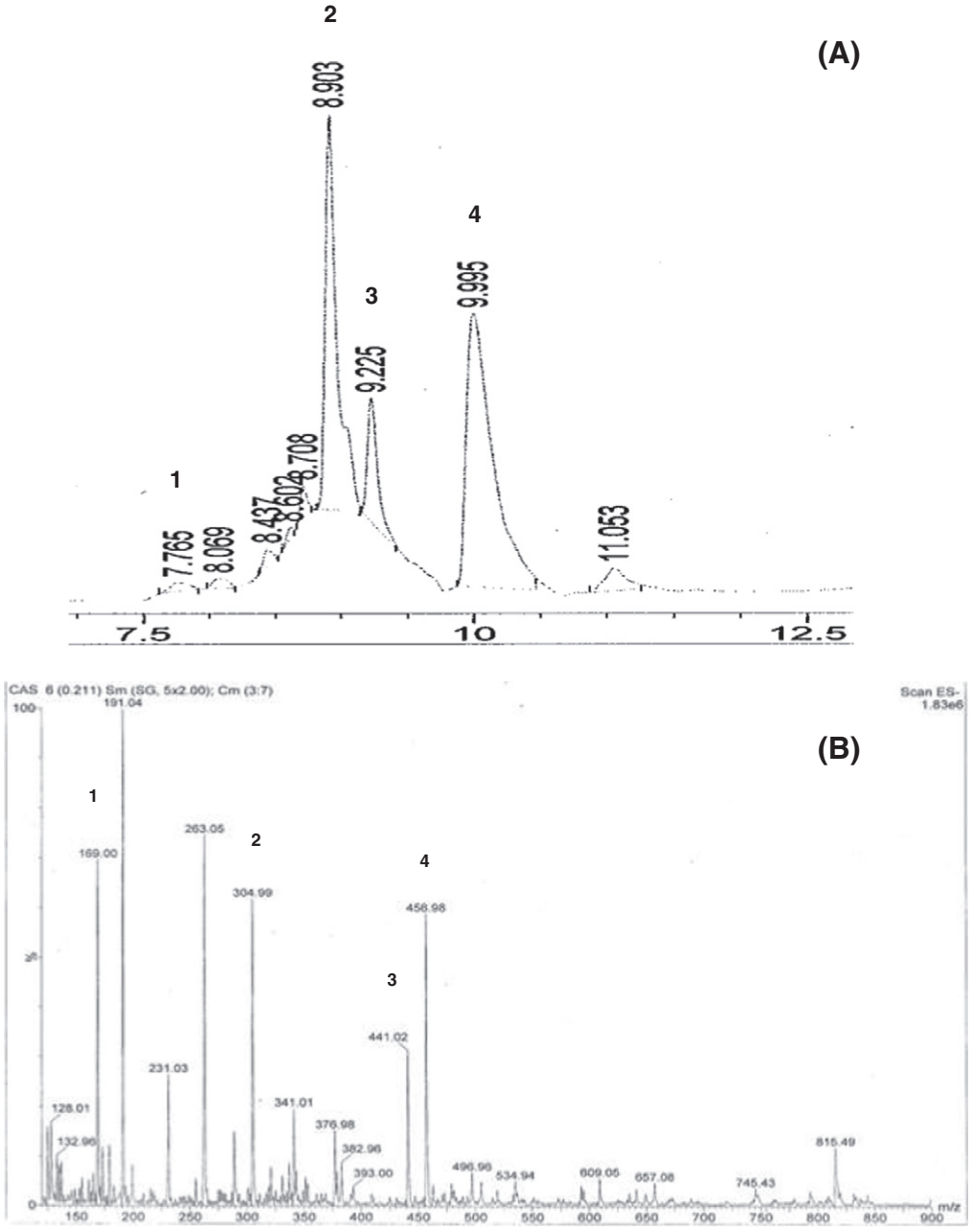


**Fig. 1.** (A, B): HPLC-DAD (A) and ESI-MS (B) chromatograms of green tea leaves methanolic extracts. Peak 1 = gallic acid, peak 2 = gallocatechin, peak 3 = epicatechin gallate, and peak 4 = epigallocatechin gallate.

this extract is in agreement with the finding of Goksu and Poyrazoglu [36], who detected similar compounds in green tea leaves by HPLC in caffeinated and decaffeinated teas. The HPLC peak of gallic acid (peak 1) was very small, while that of gallocatechin (peak 2), epigallocatechin gallate (peak 4), and epicatechin gallate (peak 3) were high. This generally reflects the abundance of each compound in the extract. The fact that green tea leaves gallic acid content is very small and that of epigallocatechin gallate and epicatechin gallate abundant has previously been reported by Dionex [37]. However, the abundance of gallocatechin, which reflected its higher amount in the studied extract, is not in agreement with the findings of Goksu and Poyrazoglu [36] and Dionex [37], which obtained very small peak heights of this compound in green tea leaves extract. This difference could be due to many factors such as genetic characteristics of the plant, environmental differences, extraction procedure, and its state of maturity. Generally, these results are also supported by the finding of Kajiya et al. [14], who reported that green tea leaves phenolic compounds are gallic acid, epicatechin, gallocatechin, epigallocatechin epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate. They have also mentioned these compounds, especially epigallocatechin gallate, to be responsible for the good antioxidant activity of tea leaves.

In order to confirm the HPLC-DAD results, ESI-MS was also conducted to detect the presence of compounds on the basis of their molecular weight. The different fragments obtained are presented in Fig. 1B. By examining the mass spectra, the molecular peak 1 [$m/z$ (M-1) = 169)] was found to be in agreement with deprotonated gallic acid (MW: 170.12 g/mol), peak 2 ($m/z$ = 304.99) to be deprotonated gallocatechin (MW = 306.24 g/mol), peak 3 ($m/z$ = 441.02) to be deprotonated epicatechin gallate (MW = 442.37 g/mol), and peak 4 ($m/z$ = 456.98) to be deprotonated epigallocatechin gallate (MW = 458.37 g/mol). These results confirm the presence of gallic acid and catechin derivatives in this extract.

### 4.2. Preliminary antioxidant tests

#### 4.2.1. 2,2-Diphenyl-1-picryl hydrazyl test (DPPH)

The measurement of the ability of a molecule or substance to scavenge free radicals has become routine in testing the antioxidant property of plant extracts. This mechanism of action is the fingerprint of primary antioxidants. The DPPH radical has the property to abstract labile hydrogen atoms, and the ability of an antioxidant to stabilize this radical by donating its hydrogen is related to its potential capability to inhibit lipid oxidation [38]. The ability of tea leaves extract to scavenge the DPPH radical in comparison to the BHT is presented in Fig. 2A. It is observed that the radical scavenging power of both the extract and BHT increases with the concentration. No significant difference ($p > 0.05$) was observed in the antioxidant activity of both products at concentrations of 12.5, 25, and 200 μg/ml. It is clear from these results that our plant extract, like BHT, is a powerful free radical scavenger. The phenolic antioxidants previously detected might be responsible for the observed effect. In many studies, these phenolic compounds have been reported to be related to the antioxidant activity of plant extracts through this mechanism of action [39]. It is also generally believed that the total number of hydroxyl groups present in the aromatic constituents of the extract, as well as their positions, offer better antioxidative properties. Similar results have been obtained by Izzreen and Fadzelly [33] and Goksu and Poyrazoglu [36].

#### 4.2.2. Ferric reducing antioxidant power (FRAP)

The efficacy of a molecule or substance to reduce $Fe^{3+}$ into $Fe^{2+}$ by donation of its electron is also known as a good indicator of its antioxidant activity. The reaction leads to the formation of a pearl's Prussian blue, which is absorbed at 700 nm [40]. FRAP activity of tea leaves in comparison to BHT and catechin is presented in Fig. 2B. Catechin has been used as a control because it is a recognized powerful ferric reducer. It is noted from the figure that the FRAP activity is

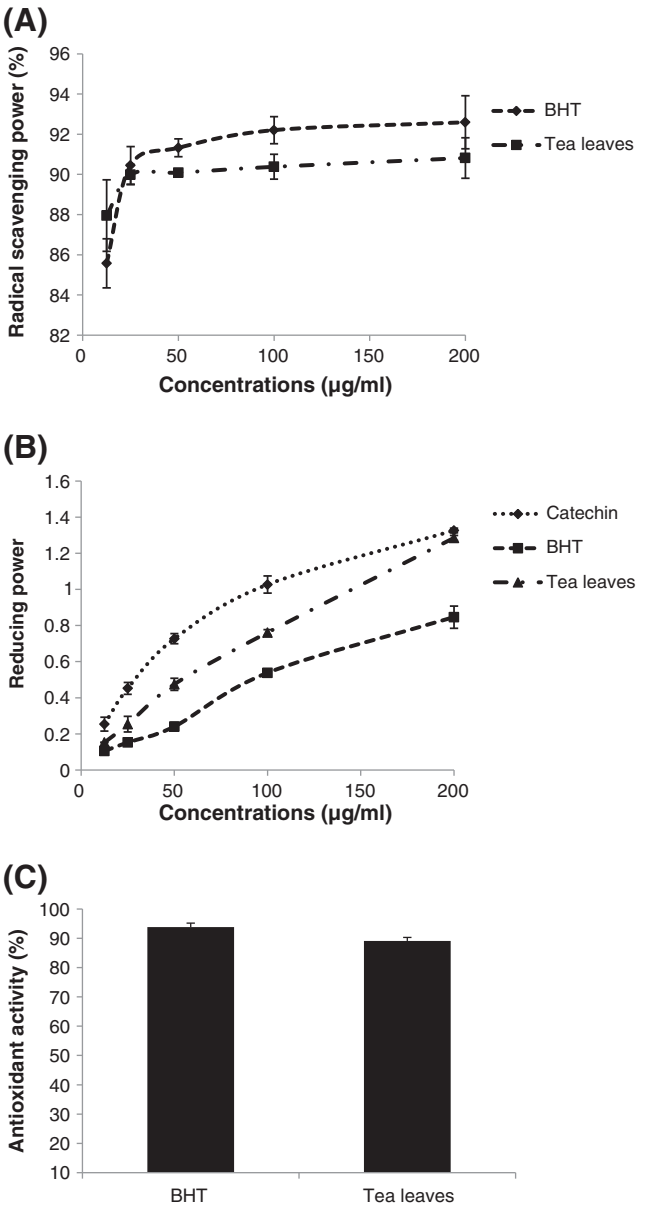


**Fig. 2.** (A–C): Antioxidant tests: DPPH radical scavenging activity (A), ferric reducing antioxidant power (B), and antioxidant activity in linoleic acid system (C) of the extract.

concentration-dependent. At all concentrations, catechin has exhibited the highest FRAP activity, followed by tea leaves extract and BHT. This indicates that our plant extract is also a powerful ferric reducer. From these results, it can be seen that BHT is a lower metal reducer, its activity was significantly lower ($p < 0.05$) than that of the other products. The FRAP activity of the extract might be attributed to the presence of phenolic compounds detected in the extract, mainly their flavonoids, as it has been already proven that flavonoids are powerful metal reducers [41]. These results are in agreement with those of Izzreen and Fadzelly [33], who also demonstrated that shoots, young and mature green tea leaves are powerful ferric reducers, with the respective ferric reducing powers ranging within 13.03–14.83 μmol $Fe_2SO_4$, 7 $H_2O$/ml.

#### 4.2.3. Antioxidant activity in the linoleic acid system

The effect of tea leaves extract in inhibiting linoleic acid peroxidation in comparison to BHT is illustrated in Fig. 2C. The inhibition percentages were found to be 93.8% and 89.0% for BHT and the extract, respectively. In fact, both percentages were quite comparable because the differences were statistically non-significant ($p < 0.05$). This is the proof of the

ability of tea leaves extract antioxidants to protect linoleic acid from oxidation at the same level as BHT, by donating its hydrogen atoms for the stabilization of formed free radicals. This activity might be attributed to the presence of gallic acid and catechin derivatives previously detected by HPLC-DAD and ESI-MS. These results are in agreement with the findings of Anwar et al. [7,42] and Iqbal and Bhanger [4], who have reported that the total phenolic content of various fruits, vegetables, herbs, spices, and agrowastes contained potential antioxidant activities against the linoleic acid peroxidation system.

### 4.3. Effect of tea leaves extract on palm olein stability

#### 4.3.1. Rancimat test

The Rancimat test is a technique based on the conductometric determination of volatile degradation products, which is currently used for the evaluation of the oxidative stability of oils and fats as well as to the study of antioxidant potentiality of new molecules [43]. Longer induction times indicate higher resistance to oxidation or good efficiency of the added antioxidants. The effects of the extract on the induction times and protection factors of palm olein in comparison with the control and oil enriched with BHT are presented in Table 1. It was clearly observed that the added extract, at all concentrations, had significantly ($p < 0.05$) prolonged the induction time (IT) of oxidation and protection factors (PF) of palm olein (IT ≥ 24.8 h; PF ≥ 1.2), compared to control (IT = 20.1 h; PF = 1.0), and PO + $BHT_{200\ ppm}$ (IT = 22.7 h; PF = 1.1). Its activity increased with its concentration. Similar results have been reported by Abd-El Ghany et al. [44], who showed that sunflower oil supplemented with olive cakes extract at concentrations of 100, 200, 400, and 600 ppm exhibited Rancimat induction times of 10.6, 12.5, 17.8, and 25.5 h, respectively, compared to the control (8.0 h) and sunflower oil enriched with BHT at a concentration of 200 ppm (10.0 h). These observations might be due to the good thermal stability of the antioxidant detected in that extract compared to BHT. In fact, the instability of BHT and BHA at elevated temperatures has been previously reported by Chang et al. [5] and Thorat et al. [6].

#### 4.3.2. Schaal oven test

*4.3.2.1. Peroxide value.* Peroxide value is commonly used to assess the primary oxidation state of oils and fats. A continuous increase in peroxide values with the increase of the storage time was observed for all the samples (Table 1). This increment could be attributed to the formation of hydroperoxides, which are the principal markers of primary oxidation of lipids. Palm olein without antioxidant (control), exhibited the highest peroxide value ($p < 0.001$) than those containing BHT or tea leaves extract (Ca.S) as preservatives during the complete period of storage. The lowest peroxide value was obtained with PO + $Ca.S_{1800\ ppm}$ and PO + $BHT_{200\ ppm}$. No significant difference ($p > 0.05$) was noted in the PV values of palm olein supplemented with the extract at concentrations 1400, 1000, 600, and 200 ppm from days 0 to 30, and their peroxide values were not far from that of PO + $BHT_{200\ ppm}$. Significantly lower PV ($p < 0.001$) in palm olein containing our natural antioxidant in comparison with the control indicated the highest antioxidant activity of this extract in retarding peroxide formation during storage. This activity could be attributed to the detected phenolic antioxidant compounds through their ability to stop free radicals by donating their hydrogen atoms or electrons. These results are in accordance with those of Navas et al. [12], where similar activity in corn oil with black tea leaves extracts was reported. In fact, these authors demonstrated that during the storage of corn oil for 7 days at 55 °C and 2 days at 140 °C, oil supplemented with black tea leaves extract exhibited lowest peroxide values (ranging between 2–5 and 25–50 meq/kg for storage at 55 °C and 140 °C, respectively) compared to the same oil without antioxidants (ranging between 2–60 and 100–250 meq/kg for storage at 55 °C and 140 °C, respectively). Many other authors have proven the same activity with other plants methanolic extracts in soybean and sunflower oils [8,21,42].

*4.3.2.2. p-Anisidine value.* The data of *p*-anisidine value, which generally reflect the amount of aldehydic secondary oxidation products in oils, is presented in Table 1. The control oil sample exhibited the significantly highest ($p < 0.001$) *p*-anisidine value compared to oil samples containing BHT or different concentrations of tea leaves extract as antioxidants. This indicates the higher rate of secondary oxidation in control samples. From days 0 to 20, no significant differences ($p > 0.05$) were observed in *p*-anisidine values of stabilized palm olein samples. At the 30th day, a significant increase in anisidine value was noted in PO + $BHT_{200\ ppm}$; while the anisidine values of the other stabilized samples remained similar, showing the rapid formation of secondary oxidation product in PO + $BHT_{200\ ppm}$ than PO samples containing the natural extracts. From these results, it is clear that this extract is also most active in retarding secondary oxidation products formation in palm olein. This activity could be due to the variable phenolic antioxidants identified as present in the extract, as in previous studies, the ability of these compounds in retarding secondary oxidation products formation in edible oils have been demonstrated [7,9].

*4.3.2.3. TOTOX value.* Table 1 also presents the evolution of total oxidation of palm olein samples during storage. The TOTOX values of all the samples were increasing in a regular pattern over the storage time. As previously observed with peroxide and *p*-anisidine values, the controls exhibited the highest total oxidation. Palm olein containing BHT and tea leaf extracts were much less oxidized than the controls. However, TOTOX values of palm olein samples supplemented with methanolic extract of tea leaves were not far from that of BHT, meaning that this

**Table 1**
Induction time, protection factor of oil samples and their changes in peroxide, *p*-anisidine, and TOTOX values during 30 days of storage at 70 °C.

| Characteristic | Day | Control | PO + $BHT_{200\ ppm}$ | PO + $Ca.S_{200\ ppm}$ | PO + $Ca.S_{600\ ppm}$ | PO + $Ca.S_{1000\ ppm}$ | PO + $Ca.S_{1400\ ppm}$ | PO + $Ca.S_{1800\ ppm}$ |
|---|---|---|---|---|---|---|---|---|
| Induction time (h) | / | $20.1 \pm 0.14^{a}$ | $22.7 \pm 0.36^{b}$ | $24.8 \pm 1.03^{c}$ | $26.2 \pm 0.66^{c}$ | $27.9 \pm 0.21^{d}$ | $28.8 \pm 0.48^{d}$ | $28.9 \pm 1.13^{d}$ |
| Protection factor | / | $1.0 \pm 0.00^{a}$ | $1.1 \pm 0.00^{b}$ | $1.2 \pm 0.04^{c}$ | $1.3 \pm 0.00^{c}$ | $1.4 \pm 0.00^{d}$ | $1.4 \pm 0.03^{d}$ | $1.4 \pm 0.04^{d}$ |
| Peroxide value (meq/kg) | 0 | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ | $8.9 \pm 1.23^{a}{}_{A}$ |
| | 10 | $27.7 \pm 0.26^{a}{}_{B}$ | $13.8 \pm 0.00^{b}{}_{B}$ | $18.8 \pm 0.47^{c}{}_{B}$ | $17.8 \pm 2.75^{c}{}_{B}$ | $16.7 \pm 1.23^{c}{}_{B}$ | $16.5 \pm 0.63^{c}{}_{B}$ | $12.3 \pm 0.22^{b}{}_{B}$ |
| | 20 | $27.9 \pm 1.13^{a}{}_{B}$ | $18.8 \pm 0.32^{b}{}_{C}$ | $21.1 \pm 0.10^{c}{}_{BC}$ | $21.4 \pm 0.76^{c}{}_{C}$ | $21.4 \pm 0.30^{c}{}_{C}$ | $21.4 \pm 0.53^{c}{}_{C}$ | $18.0 \pm 0.60^{d}{}_{C}$ |
| | 30 | $30.3 \pm 0.17^{a}{}_{C}$ | $23.3 \pm 0.64^{b}{}_{D}$ | $23.6 \pm 1.08^{b}{}_{C}$ | $23.9 \pm 0.92^{b}{}_{C}$ | $22.4 \pm 0.78^{b}{}_{C}$ | $21.8 \pm 0.63^{c}{}_{C}$ | $19.7 \pm 1.26^{c}{}_{C}$ |
| *p*-Anisidine value | 0 | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{A}$ | $7.8 \pm 0.77^{a}{}_{AB}$ |
| | 10 | $12.0 \pm 0.93^{a}{}_{B}$ | $7.8 \pm 0.24^{b}{}_{A}$ | $7.2 \pm 0.15^{b}{}_{A}$ | $7.3 \pm 0.15^{b}{}_{A}$ | $7.0 \pm 1.20^{b}{}_{A}$ | $7.5 \pm 0.00^{b}{}_{A}$ | $7.1 \pm 0.00^{b}{}_{A}$ |
| | 20 | $12.0 \pm 0.00^{a}{}_{B}$ | $8.5 \pm 0.72^{b}{}_{A}$ | $7.2 \pm 1.60^{b}{}_{A}$ | $7.6 \pm 1.28^{b}{}_{A}$ | $7.9 \pm 0.00^{b}{}_{A}$ | $7.4 \pm 0.00^{b}{}_{A}$ | $7.6 \pm 0.00^{b}{}_{B}$ |
| | 30 | $14.6 \pm 0.00^{a}{}_{C}$ | $11.8 \pm 0.06^{b}{}_{B}$ | $7.9 \pm 0.11^{c}{}_{A}$ | $8.7 \pm 1.02^{c}{}_{A}$ | $8.4 \pm 0.00^{c}{}_{AB}$ | $7.6 \pm 1.00^{c}{}_{A}$ | $7.8 \pm 1.00^{c}{}_{B}$ |
| TOTOX value | 0 | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ | $25.6 \pm 1.31^{a}{}_{A}$ |
| | 10 | $67.2 \pm 1.47^{a}{}_{B}$ | $34.8 \pm 0.73^{b}{}_{B}$ | $45.7 \pm 1.10^{c}{}_{B}$ | $42.8 \pm 3.67^{c}{}_{B}$ | $40.5 \pm 2.50^{c}{}_{B}$ | $40.6 \pm 1.27^{c}{}_{B}$ | $31.7 \pm 0.44^{d}{}_{B}$ |
| | 20 | $69.7 \pm 2.27^{a}{}_{B}$ | $46.0 \pm 0.90^{b}{}_{C}$ | $49.5 \pm 1.81^{b}{}_{BC}$ | $50.4 \pm 2.81^{b}{}_{BC}$ | $50.6 \pm 0.05^{b}{}_{C}$ | $50.2 \pm 1.07^{b}{}_{C}$ | $43.5 \pm 1.21^{c}{}_{C}$ |
| | 30 | $75.3 \pm 0.35^{a}{}_{C}$ | $58.3 \pm 1.34^{b}{}_{D}$ | $55.1 \pm 2.28^{bc}{}_{C}$ | $56.4 \pm 2.86^{b}{}_{C}$ | $53.1 \pm 1.56^{bc}{}_{C}$ | $51.3 \pm 2.54^{bc}{}_{C}$ | $47.2 \pm 3.91^{c}{}_{C}$ |

Data are presented as mean (±SD) ($n = 3$) $^{(a–d)}$ Means within each row for each parameter with different superscripts are significantly ($p < 0.05$) different. (A–C) Means within each column for each parameter with different superscripts are significantly ($p < 0.05$) different. (Control: palm olein without antioxidant; PO + BHT 200 ppm: palm olein containing BHT as antioxidant at concentration of 200 ppm; PO + $Ca.S_{200}$: palm olein supplemented with the extract at a concentration of 200 ppm).

extract significantly limited the oxidation of palm olein during storage. As previously mentioned, antioxidant compounds detected in this extract by HPLC and ESI-MS could be responsible of the observed protective effect. These results are in accordance with those of Nyam et al. [9], who have proven that extracts of kenaf and roselle (at 1500 ppm) significantly limit the total oxidation of sunflower oil during an accelerated storage at 65 °C for 24 days.

*4.3.2.4. Thiobarbituric acid value.* Thiobarbituric acid assay is the most widely used method for the measurement of secondary oxidation products, which may contribute to the off-flavour in oxidized oils [4]. The changes in TBA value of stabilized and control palm olein samples are presented in Table 2. A continuous increase in TBA value was noted for all the samples with increasing heating time. It is noted that at all stages of analyses during the storage, controls exhibited the highest TBA value, while the values in palm olein samples supplemented with synthetic or natural antioxidants were significantly lower ($p < 0.001$). TBA values of oil samples containing tea extracts as antioxidants were near those of PO + $BHT_{200\ ppm}$ during the storage; proof of the low formation of malonaldehyde in these samples, the main compound detected by this method. These results confirm the efficiency of our natural antioxidant in limiting the formation of secondary oxidation products in palm olein at the same level as BHT. The antioxidants found in this extract might also be responsible for the inhibition of malonaldehyde formation. Similar results were obtained by Iqbal and Bhanger [4] and Iqbal et al. [21] in sunflower oil supplemented with garlic and pomegranate methanolic extracts, respectively, during accelerated storage at 65 °C for 24 days.

*4.3.2.5. Iodine value.* The iodine value of control and stabilized palm olein samples over an incubation period of 30 days at 70 °C is shown in Table 2. It is observed that this parameter decreased gradually during the storage. The rate of decrement in control was significantly higher ($p < 0.001$) than that of oil containing BHT and tea leaf extracts. The lowest decrement was noted in PO + $Ca.S_{1800\ ppm}$. A decrement of the iodine value of oils is generally attributed to the destruction of the fatty acid double bonds caused by oxidation reactions [45]. The lowest decrement of the iodine value in stabilized palm olein samples compared to the control might be the consequence of the protective action of these antioxidants on the unsaturated fatty acids of oil. Womeni et al. [8] have shown that some Cameroonian spices methanolic extracts and powders were able to limit the alteration of fatty acid double bonds of soybean oil during an accelerated storage at 65 °C for 24 days. Similar results have also been reported by Nyam et al. [9] with sunflower oil after the addition of roselle and kenaf extract and heating for 24 days at 65 °C.

*4.3.2.6. Effects of tea leaf extract on the linoleic acid profile of palm olein.* The effect of tea leaves on the linoleic acid profile of palm olein during storage is presented in Table 2. At the start, the linoleic acid percentage of non-heated palm olein was 11.0%. After 30 days of heating, these values significantly decreased until 10.0%, 9.2%, 10.4%, 10.6%, 10.9%, 10.9% and 10.9%, respectively, for PO, PO + $BHT_{200\ ppm}$, PO + $Ca.S_{200\ ppm}$, PO + $Ca.S_{600\ ppm}$, PO + $Ca.S_{1000\ ppm}$, PO + $Ca.S_{1400\ ppm}$, and PO + $Ca.S_{1800\ ppm}$. As previously mentioned with the iodine value, this decrement might be the consequence of the rapid oxidation of linoleic acid in favour of primary and secondary oxidation products. It appears clear that the linoleic acid percentage of oil containing the extract was significantly higher than that of the control and PO + $BHT_{200\ ppm}$ at the 30th storage day, showing that linoleic acid was most preserved by the natural extract from oxidative degradation. Similar results have been reported by Che Man and Tan [16] who showed that, after 7 days of frying potatoes chips, the linoleic acid content of palm olein supplemented with BHA, BHT, oleoresin rosemary, and sage extracts were 5.89%, 6.25%, 6.25%, and 6.11%, respectively, compared to that of the same oil without antioxidant, which was 2.93%.

## 5. Conclusion

From the present study, it can be concluded that old Cameroonian green tea leaf methanolic extracts, at concentrations of 200, 600, 1000, 1400, and 1800 ppm, can stabilize palm olein. Its efficiency was higher than that of BHT in the Rancimat test. This might be the proof of its good thermal stability. In the Schaal oven test, this efficiency was similar to that of BHT. It was also observed that these extracts were efficient in limiting linoleic acid degradation during storage. Therefore, on behalf of this study, old Cameroonian green tea leaves can be recommended as a potent source of natural antioxidants for stabilization of palm olein and other oils.

## Conflict of interest

None.

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**Table 2**
Changes in TBA, iodine values and linoleic acid content of RBD palm olein during 30 days of storage at 70 °C.

| Characteristic | Day | Control | PO + $BHT_{200\ ppm}$ | PO + $Ca.S_{200\ ppm}$ | PO + $Ca.S_{600\ ppm}$ | PO + $Ca.S_{1000\ ppm}$ | PO + $Ca.S_{1400\ ppm}$ | PO + $Ca.S_{1800\ ppm}$ |
|---|---|---|---|---|---|---|---|---|
| TBA value (ppm) | 0 | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ | $0.85 \pm 0.00^{a}{}_{A}$ |
| | 10 | $1.94 \pm 0.01^{a}{}_{B}$ | $0.93 \pm 0.09^{bc}{}_{A}$ | $1.07 \pm 0.01^{b}{}_{B}$ | $0.96 \pm 0.00^{c}{}_{B}$ | $0.90 \pm 0.03^{c}{}_{A}$ | $0.87 \pm 0.07^{c}{}_{A}$ | $0.84 \pm 0.01^{c}{}_{A}$ |
| | 20 | $2.00 \pm 0.00^{a}{}_{B}$ | $1.00 \pm 0.17^{b}{}_{A}$ | $1.13 \pm 0.02^{b}{}_{C}$ | $0.98 \pm 0.02^{b}{}_{B}$ | $0.90 \pm 0.08^{b}{}_{A}$ | $0.92 \pm 0.00^{b}{}_{A}$ | $0.87 \pm 0.00^{b}{}_{A}$ |
| | 30 | $2.22 \pm 0.06^{a}{}_{C}$ | $1.09 \pm 0.02^{b}{}_{A}$ | $1.36 \pm 0.00^{c}{}_{D}$ | $1.16 \pm 0.01^{b}{}_{C}$ | $1.16 \pm 0.00^{b}{}_{B}$ | $0.91 \pm 0.02^{d}{}_{A}$ | $0.90 \pm 0.07^{d}{}_{A}$ |
| Iodine value (g $I_2$/100 g) | 0 | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ | $57.7 \pm 0.04^{a}{}_{A}$ |
| | 10 | $56.9 \pm 0.00^{a}{}_{B}$ | $57.5 \pm 0.10^{b}{}_{B}$ | $57.2 \pm 0.07^{c}{}_{B}$ | $57.5 \pm 0.10^{b}{}_{B}$ | $57.7 \pm 0.07^{b}{}_{A}$ | $57.9 \pm 0.12^{b}{}_{A}$ | $57.9 \pm 0.01^{b}{}_{B}$ |
| | 20 | $55.9 \pm 0.03^{a}{}_{C}$ | $57.1 \pm 0.05^{bc}{}_{C}$ | $57.1 \pm 0.03^{bc}{}_{B}$ | $57.1 \pm 0.12^{bc}{}_{C}$ | $57.5 \pm 0.07^{d}{}_{A}$ | $57.1 \pm 0.15^{bc}{}_{B}$ | $57.0 \pm 0.08^{c}{}_{C}$ |
| | 30 | $55.2 \pm 0.07^{a}{}_{D}$ | $56.4 \pm 0.05^{b}{}_{B}$ | $56.8 \pm 0.10^{c}{}_{C}$ | $56.6 \pm 0.08^{d}{}_{D}$ | $57.1 \pm 0.07^{e}{}_{B}$ | $56.1 \pm 0.05^{f}{}_{B}$ | $57.0 \pm 0.01^{e}{}_{C}$ |
| Linoleic acid profile (%) | 0 | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ | $11.0 \pm 0.03^{a}{}_{A}$ |
| | 10 | $10.4 \pm 0.01^{a}{}_{B}$ | $10.9 \pm 0.06^{b}{}_{A}$ | $10.6 \pm 0.02^{c}{}_{B}$ | $10.0 \pm 0.07^{d}{}_{B}$ | $11.1 \pm 0.07^{b}{}_{B}$ | $10.7 \pm 0.03^{c}{}_{B}$ | $10.7 \pm 0.02^{c}{}_{B}$ |
| | 20 | $10.1 \pm 0.00^{a}{}_{C}$ | $10.5 \pm 0.03^{b}{}_{B}$ | $10.6 \pm 0.01^{b}{}_{B}$ | $10.4 \pm 0.04^{b}{}_{C}$ | $10.6 \pm 0.04^{b}{}_{C}$ | $10.6 \pm 0.13^{b}{}_{B}$ | $10.6 \pm 0.05^{b}{}_{C}$ |
| | 30 | $10.0 \pm 0.05^{a}{}_{C}$ | $9.2 \pm 0.06^{b}{}_{C}$ | $10.6 \pm 0.08^{c}{}_{C}$ | $10.6 \pm 0.06^{d}{}_{D}$ | $10.9 \pm 0.04^{e}{}_{A}$ | $10.9 \pm 0.01^{e}{}_{A}$ | $10.9 \pm 0.01^{e}{}_{D}$ |

Data are presented as mean (±SD) ($n = 3$) $^{(a-e)}$ Means within each row for the same parameter with different superscripts are significantly ($p < 0.05$) different. (A–D) Means within each column for the same parameter with different superscripts are significantly ($p < 0.05$) different. (Control: palm olein without antioxidant; PO + BHT 200 ppm: palm olein containing BHT as antioxidant at concentration of 200 ppm; PO + $Ca.S_{200}$: palm olein supplemented with the extract at concentration of 200 ppm.

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