

OXIDATIVE STABILITY OF COTTONSEED OIL ENRICHED WITH CAMEROONIAN PLANT LEAVES EXTRACTS

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Abstract

This work aimed to assess the oxidative stability of cottonseed oil enriched with natural antioxidants from Cameroonian plant leaves extracts. Total polyphenols, flavonoids, and antioxidant activity via DPPH radical scavenging assay were assessed in ethanolic extracts. These extracts were added to cottonseed oil at concentrations of 200, 500, 1250, and 2000 ppm. The cottonseeds' oil oxidative stability was evaluated by determining their induction/stability times on Rancimat (120±1.6°C) and by measuring their oxidative state in Schaal oven during 30 days (samples were removed after 10 days) of storage at 60°C. The outcomes showed that *Canarium schweinfurthii*, *Trichoscypha acuminata*, *Dacryodes edulis*, and *Psidium guajava* leaves extracts are rich in polyphenols (18.56 to 42.70 mg/g) and flavonoids (2.14 to 5.36 mg/g). The ethanolic extracts, above 1250 ppm, were also efficient like synthetic antioxidants in inhibiting the oxidation of cottonseeds oil at high temperatures. The evaluation of oxidation parameters of enriched cottonseeds oil showed that different extracts at 2000 ppm can limit the appearance of both primary and secondary oxidation products in oils.

Keywords: plant leaves extract, antioxidant, cottonseeds oil, oxidation, Rancimat test, phytochemical content

Introduction

Nowadays, the food needs to be healthy and cheaper, perceived as natural, with a long shelf life, and at the same time it must preserve nutritional and sensory properties. In this context, many agri-food industries wish to develop products that

meet social demand and public health recommendations (Roman, 2012). Thus, food additives are used to improve food quality. They are added to perform certain technological purposes (safety, freshness, taste, color, texture, appearance), which consumers often take for granted. Food ingredients can be derived from plants, animals, minerals, or synthetic (Roca *et al.*, 2010). Although the food additive industry is more devoted to developing synthetic molecules due to their stability, attractive color, and low cost, natural food additives are gradually being preferred due to the changing consumers' lifestyles and increased concerns about potential adverse health effects and environmental damage caused by synthetic (Hou, 2003; Albuquerque *et al.*, 2020). For instance, recent reports reveal those synthetic antioxidants such as tert-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), that are used to overcome the stability problem of oils are involved in several health hazards, such as cancer and carcinogenesis (Botterweck *et al.*, 2000; Hou, 2003). Due to these safety concerns, industries are increasingly interested in finding natural sources of food ingredients. The use of plant extracts containing bioactive compounds is a burgeoning approach to the fortification of products.

Agriculture provides a wide variety of fruits and vegetables, rich sources of nutrients and phytochemical compounds, intended for consumption (Dauchet *et al.*, 2006; Slavin and Lloyd, 2012). However, agriculture and agri-foods by-products such as leaves, peels, seeds, and others which are often neglected, are also excellent sources of food additives like polysaccharides, dietary fibers, natural pigments (chlorophyll, carotenoids, anthocyanins), polyphenols, antioxidants, and antimicrobial compounds (Ayala-Zavala *et al.*, 2010). These phytochemical substances act as systems of natural defenses for their plants, protecting them from infections and microbial invasions and guests, giving them color, aroma, and flavor (Martillanes *et al.*, 2018). They are natural, safe, and may have antioxidant and coloring activities in food applications (Sagar *et al.*, 2018). Despite many plant sources already explored to find effective food additives, very few are used in industry, hence the need to find more.

The fruits of *Trichoscypha acuminata*, *Canarium schweinfurthii*, *Dacryodes edulis*, and *Psidium guajava* are widely consumed in Cameroon, and the leaves of trees are abandoned. The valorization of these agricultural by-products via green chemistry is not yet complete because several studies focused on the pharmacological effects.

This work aimed to assess phytochemical content, antioxidant activity and the ability of these by-products to prevent cottonseed oil oxidation.

Materials and methods

Chemicals and plant materials

Cottonseed oil without additives was obtained from SODECOTON Company Ltd (Garoua, North-Cameroon). Fresh leaves of *Trichoscypha acuminata*, *Canarium schweinfurthii*, *Dacryodes edulis*, and *Psidium guajava* were collected at Dschang,

Menoua Division, West-Cameroon, in April 2018. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium nitrite, sodium carbonate, aluminum chloride, sodium hydroxide, gallic acid, and potassium persulfate achieved from Sigma Aldrich Steinheim, Germany. All the used chemicals and reagents were of analytical reagent grade.

Drying treatment and ultrasound-assisted extraction

Fresh leaves were subjected to oven drying at 45°C until there was no significant weight loss.

The dry leaves were grounded and sieved to obtain particles of 1.0 mm in size. The ground powder obtained was kept at room temperature in polyethylene containers until the extracts obtaining.

The ground powder (50 g) was placed in a tube and mixed separately with ethanol (70%). The mixture was subjected to ultrasound treatment in a water bath (MRC Scientific Instruments, Israel) for 30 min at a temperature of 40-42°C. After extraction, the sample was centrifuged at 5000 × g for 30 min, and the supernatant was collected and stored at 4°C for further analysis.

Phytochemical characterization of the extracts

Total polyphenols content

Folin-Ciocalteu colorimetric method was used for total phenols content determination, as described by Turturica *et al.* (2016). Briefly, 0.2 mL of diluted ethanolic plant extract (1:10) was combined with 1 mL of Folin-Ciocalteu reagent and 15.8 mL of distilled water. The mixture obtained was incubated for 10 minutes at room temperature. After that, 3 mL of 20% sodium carbonate was added, followed by a 1 hour incubation at room temperature. The mixture absorbance was measured at a wavelength of 765 nm. The total polyphenols content calculated was expressed as mg of gallic acid equivalents (GAE) per gram dry weight (D.W.) of extract.

Total flavonoids content

Total flavonoid content was assessed corresponding to the colorimetric method described by Dewanto *et al.* (2002) using catechin as a standard with some modification. A volume of 0.25 mL ethanolic plant extract (diluted 1:10) was added to 1.25 mL distilled water. On the mixture, a volume of 0.075 mL NaNO₂ 5% was added and stored for 5 min at 25°C. After that, a 0.15 mL AlCl₃ 10% was added and stored for 6 min. In the end, the mixture was treated with 0.5 mL (1 mM NaOH) and was diluted to 0.775 mL with distilled water. The absorbance was measured spectrophotometrically at a wavelength of 510 nm. Results were expressed as mg of catechin equivalents (CE) per gram dry weight (D.W.) of extract.

DPPH scavenging activity

The radical scavenging ability of ethanolic extracts was assessed following a colorimetric procedure using a modification of the method described by Almela *et al.* (2006). Briefly, 3.9 mL of DPPH solution was added to 0.1mL of samples (1mg/mL), and the samples were stored in the dark at room temperature for 30

minutes. The absorbance was measured spectrophotometrically at a wavelength of 515 nm. The results were expressed as percentage DPPH RSA (Equation 1).

$$DPPH\ RSA\ \% = \frac{(Absorbance\ control - Absorbance\ Sample)}{Absorbance\ control} \times 100 \quad (1)$$

Rancimat test

The oxidative stability was determined as described by Raikos *et al.* (2016) with some modifications using 892 Professional Rancimat (Metrohm Ltd., Herisau, Switzerland). The ethanolic (70%) extracts of different plants were added to 5 g of refined cottonseed oil at 200, 500, 1250, and 2000 ppm. The samples' induction time was calculated in order to determine the oxidative stability. The samples were maintained to a constant airflow of 20 L/h at 120°C. The stability time was also measured. Synthetic antioxidants (BHT and BHA, 200 ppm) were used to compare with the natural antioxidants' efficacy (Duh and Yen, 1997). A cottonseeds oil sample was used as a control without extracts.

Schaal oven test and analysis of oils

Schaal oven test was done to evaluate oil stability under the accelerated storage condition, as described by Tinello and Lante (2020). In brief, the cotton oil samples without any addition (Control), with the addition of BHT and BHA (200 ppm) and enriched with plant extracts (2000 ppm) were weighed at 40 g each and placed in open glass tubes without headspace and stored in an oven at 60°C for 30 days with forced air circulation. After every ten days, samples were removed from the oven and kept at -18°C before analysis.

The oil sample stability was evaluated by performing the following analysis: peroxide value (PV) and *p*-anisidine value (*p*-AV) using spectrophotometric methods (AOCS Cd 8b-90, 2011; AOCS Cd 18-90, 2011).

The total oxidation value (*Totox* value) of each sample was expressed using equation 2 (Shahidi and Wanasundara, 2008)

$$TOTOX\ value = 2PV + p-AV \quad (2)$$

Statistical analysis

All the measurements were made in triplicate. The one-way analysis of variance (ANOVA) was used and Dunnet and Student-Newman-Keuls tests were applied by using Graphpad-InStat v. 3.05. Mean differences were determined for separation of means showing significant differences at $p < 0.05$.

Results and discussion

Total polyphenols, flavonoids, and DPPH scavenging activity of extracts

Table 1 shows the total polyphenols, flavonoids, and the leaf extract plants' free radical scavenging capacities. *Canarium schweinfurthii* extract showed the highest

polyphenols and flavonoid content ($p < 0.001$), followed by *Dacryodes edulis* and *Psidium guajava* extracts. These results also indicated that extracts with higher polyphenols and flavonoid contents also showed the best antioxidant activities. It is well known that the antioxidant activity of extracts is usually correlated with their content of phenols and flavonoids (Katalinic *et al.*, 2006; Kevers *et al.*, 2007). Indeed, the degree of hydroxylation or total phenol content is essential for good antioxidant efficiency (Roginsky and Lissi, 2005). The predominant mode of antioxidant activity of phenolic compounds is radical scavenging via hydrogen donation (Reyes-Carmona *et al.*, 2005). The results showed that extracts from the plant leaves studied would indicate a strong suspicion that they could constitute a potential source of natural antioxidants used to limit oxidation.

Table 1. Total polyphenols, flavonoids, and DPPH scavenging activity of ethanolic (70%) extract.

Samples	Total polyphenols (mg GAE/g dw)	Total flavonoids (mg CE/g dw)	DPPH RSA (%)
<i>Canarium schweinfurthii</i>	42.70±1.37 ^d	5.368±0.18 ^c	81.36±0.31 ^b
<i>Trichoscypha acuminata</i>	18.56±0.45 ^a	2.140±0.00 ^a	70.44±5.80 ^a
<i>Dacryodes edulis</i>	35.02±1.58 ^c	2.886±0.24 ^b	82.34±1.16 ^b
<i>Psidium guajava</i>	27.92±2.70 ^b	2.596±0.23 ^b	82.40±0.0 ^b

Means with different letters within a column are significantly different ($P < 0.05$).

Oxidation stability of cottonseed oil (CO) supplemented with Cameroonian plant leaves extracts

CO is an ingredient for cake icings and baked goods and is employed for domestic frying and preparation of margarine (butter substitute) (Sekhar and Rao, 2011). CO is a good source of an essential fatty acid (linoleic acid) and contains a reasonable amount of oleic acid (O'Brien *et al.*, 2005). This oil, because of its richness in unsaturated fatty acids, can oxidize at high temperatures. For this purpose, the plant extracts studied were added to evaluate their capacity to limit the oxidation of cottonseed oil at high temperatures. Table 2 shows the induction period and stability time of oils enriched with extracts compared to the oil sample without additives and oils containing BHA and BHT. These results show that the increased parameters evaluated are concentration-dependent because the oil samples enriched with extracts at 2000 ppm exhibited the highest protection factors and induction times. It was also noted that the control has the lowest induction time and stability time with general values that differ significantly ($p < 0.001$) from that of the oil samples enriched at 1250 and 2000 ppm. Above 1250 ppm, oils enriched with extracts exhibited similar induction periods ($p > 0.05$) to oils enriched with synthetic antioxidants. Long induction periods testify to the oil's good resistance to oxidation and a high antioxidant protection factor. This protective action of the extracts, which is more significant with the increase in the concentration of the extract, is explained by the quantitative presence of phenolic compounds demonstrated by the assay of total phenols and flavonoids. In addition, these extracts have previously

proven their effectiveness as antioxidants via their ability to scavenge DPPH radicals. These results corroborate those of Womeni *et al.* (2016a,b) and Teboukeu *et al.* (2018), who showed that adding plant extracts rich in phenolic compounds increased the induction period and the protective factor of palm olein. The values of the induction periods of the different cottonseeds oil samples analyzed are very close to those obtained by Kouzer *et al.* (2015) and oscillate between 3.19 and 3.61h.

Table 2. Induction and stability time of refined cotton oil samples enriched with plant extracts.

Samples	Induction time (h)	Stability time (h)
Control (C)	3.60±0.02 ^a	4.14±0.031 ^a
CO + BHT _{200ppm}	3.98±0.04 ^{bc}	4.56±0.05 ^{de}
CO + BHA _{200ppm}	4.06±0.04 ^c	4.29±0.02 ^b
CO + CS _{200ppm}	3.72±0.05 ^a	4.11±0.03 ^a
CO + CS _{500ppm}	3.91±0.01 ^b	4.31±0.01 ^b
CO + CS _{1250 ppm}	3.89±0.02 ^b	4.36±0.01 ^c
CO + CS _{2000ppm}	4.06±0.09 ^c	4.26±0.02 ^b
CO + TA _{200ppm}	3.64±0.04 ^a	4.17±0.01 ^a
CO + TA _{500ppm}	3.70±0.04 ^a	4.28±0.03 ^b
CO + TA _{1250 ppm}	3.69±0.06 ^a	4.18±0.04 ^a
CO + TA _{2000ppm}	3.88±0.01 ^b	4.37±0.01 ^c
CO + DE _{200ppm}	3.62±0.07 ^a	4.12±0.07 ^a
CO + DE _{500ppm}	3.67±0.07 ^a	4.12±0.03 ^a
CO + DE _{1250 ppm}	3.68±0.02 ^a	4.51±0.02 ^d
CO + DE _{2000ppm}	3.97±0.01 ^b	4.61±0.05 ^e
CO + PG _{200ppm}	3.70±0.02 ^a	4.40±0.05 ^c
CO + PG _{500ppm}	3.71±0.04 ^a	4.43±0.01 ^c
CO + PG _{1250 ppm}	3.88±0.04 ^b	4.52±0.03 ^d
CO + PG _{2000ppm}	3.91±0.06 ^b	4.58±0.02 ^e

Means with different letters within a column are significantly different ($P < 0.05$).

CO + BHT_{200ppm}: Cotton oil containing BHT (Butylated hydroxytoluène) as antioxidant at concentration of 200 ppm; CO + BHA_{200ppm}: Cotton oil containing BHA (Butylated hydroxyanisole) as antioxidant at concentration of 200 ppm; CO + CS: Cotton oil containing *Canarium schweinfurthii* (CS) extract as antioxidant at concentration of 200 ppm, 500 ppm, 1250 ppm and 2000 ppm; CO + TA: Cotton oil containing *Trichoscypha acuminata* (TA) extract as antioxidant at concentration of 200 ppm, 500 ppm, 1250 ppm and 2000 ppm; CO + DE: Cotton oil containing *Dacryodes edulis* (DE) extract as antioxidant at concentration of 200 ppm, 500 ppm, 1250 ppm and 2000 ppm; CO + PG: Cotton oil containing *Psidium guajava* (PG) extract as antioxidant at concentration of 200 ppm, 500 ppm, 1250 ppm and 2000 ppm.

Oil stability evaluation

Peroxide value

The degree of primary oxidation of enriched and control samples was assessed by measuring their peroxide values (Table 3). The peroxide values were continuously increasing in all the samples during the storage. Control has exhibited the highest peroxide value ($p < 0.01$) at all the storage stages. However, cotton oil samples

containing extracts at different concentrations and synthetic antioxidants 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 hydroperoxides, which are the principal markers. A low concentration of peroxide obtained in oils enriched with synthetic antioxidants and extracts indicates the preservative effect of these additives. The phenolic antioxidants present in these extracts might be responsible for the registered activity. These antioxidants can provide a hydrogen atom to stabilize the free radicals present in oil and consequently increase its oxidative stability (Gordon, 1990). Similar results were reported by Womeni *et al.* (2016b) with palm olein supplemented with tea leaves extracts.

p-Anisidine value

Table 3 also shows the relative increase in *p*-anisidine value of oil samples fortified with antioxidants and control. *p*-anisidine value increased in all the samples with the storage time. This increment was significantly pronounced in control ($P < 0.05$) compared to oil samples enriched with extracts. The extracts' activity was not significantly different from that of the synthetic antioxidants. The second stage of oil oxidation is the decomposition of hydroperoxides into secondary oxidation products, mainly carbonyls (2-alkenal and 2, 4-alkadienal) (Chan *et al.*, 1976). The highest *p*-anisidine value of control shows a higher degree of secondary oxidation due to the absence of antioxidants. *Canarium schweinfurthii*, *Trichoscypha acuminata*, *Dacryodes edulis* and *Psidium guajava* leaf extracts inhibited secondary oxidation products' formation and effectiveness could be related to their polyphenols content which, by inhibiting peroxide formation, reduces in the same way the amount of secondary oxidation products in oils. Hydroperoxides are a thermolabile species and their breakage causes secondary products. Consequently, the limitation of peroxide formation by extracts leads to inhibiting the formation of 2-alkenal and 2, 4-alkadienal. These results were found to agree with the studies of Asma *et al.* (2005) and Womeni *et al.* (2016a), which found that *Moringa oleifera* and tea leaf extracts delay the formation of secondary oxidation products in oils.

Total oxidation value

The changes in the total oxidation value of control and enriched cotton oil samples during the storage are presented in Table 3.

As previously observed with the peroxide and *p*-anisidine-value, total oxidation was significantly increased in all the samples. The oxidation rate was significantly higher ($p < 0.05$) in control, while it was very low in oil samples supplemented with antioxidants. The measured Totox gives a better evaluation of the oxidative deterioration of oils by quantification of hydroperoxides and their breakdown products (Womeni *et al.*, 2016b). The results obtained during this investigation show that the used extracts have an excellent antioxidant activity toward cottonseeds oil oxidation. These results are in accordance with those reported in previous work (Nyam *et al.*, 2013; Womeni *et al.*, 2016a) proving that natural plant extract can limit the total oxidation of vegetable oils.

Table 3. Changes in peroxide, *p*-anisidine, and total oxidation (Totox) values during storage of enriched cotton oil.

	Days	Control	CO + BHT _{200ppm}	CO + BHA _{200ppm}	CO + CS _{200ppm}	CO + TA _{200ppm}	CO + DE _{200ppm}	CO + PG _{200ppm}
Peroxide value (ppm)	0	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}	4.97±0.01 ^{aA}
	10	12.78±0.14 ^{bB}	6.98±0.00 ^{aB}	6.98±0.00 ^{aB}	7.02±0.04 ^{aB}	7.09±0.04 ^{aB}	7.07±0.04 ^{aB}	7.12±0.03 ^{aB}
	20	36.84±0.04 ^{cC}	10.06±0.07 ^{aC}	11.06±0.03 ^{bC}	12.11±0.01 ^{cC}	12.16±0.02 ^{dC}	12.19±0.01 ^{dC}	12.21±0.02 ^{dC}
	30	64.88±0.05 ^{dD}	14.28±0.04 ^{aD}	14.25±0.05 ^{aD}	17.36±0.04 ^{dD}	16.25±0.05 ^{bD}	16.29±0.01 ^{dD}	17.34±0.04 ^{dD}
<i>p</i>-Anisidine value	0	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}	1.20±0.00 ^{aA}
	10	2.07±0.20 ^{bB}	1.36±0.04 ^{aA}	1.38±0.04 ^{aA}	1.35±0.02 ^{aA}	1.41±0.00 ^{aA}	1.32±0.01 ^{aA}	1.36±0.01 ^{aA}
	20	5.32±0.03 ^{cC}	2.32±0.03 ^{abB}	2.35±0.02 ^{abB}	2.02±0.08 ^{aB}	2.68±0.06 ^{cB}	2.53±0.02 ^{bB}	2.72±0.05 ^{bB}
	30	8.44±0.04 ^{dD}	3.6±0.11 ^{aC}	3.48±0.10 ^{aC}	3.50±0.12 ^{aC}	3.90±0.02 ^{bC}	3.73±0.03 ^{abC}	3.82±0.01 ^{bC}
Totox value	0	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}	11.14±0.02 ^{aA}
	10	27.64±0.28 ^{bB}	15.33±0.05 ^{aB}	15.35±0.01 ^{aB}	15.39±0.09 ^{aB}	15.60±0.12 ^{aB}	15.47±0.04 ^{aB}	15.61±0.14 ^{aB}
	20	79.01±0.08 ^{cC}	22.44±0.15 ^{aC}	24.48±0.17 ^{bC}	26.24±0.24 ^{cC}	27.00±0.06 ^{dC}	26.91±0.04 ^{dC}	27.14±0.05 ^{dC}
	30	138.20±0.11 ^{dD}	32.16±0.09 ^{aD}	31.98±0.11 ^{aD}	38.23±0.07 ^{dD}	36.40±0.12 ^{bD}	36.31±0.03 ^{bD}	38.5±0.09 ^{dD}

(a- e) Means within each row with different superscripts are significantly ($P < 0.05$) different. (A-D) Means within each column with different superscripts are significantly ($P < 0.05$) different. (Control: cotton oil without antioxidant; CO + BHT_{200 ppm}: cotton oil containing BHT as antioxidant at concentration of 200 ppm; CO + BHA_{200 ppm}: cotton oil containing BHA as antioxidant at concentration of 200 ppm; CO + CS_{200ppm}: Cotton oil containing Canarium schweinfurthii (CS) extract as antioxidant at concentration of 2000 ppm; CO + TA_{200ppm}: Cotton oil containing Trichoscypha acuminata (TA) extract as antioxidant at concentration of 2000ppm; CO + DE_{2000ppm}: Cotton oil containing Dacryodes edulis (DE) extract as antioxidant at concentration of 2000ppm; CO + PG_{2000ppm}: Cotton oil containing Psidium guajava (PG) extract as antioxidant at concentration of 2000ppm).

Conclusions

Results showed that *Canarium schweinfurthii*, *Trichoscypha acuminata*, *Dacryodes edulis*, and *Psidium guajava* leaves extracts are rich in phenolic compounds with a high potential free radical scavenger. These extracts can increase the oxidative stability of cottonseeds oil at high temperatures and limit the appearance of oxidation products. These extracts can be used as antioxidants substitute to stabilize cottonseeds oil and other oil matrices.

Author Contributions

GBT, GR, and HMW conceived and designed the experiments and reviewed the final manuscript; GBT, GP, OEC performed the experiments, analyzed the data, and prepared the manuscript; MPK collected samples and prepared powders. All authors have read and agreed to the published version of the manuscript.

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