



Antioxidant Potential of *Ocimum basilicum* (Lamiaceae) Essential Oil as Preservation of the Physicochemical Properties of Palm Oil During One Month

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Abstract: The present study aimed to determine ability of essential oil (EO) of *Ocimum basilicum* as protectant of stored crude palm oil (CPO) in Cameroon. Twenty nine constituents were identified representing over 95.9% of *O. basilicum*. The physicochemical properties of CPO sample change significantly ($p < 0.000$) during storage period, except moisture content. When EO was added in this CPO, there are not significant variations for this physicochemical properties during storage period. The total phenol and β -carotene contents showed any significant variations during storage period for the formulation with the EO or citric acid. For concentrations of EO ranging from 1 to 10 mg/L, the antioxidant activity rises from 23.07 to 92.20 and from 10.37 to 73.02% respectively for the first day and one month storage sample. The ET_{50} for conjugated dienes of CPO-EO vary from 6 days to 21 days at the concentrations going from 200 ppm to 1000 ppm.

Keywords: Crude Palm Oil, Storage, *O. basilicum*, Physicochemical Properties

1. Introduction

In recent years, global demand for palm oil has increased significantly. This expansion is also due to increased consumption in emerging countries such as China, India and other Asian countries, where palm oil is mainly used as a frying oil [1]. The palm oil comes from mesocarp of the fruit of *Elaeis guineensis* when undergoes pressing process. It contains more than 50% saturated fats and 40% unsaturated fats [2]. Crude Palm oil (CPO) and its derivatives are used (80%) for human consumption [3]. During storage of CPO, degradations occur affecting the organoleptic and nutritional qualities. These deterioration depend on several factors such as presence of enzymes, temperature rise, light, presence of oxygen and the presence of metals [4] and microbial

infestation before precessing [5].

However, previous studies tend to demonstrate that, the product obtained from the process contains several compounds such as carotenoids, tocopherols, vitamin E, sterols, phosphatides, triterpenes and aliphatic alcohols, phenolic and flavonoids [6]. The pronounced red color of the CPO is due to its particular richness in carotenoids [7]. The phenolic acids and flavonoids have antioxidant properties [8]. These antioxidant activity is influenced by position and degree of hydroxylation, polarity, solubility, and stability of the phenoxy radical [8].

Industrial palm oil production in Cameroon requires the addition of a chemical preservative so the citric acid is one of the most used. Citric acid was widely used in food industry. Tulin *et al.* [9] studies showed that liver of mice treated with citric acid has necrotic changes after injection during 10 days. Some countries like France describe synthetic citric

acid as the most dangerous/cancerous additive and have given to E330 the title « E poison in food » [10].

Many researches done on natural products drifted of aromatic plants as alternative to the conventional additives for the control of food stability [11]. Essential oils of several plants have been used more widely as the alternative additives [12]. These plants are also used in traditional pharmacopoeia against some affections or for the cooking and for increasing the shelf life of food products [12]. Secondary metabolites is found in EO extract from aromatic plants. These essential oils, when they are used for the food protection, leave characteristic aromas of these plants. These aromas contain bioactive compounds that play many biological roles such as their antimicrobial effect, their antioxidant potential which can prevent the formation of free radicals by the oxidation of fatty acids within food on which they are applied [13]. *Ocimum basilicum* (Lamiaceae) is one of this aromatic plant which exhibited higher antioxidant activity [14, 15, 16]. Data on the potential activities of EO to preserve CPO stay uncharted. In this work, the antioxidant properties of EO of *O. basilicum* to preserve physicochemical properties of CPO were studied.

2. Materials and Methods

2.1. Chemicals and Instruments

EO was obtained by the hydrodistillation of fresh leaves of *O. basilicum* collected from Maroua market in Far-North Cameroon in January 2016.

CPO sample was collected from the tank after delivery by a partnership instead. It is produced artisanally by small producers, with rudimentary equipment. The CPO sample was stored at 27 °C in small galvanized tank.

All reagents and solvents used were analytical grade.

Absorbance measurements were recorded by a PharmaSpec MODEL UV-1700 UV-Visible Reading spectrophotometer and Hitachi U-2001 UV-Invisible spectrophotometer using disposable and quartz cuvettes.

2.2. Analysis of Chemical Composition of *Ocimum basilicum* Essential Oil

The volatile components of *O. basilicum* were identified on the basis of GC/FID (Chromatograph Agilent HP-6820). The GC/FID was carried out with HP-5MS column (5% phenyl methyl siloxane) with 30 m length and 250 µm in diameter and 1 µm of thickness. The oven temperature was programmed from 40°C to 230°C at 5°C/min and the final temperature was held for 10 min. Samples were injected with an autosampler. Individual compound identifications were made by matching spectra with those from reference data [17, 18].

2.3. Physico-chemical Analysis of Crude Palm Oil and Formulation Crude Palm Oil-Essential Oil

2.3.1. Total Phenolics Content

Total phenolics content was determined by the Folin-Ciocalteu method as modified by Gao *et al.* [19]. Total phenolics content was expressed as mg gallic acid

equivalents (GAE)/g dry matter.

2.3.2. β -carotene Amount

The amount of β -carotene content in sample was determined according to Ainie *et al.* [20] method.

2.3.3. Free Fatty Acid

Estimation of the percentage free fatty acids as oleic acid was done, following the method of Pearson [21].

2.3.4. Deterioration of Bleachability Index

Deterioration of bleachability index (DOBI) was measured using the PORIM Test Methods [22].

2.3.5. Moisture Content

The moisture content was determined using AFNOR [23] method.

2.3.6. Peroxide Value

The peroxide value was determined using Alektor *et al.* [24] method.

2.4. Antioxidant Activities of Crude Palm Oil and Formulation Crude Palm Oil-Essential Oil

2.4.1. β -Carotene-Linoleate Method

The cooxidation of β -carotene of CPO in the presence of EO was evaluated by the β -carotene-linoleate model system [25]. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of CPO or formulation CPO-EO at different concentration (1, 2, 5 and 10 mg.L⁻¹). After 2 hours, the absorbance was measured at 470 nm, using a PharmaSpec MODEL UV-1700 spectrophotometer. Citric acid was used as positive control.

2.4.2. DPPH Radical-Scavenging Activity

The antioxidant activity of the CPO in the presence of EO, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* [26]. About 0.004% MeOH solution of DPPH was prepared and 2 mL of the solution was added to 3 mL of the CPO, CPO-EO at different concentration (1, 2, 5 and 10 mg.L⁻¹). Citric acid was used for comparison. Half an hour later, the absorbance at 517 nm was determined using a PharmaSpec MODEL UV-1700 spectrophotometer.

2.4.3. Conjugated Dienes Method

The amount of conjugated dienes was determined using Frankel *et al.* [27] method. Different quantity of EO (0.02, 0.04, 0.8 and 1 mg) were mixed with 20 mg of CPO and 2 mL of iso-octane. The mixture was placed in darkness at 50 °C to accelerate oxidation. After incubation, the absorbance of the mixture was measured every three days during four weeks at 234 nm against a blank in spectrophotometer. The same experiment without EO were carried on CPO and CPO-citric acid. The antioxidant was determined.

2.5. Statistical Analysis

Experimental results were expressed as means with standard deviation. Data were analyzed statistically by

analysis of variance (ANOVA) ($p < 0.05$) followed by the Duncan test, with the level of significance set at 5%, with XLSTAT 2017 software. The ET_{50} were calculated from linear regression analysis using PROINRA 2.0 software.

3. Result

3.1. Volatil Composition of *Ocimum Basilicum* Essential Oil

The essential oil yield obtained for two days dried leaves is

Table 1. Chemical composition obtained by GC-FID of the crude essential oil of *Ocimum basilicum* leaves collected in the Far-North of Cameroon in January 2016.

N°	KI*	Composés	%	N°	KI*	Composés	%
1	926	α -thujene	0.11	16	1169	Terpine-4-ol	2.01
2	938	α -pinene	0.35	17	1238	Neral	0.13
3	947	Camphene	0.32	18	1288	Thymol	4.52
4	970	Sabinene	0.21	19	1295	Carvacrol	1.47
5	982	β -pinene	0.52	20	1352	Eugenol	2.86
6	987	Myrcene	0.01	21	1357	Farnesene	3.02
7	1013	α -terpinene	0.91	22	1377	α -copaene	1.65
8	1026	ρ -cymene	1.86	23	1389	β -elemene	1.92
9	1028	Limonene	27.65	24	1422	β -caryophyllene	0.23
10	1031	β -phellandrene	14.86	25	1455	α -humulene	0.14
11	1035	1,8-cineole	0.1	26	1496	Germacrene-D	2.02
12	1042	(ϵ)- β -ocymene	1.81	27	1518	(ϵ)- α -bisabolene	0.53
13	1082	Terpinolene	0.21	28	1525	δ -cadinene	1.04
14	1085	Fenchone	0.25	29	1642	Cardinol	3.68
15	1086	Linalool	21.51				
Total						95.9	

RI: Retention Index

* The compounds presented in this Table are those having a proportion higher or equal than 0.1%.

3.2. Physico-chemical Analysis of Crude Palm Oil and Formulation Crude Palm Oil-Essential Oil

The results of some physicochemical properties during storage, total phenol (mg/100g) and β -carotene contents present in CPO are presented in the Table 2.

This Table shows that physicochemical properties of CPO sample change significantly ($p < 0.000$) during storage period,

Table 2. Physicochemical qualities, total phenol and β -carotene contents of crude palm oil extracted traditionally and formulation crude palm oil and *O. basilicum* essential oil.

Physicochemical proprieties	CPO		CPO With EO		CPO With Citric acid	
	First day	4 weeks	First day	4 weeks	First day	4 weeks
FFA (%)	6.85 \pm 0.05 ^b	7.73 \pm 0.03 ^a	4.95 \pm 0.03 ^c	5.00 \pm 0.05 ^c	3.93 \pm 0.02 ^d	4.00 \pm 0.02 ^d
DOBI	1.95 \pm 0.03 ^b	1.85 \pm 0.04 ^c	2.02 \pm 0.01 ^b	2.01 \pm 0.01 ^b	2.27 \pm 0.05 ^c	2.23 \pm 0.04 ^c
PV (Meq Peroxide/kg)	4.92 \pm 0.05 ^b	5.68 \pm 0.04 ^a	4.80 \pm 0.04 ^b	4.58 \pm 0.05 ^b	4.86 \pm 0.08 ^b	4.46 \pm 0.06 ^b
MIV (%)	0.35 \pm 0.01 ^a	0.34 \pm 0.01 ^a	0.35 \pm 0.01 ^a	0.35 \pm 0.01 ^a	0.35 \pm 0.01 ^a	0.35 \pm 0.01 ^a
Total Phenol (mgGAE/100g)	393.25 \pm 2.64 ^a	369.73 \pm 2.10 ^b	391.37 \pm 0.20 ^a	390.14 \pm 1.25 ^a	391.52 \pm 0.09 ^a	390.78 \pm 0.38 ^a
β -carotene content (mg/Kg)	583.50 \pm 1.07 ^a	552.55 \pm 1.84 ^b	584.86 \pm 2.26 ^a	581.61 \pm 1.07 ^a	584.36 \pm 1.58 ^a	583.28 \pm 0.61 ^a

Averages followed by the same letter in the same line are not different significantly with $P < 0.05$ (Test of Duncan).

FFA: Free Fatty Acid; DOBI: bleaching index; MIV: moisture contents; PV: Peroxide Values

The total phenol contents of CPO at the first day and after four weeks were different significantly ($p < 0.000$) and were found to be 393.25 \pm 2.64 and 369.73 \pm 2.10 mgGAE/g extract, respectively. With the EO or citric acid, the total phenol contents showed any significant differences during storage period.

2.32 \pm 0.14%. Twenty nine constituents were identified corresponding to 95.9% of *O. basilicum* essential oil with three major components: Limonene (27.65%), linalool (21.51%) and β -phellandrene (14.86%) (Table 1). Monoterpenes hydrocarbons was the predominant chemical group (46.8%) in *O. basilicum*, followed by the oxygenated monoterpenes (31.91%) and hydrocarbons sesquiterpenes (10.455%), while the oxygenated sesquiterpenes (3.68%) had a minor share in the essential oil profile.

except moisture content. This physicochemical parameters of CPO was increased from 6.85 at 7.73 and from 4.95 at 5.68 respectively for FFA and PV, and decreased from 1.95 at 1.85 for DOBI parameter within four weeks time. When EO was added for this CPO, there are not significant variations for this physicochemical properties during storage period.

The carotene contents of CPO decreased significantly ($p < 0.001$) with the storage time from 583.50 \pm 1.07 to 552.55 \pm 1.84, respectively at the first day and after four weeks. There are not significant variations between both periods, when essential oil or citric acid are added in the CPO.

3.3. Antioxidant Activity

Table 3. Antioxidant activity (%) of CPO storage during four weeks with essential oil of *O. basilicum* in β -carotene-linoleate system.

Analysis Time	CPO with HE (C (mg/L))					CPO with Citric acid
	CPO	1	2	5	10	
First day	13.39±0.66 ^d	23.07±0.54 ^d	42.46±0.97 ^c	73.62±1.27 ^b	87.85±1.97 ^a	96.05±0.28
A week	9.04±0.40 ^c	18.07±0.54 ^d	37.94±0.45 ^c	67.30±2.04 ^b	81.20±0.49 ^a	
2 weeks	6.30±0.54 ^c	16.35±0.77 ^d	32.10±1.48 ^c	53.64±0.93 ^b	72.55±0.99 ^a	
4 weeks	4.02±1.22 ^c	12.57±0.68 ^d	28.80±1.35 ^c	46.72±1.28 ^b	63.01±0.33 ^a	95.87±0.42

Averages followed by the same letter in the same line are not different significantly with $P < 0.05$ (Test of Duncan).

The results of Table 3, 4 and 5 shows the antioxidant activity of CPO storage during four weeks with EO of *O. basilicum*. CPO sample without EO has the lower antioxidant activity at the first day of storage (13.39%) which significantly ($p < 0.001$) decreased with storage time (4.02% after four weeks). The addition of EO of *O. basilicum* to

CPO at different concentrations prevented significantly ($p < 0.001$) the bleaching of β -carotene (Table 3). For concentrations of EO ranging from 1 to 10 mg/L, the antioxidant activity increases from 23.07 to 87.85 and to 12.57 to 63.01% respectively for the first day sample and those storage during one month.

Table 4. Antioxidant activity (%) of CPO storage during one month with essential oil of *O. basilicum* using DPPH system.

Analysis Time	CPO with HE (C (mg/L))					CPO Citric acid
	CPO	1	2	5	10	
First day	15.86±0.93 ^c	27.35±0.70 ^d	46.43±0.88 ^c	71.19±0.47 ^b	92.20±0.92 ^a	95.86±0.87
A week	13.13±1.31 ^c	23.00±0.77 ^d	41.06±0.28 ^c	66.50±0.77 ^b	85.03±0.54 ^a	
2 weeks	10.95±0.14 ^c	20.22±0.76 ^d	36.58±0.67 ^c	60.15±0.98 ^b	79.98±0.18 ^a	
4 weeks	7.48±1.46 ^c	17.75±0.36 ^d	30.25±0.85 ^c	54.47±0.83 ^b	73.02±0.90 ^a	

Averages followed by the same letter in the same line are not different significantly with $P < 0.05$ (Test of Duncan).

The Table 4 showed that all sample of CPO used in this study were able of scavenging DPPH free radicals. The CPO without EO and citric acid inhibit 15.86% of DPPH free radical contained in the solution at the first day. After one month storage this antioxidant activity decreased at 7.48%. With EO added, CPO showed a significant variations ($p < 0.001$) concentration-dependent antiradical activity by inhibiting from 27.35% to 92.20% and from 17.75% to 73.02% DPPH free radical at the concentration range from 1 to 10 mg/L, respectively at the first and four weeks storage. No significant different was observed between the antioxidant activity of citric acid at the first to last day of conservation. The CPO and formulation CPO-EO showed the weak activity compared to citric acid a known antioxidant

product used in CPO in Cameroon.

Using the conjugated diene method, the storage CPO with EO of *O. basilicum* showed an antioxydant activity which depend to concentraion of EO and storage time (Table 5). At 50 °C, CPO, CPO-EO, and CPO-citric acid were prooxidant and increased the formation of hydroperoxide from 200 ppm to 1000 ppm during storage time. The CPO sample upgraded hydroperoxide formation, with an ET_{50} corresponding to 3 days 23 hours and 38.07 minutes. The ET_{50} of formulation CPO-EO vary from 6 days to 21 days at the concentrations going from 200 ppm to 1000 ppm. Formulation with citric acid which is the control, had lower prooxidant activity (ET_{50} =28 days) than the formulation of CPO-EO at 1000 ppm.

Table 5. ET_{50} values (days) of crude palm oil storage during four weeks with essential oil of *O. basilicum* leaves from conjugated dienes method.

ET_{50}		
CPO		
3d23h38.07mn		
Concentrations	CPO with HE	CPO with Critic acid
200 ppm	6d21h18.3mn	28j21h52.5mn
400 ppm	11d18h12.2mn	
800 ppm	17d11h28.3mn	
1000 ppm	21jd3h41.5mn	

D: days; h: hour; mn: minutes.

4. Discussion

The physicochemical properties of samples increased when CPO is storage for four weeks. During storage, edible

oils undergo physical and chemical changes due to several factors such as light, high temperatures, traces of metals, water and miroorganisms [28, 29]. The common pathways of degradation are isomerization, oxidation and hydrolysatation.

Light, enzymes, pro-oxidant metals in the presence of unsaturated lipids, induce oxidation [28]. These reactions therefore lead to a decrease in DOBI and an increase in acidity [13]. During storage there is a gradual accumulation of oxidation products, which tends to reduce the melting point and increase the peroxide value of stored oils [30].

When essential oil of *O. basilicum* was added to CPO, the variation between physicochemical parameter was not significant. This non significant variation of physicochemical parameters was due to actives compounds of these essential oil which protect foods against pathogenic and spoilage microorganisms [12]. Sienkiewicz *et al.* [31] shown that, *O. basilicum* essential oil was more active against microorganisms. These antimicrobial activity may be an important factor for stability of study CPO. When EO was added to a food product, it is enclosed into nanoemulsions, and stability of volatile components increases for the long time effect on microorganisms [32]. Udensi and Irogbu [2] report that low levels of carotene in the oil sample indicate low levels in vitamin A, and consequently indicates a long storage period of the oil sample.

The study CPO sample possessed higher total phenolic contents. Phenolic compounds play antioxidant role which act as free radical terminators and reduced oxidative degradation of lipids [33, 34]. Bouhdid *et al.* [35] shown that essential oils rich in thymol, borneol, carvacrol, cymene and terpinolene inhibit more than 74.5% oxidation of linoleic acid. The reductive activity of the DPPH radical *O. basilicum* EO was due to the phenolic compounds and non-phenolics such as linalool, as shown by Judic and Milos [36] and Zohra [37].

According to their compounds, essential oils have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts [11, 38]. In our present study, EO of *O. basilicum* was antioxidant in CPO and strongly reduce peroxides formation.

5. Conclusion

EO of *O. basilicum* leaves has an antioxidant activity which preserves physicochemical properties of the CPO from degradation during one month of exposure. This antioxidant activity, although below that of citric acid, may be useful in the conservation of vegetable oils intended for human consumption.

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