

# Effects of Degumming on the Antioxidants Properties of Some Non-conventional Seed Oils

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**Abstract** This study examined the effect of degumming process on antioxidants properties and oxidative stability of six non-conventional oils in Nigeria extracted from the seeds and flesh of *Terminalia catappa* (seed), *Irvingia gabonensis* (seed), *Glycine max* (seed), *Persea americana* (flesh), *Tithonia diversifolia* (seed), and *Dacryodes edulis* (flesh). DPPH scavenging activity of the oils decreased after degumming except for *D. edulis* with increased DPPH scavenging activity. Similarly, total antioxidant capacity (TAC) decreased for *G. max*, *T. diversifolia* and *I. gabonensis* after degumming but increased for *T. catappa*, *P. americana* and *D. edulis*. The degummed oil showed high peroxidation under the different light intensities (under direct sunlight, fluorescent light, and daylight) except *I. gabonensis* oil (PV: 5.7 meq/kg) and *T. catappa* oil (PV: 11 meq/kg) with improved stability in the dark for the period of study (84 days).

**Keywords:** non-conventional seedoils, antioxidant properties, degumming, oxidative stability

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## 1. Introduction

Antioxidants are compounds that extend the induction period of oxidation or slow down the oxidation rate. Antioxidants scavenge free radicals such as lipid alkyl radicals or lipid peroxy radicals, control transition metals, quench singlet oxygen, and inactivate sensitizers [1]. Antioxidants act by prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical-scavenging [2]. 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) is a stable free radical that can easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is typically used as a substrate to evaluate the antioxidants potential of seed oils.

Antioxidants can be classified to natural or synthetic. The synthetic compounds are cheaper and often more effective, but there is a growing demand for natural compounds. Much of the large and growing food industry would not be possible without antioxidants of some kind [3]. Synthetic antioxidants (e.g. BHA, BHT, TBHQ, PG) are solid compounds and may be conveniently used as solutions in propylene glycol, monoacylglycerols or vegetable oils. The synthetic antioxidants are mono or dihydric phenols and react with a peroxy radical to give a phenoxy radical (ArO $\cdot$ ), stabilised by extensive delocalisation of the odd electron over the aromatic system [3].

Degumming involves treatment with water or dilutes acid (phosphoric or citric) producing a gum containing phospholipids (or gum) and trace metals which can be separated with a centrifuge. It can also be defined as the treatment of oils with additives that are used for the removal of waxes, phosphates and other impurities in oil and some of these additives include salts solutions, water, dilute acids or alkalis [4].

The effect of light intensities on oil is associated with a series of reactions such as oxidation, decarboxylation, polymerization, isomerisation and hydrogenation e.t.c. [5]. Oxidation of oil is an important indicator to determine oil quality and shelf life [6] because low-molecular weight off-flavor compounds are produced during oxidation. It also destroys essential fatty acids and produces toxic compounds and oxidized polymers [1]. Oxidation of oil is very important in terms of palatability, nutritional quality, and toxicity of edible oils [1]. Oxidative stability of oils is the resistance to oxidation during processing and storage [7]. Resistance to oxidation can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process [8]. The oxidation of oils is influenced by the fatty acid composition of the oil, oil processing, energy of heat or light, the concentration and type of oxygen, and free fatty acids, mono- and diacylglycerols, transition metals, peroxides, thermally oxidized compounds, pigments, and antioxidants. These factors interactively affect the oxidation of oil and it is not easy to differentiate the individual effect of the factors [1].

Recently, several authors have investigated the effect of degumming on the antioxidants present in oil; and other minor components such as trace metals, iodine value, fatty acid composition and peroxide value of some conventional seed oils such as rapeseed, soybean, olive and sesame oil. Also some authors have studied the influence of microwave and oven at different temperature on these oil seeds after degumming. However, there are relatively few studies in literature concerning the effects of degumming on the antioxidant properties and oxidative stability of non-conventional seed oils

Since pure oil is produced through degumming steps, oxidative stability, natural antioxidants and fatty acid contents of oils may be affected during these processes. This study therefore seeks to investigate the effects of degumming on the natural antioxidant content and oxidative stability (peroxide values) of some non-conventional seedoils. Five non-conventional oil sources (including their seeds and flesh) indigenous to the tropics (Nigeria) were analysed. They are *Dacryodes edulis* flesh, *Persea americana* flesh, *Irvingia gabonensis* seed, *Terminalia catappa* seed, *Tithonia diversifolia* seed. *Glycine max* is a conventional oil source used for a comparative study with the above non-conventional oil sources. Degumming process was carried out on the oils for removal of phospholipids. The effect of degumming process was established on the antioxidant properties of both raw and degummed oils. Effect of different intensity of light on the quality and stability of the oils (raw and degummed) was monitored for eighty-four days. The effect of different light intensity was monitored using one chemical parameter (peroxide value).

## 2. Materials and Methods

### 2.1. Extraction of Oils

The extraction was carried out using the Soxhlet extractor method [9]. About 20 g of the milled samples was weighed at a time and packed into a cellulose thimble prewashed with acetone/*n*-hexane mixture and allowed to dry in an oven at a temperature of 70 °C for 2 h before use. The extraction of the oils with *n*-hexane lasted 7 h on the average. After extraction, the content in the flask was concentrated by distilling off the solvent content to obtain a solvent-free crude oil. The flask was later placed in the oven maintained at 70 °C to drive off traces of the solvent left. The concentrated oil sample was then stored in a bottle in a cool dry place before the degumming process was carried out.

### 2.2. Degumming Operation

Accurately measured 5 mL of raw oil was mixed with 1 mL of the 300 µg/mL NaCl solution. The oil and NaCl solution mixture was agitated for 1 hour at 60 °C on magnetic stirrer to render fat-soluble phosphatides insoluble. These insoluble phosphatides are then separated by centrifugation at 1000 rpm for 30 mins. During the agitation process, a colloidal mixture beneath the oil layer was formed. This was believed to be a mixture of the fat-soluble impurities (phospholipid lecithin complexed with metals) contained earlier in the oil. The oil was separated from the colloidal mixture by decanting [10].

### 2.3. Effects of Different Intensity of Light on Oil Stability

Duplicate 10 mL sample of oil, was transferred to a series of transparent bottles having 0.5 mL cross sectional area and a volume of 20 mL. The bottles were loosely capped, enabling direct contact between oil and atmospheric air. The oils were exposed to four oxidative conditions which are:

- i. Leaving in the dark
- ii. Exposure to daylight
- iii. Exposure to 40 W fluorescent lamp (oil samples were placed 50 cm beneath the fluorescent lamp).
- iv. Exposure to direct sunlight.

Peroxide values of the oils in duplicate were determined using the [9] method. Accurately weighed 5.0 g of each oil sample was placed in a conical flask. A solvent mixture containing 12 mL chloroform and 18 mL glacial acetic acid (i.e ratio 2:3 v/v) was added to dissolve the oil. A saturated aqueous potassium iodide solution (0.5 mL) was added; the flask was stoppered and allowed to stand for 1 minute. Accurately measured 30 mL of water was added and the solution was titrated with 0.1 M sodium thiosulphate solution until the yellow colour had almost disappeared. To this was added 0.5 mL of freshly prepared starch solution and the titration continued until the blue black colour disappeared. A blank determination was also performed. The peroxide value (P.V.) was calculated from the expression:

$$PV(\text{meq/g sample}) = \frac{(S - B) \times M \times 1000}{\text{Weight of sample}} \quad (1)$$

where S = titre value (mL) Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for sample, B = titre value (mL) Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for blank, M = molarity of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. The determination was carried out every two weeks for a period of three months.

### 2.4. Antioxidant's Activities of Oils

#### 2.4.1. Determination of Total Antioxidant Capacity (TAC)

This method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH [11]. To 0.1 mL of the oil extracts (20, 40, 60, 80, 100 µg/mL) was added 1ml of the reagent solution which consisted of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95 °C for 90 mins. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695 nm against a blank which consisted of the reacting mixture containing distilled water in place of the extract. The antioxidant activities of the extracts were expressed as an ascorbic acid equivalent.

#### 2.4.2. Determination of 2,2 - Diphenyl -1 - Picryl Hydrazyl (DPPH) Assay

The radical scavenging ability of the oil was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described by [12]. The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction [13]. The change in colour from deep

violet to light yellow was measured spectrophotometrically at 517 nm.

To 1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of the oil extract in a test tube was added to 1 mL of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 mins after which the absorbance was read at 517 nm against a DPPH control containing only 1 mL methanol in place of the extract. The percent of inhibition was calculated in using the expression:

$$\%I = \left[ \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \right] \times 100 \quad (2)$$

Where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound. Sample concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph plotting inhibition percentage against extract concentration.

### 3. Results and Discussion

#### 3.1. Antioxidant Activities of Oils

Antioxidants act by prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical-scavenging [2]. 2,2 - diphenyl -1 - picryl hydrazyl (DPPH) is a stable free radical that can easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is typically used as a substrate to evaluate the antioxidants potential of seed oils.

DPPH results are expressed as  $IC_{50}$  values (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%). The lower the  $IC_{50}$  value, the higher the antioxidant capacity of the oil. The  $IC_{50}$  values of raw oils ranged from  $8.40 \pm 0.03 \mu\text{g/mL}$  in *T. diversifolia* to  $36.33 \pm 3.25 \mu\text{g/mL}$  in *D. edulis* (Table 1a). Raw vegetable oil from *D. edulis* have the lowest free radical scavenging potential among all the raw oils while *T. diversifolia* have the highest scavenging potential.

After degumming, the  $IC_{50}$  value of the oils increased suggesting that their antioxidant capacity decreased except that of *D. edulis* oil with lower  $IC_{50}$  value. The values ranged from  $6.81 \pm 0.42 \mu\text{g/mL}$  in *D. edulis* to  $52.77 \pm 3.12 \mu\text{g/mL}$  in *T. catappa* (Table 1a). Reduction in  $IC_{50}$  value of *D. edulis* may be due to the presence of additional minor components (polyphenols) which are responsible for the taste and flavour of the fruit [14], and increased the antioxidant's activities of the oil. The decrease in DPPH antioxidant capacity in degummed oils could be attributed to the effect of heat and alkali on natural antioxidants present in the oils during degumming process as reported by [15].

The results for total antioxidant activity are presented in Table 1b. The values for raw vegetable oils ranged from  $1.41 \pm 0.08 \text{ mg/mL}$  in *T. catappa* to  $34.73 \pm 2.01 \text{ mg/mL}$  in *T. diversifolia*. From the result, total antioxidant activity was high in *T. diversifolia* and low in *T. catappa* for raw oils. A significant increase in total antioxidant activity was observed in *T. catappa* ( $10.65 \pm 0.6 \text{ mg/mL}$ ) and *P. americana* ( $11.82 \pm 0.68 \text{ mg/mL}$ ) after degumming. In *D. edulis* slight increase ( $2.27 \pm 0.15 \text{ mg/mL}$ ) was observed,

while decrease in total antioxidant activity was observed in *G.max* ( $6.28 \pm 0.37 \text{ mg/mL}$ ), *T. diversifolia* ( $4.22 \pm 0.24 \text{ mg/mL}$ ) and *I. gabonensis* ( $3.54 \pm 0.20 \text{ mg/mL}$ ) after degumming process.

The result of both DPPH scavenging activity and total antioxidant activity of raw oils showed that *T. diversifolia* have the highest antioxidant capacity which means the oil is stable. After degumming process, *D. edulis* showed higher DPPH and total antioxidant activities. Decrease in antioxidant's activities of vegetable oils after degumming process can be attributed to the increase in peroxidation as observed in the present study, thus, the oils become unstable to oxidation.

**Table 1a. DPPH Scavenging activity ( $IC_{50} \mu\text{g/mL}$ )**

Sample	Raw oils	Degummed oils
<i>T. catappa</i>	$24.66 \pm 0.90$	$52.77 \pm 3.12$
<i>P. americana</i>	$14.16 \pm 1.69$	$41.81 \pm 1.23$
<i>I.gabonensis</i>	$21.55 \pm 2.14$	$47.21 \pm 0.34$
<i>G.max</i>	$14.89 \pm 2.84$	$22.17 \pm 1.04$
<i>T.diversifolia</i>	$8.40 \pm 0.03$	$40.50 \pm 1.62$
<i>D.edulis</i>	$36.33 \pm 3.25$	$6.81 \pm 0.42$

**Table 1b. Total antioxidant capacity (TAC) (mg AAE/mL)**

Sample	Raw oils	Degummed oils
<i>T. catappa</i>	$1.41 \pm 0.08$	$10.65 \pm 0.61$
<i>P. americana</i>	$6.35 \pm 0.37$	$11.82 \pm 0.68$
<i>I.gabonensis</i>	$5.56 \pm 0.32$	$3.54 \pm 0.20$
<i>G.max</i>	$6.68 \pm 0.39$	$6.28 \pm 0.37$
<i>T.diversifolia</i>	$34.73 \pm 2.01$	$4.22 \pm 0.24$
<i>D.edulis</i>	$2.25 \pm 0.13$	$2.27 \pm 0.15$

#### 3.2. Effect of Light Intensities on Raw and Degummed Vegetable Oils

The effect of light intensities on oils is associated with a series of reactions such as oxidation, decarboxylation, polymerization, isomerisation and hydrogenation etc. [5]. In this study, light induced oxidative stability of raw and degummed oils was monitored under light of different intensities. The results are presented in Table 2 below. The results showed raw oil of *I. gabonensis* to be the best grade oil in terms of stability under light conditions investigated. In the dark, the peroxide value of this oil ranged from 1.00 - 5.11 meq/Kg after 84 days. Raw oil from *T. catappa* is next to *I. gabonensis* in terms of oxidative stability. Its peroxide values in the dark ranged from 2.6 to 8.7 meq/kg after 84 days. The peroxide value of both oils still fall below the acceptable value of 10 meq/kg after 84 days, this means the oils have high level of stability to oxidative deterioration. The peroxide value of raw oil from *T. catappa* showed high peroxide value which ranged from 2.6 to 144 meq/kg under direct sunlight after 84 days. High peroxide value observed under sunlight suggest that the presence of a natural antioxidants present in the oil might be destroyed by exposure to light intensity. Raw oils from *P. americana* and *D. edulis* showed high peroxide value of 46 and 37 meq/kg respectively in the dark after 84 days. These values are higher than the acceptable value of 10 meq/kg for good oil. High peroxide value exhibited by these oils might be due to the glossy nature of the materials from which the oils were extracted.

Effect of light intensities on degummed oils showed an increase in peroxide value in all conditions during the

84 days of study. The increase in peroxide value of degummed oil may be due to the removal of phospholipids in oils as reported by researchers [16,17] that phospholipids contribute to the stability of vegetable oils through their antioxidation activity. Increase in peroxidation of degummed oils may also be due to the reduction in the scavenging activity of radicals of natural antioxidants present in the oils after degumming which may be caused by heat or the salt used during the degumming process as reported by [15].

Despite the increase in peroxide value after degumming, degummed oil of *I.gabonensis* still show case stability in the dark after 84 days (5.7 meq/kg). The value is still below 10meq/kg which is the acceptable value for good oils. The peroxide value increased drastically after 42 days under direct sunlight, fluorescent and daylight conditions. The increase may be due to the antioxidants present in the oil have been destroyed by the intensities from the light.

Degummed oil of *T. cattapa* also showed some stability in the dark after 84 days (11 meq/kg). The value is a little bit higher than the acceptable value of 10meq/kg for good oils. But the peroxide value after 70 days is 8.4meq/kg which is below the acceptable value. This means the oil is still stable after degumming process. There is drastic increase in peroxide value of the oil under fluorescent, sunlight and daylight condition after 14 days. Degummed oils of *P. americana*, *D. edulis* and *G. max* showed high peroxide value of 57.31, 23.42, 27.51 meq/kg respectively after 84 days under dark condition. These values are higher than the acceptable value for good oils. This means the oils are not stable.

Decolouration and off flavour was observed in both crude and degummed oils under fluorescent, daylight, and direct sunlight after 84 days. Light green colouration of *P. americana* and *D. edulis* turned brown and light yellow of *T. cattapa* and *I. gabonensis* turned white while the golden yellow of *G.max* turned to light yellow. Chromophoric molecules in the oils can contribute to decolouration and instability of oils as reported by [18]. According to [19], light deterioration in oils involves transfer of excitation energy from excited chromophoric entities to oxygen to give singlet oxygen. The singlet oxygen reacts directly with the double bonds of unsaturated fatty acids present in the oils producing hydroperoxides.

**Table 2a. Influence of light intensities on peroxide value of *T. cattapa* oil**

Raw oil				
Days	In the dark	Fluorescent light	Daylight	Direct sunlight
0	2.6	2.6	2.6	2.6
14	2.8	6.5	3.2	3.1
28	3.2	9.3	3.8	7.3
42	4.0	19.8	10.5	18.6
56	6.3	31.0	28.0	54.0
70	6.9	47.8	35.0	99.0
84	8.7	68.0	51.2	144.0
Degummed oil				
0	3.0	3.0	3.0	3.0
14	3.2	8.2	6.5	9.5
28	3.9	10.5	7.9	13.9
42	4.7	21.2	12.0	25.8
56	7.2	54.1	41.3	67.2
70	8.4	97.0	77.8	132.0
84	11.0	143.7	104.6	176.0

After 56 days, thin sludge was observed in both crude and degummed oils of *P. americana*, *D. edulis* and *T. cattapa* which become thicker after 70 days. It was also observed that the thickness of the oils increases as the storage time increase in both crude and degummed oils. This observation may be attributed to photolysis/photooxidation as explained by [20].

**Table 2b. Influence of light intensities on peroxide value of *P. americana* oil**

Raw oil				
Days	In the dark	Fluorescent light	Daylight	Direct sunlight
0	3.4	3.4	3.4	3.4
14	4.0	7.1	9.0	9.5
28	5.6	14.8	12.5	15.1
42	6.0	28.9	32.0	36.0
56	13.0	39.0	44.0	55.0
70	26.0	89.0	79.0	85.0
84	46.0	119.0	99.0	168.0
Degummed oil				
0	5.9	6.4	6.4	6.4
14	6.0	9.3	10.0	9.8
28	6.5	14.9	13.0	17.6
42	6.9	31.2	43.6	39.1
56	18.0	44.2	49.2	61.3
70	32.0	98.4	89.1	89.0
84	57.3	132.7	121.3	169.0

**Table 2c. Influence of light intensities on peroxide values of *I. gabonensis* oil**

Raw oil				
Days	In the dark	Fluorescent light	Daylight	Direct sunlight
0	1.0	1.0	1.0	1.0
14	1.1	2.0	3.0	2.5
28	1.5	2.9	3.5	2.8
42	1.8	5.0	4.1	5.2
56	2.1	9.8	6.2	8.4
70	3.9	20.0	11.4	21.0
84	5.1	35.0	33.8	48.0
Degummed oil				
0	1.6	1.6	1.6	1.6
14	1.7	4.0	3.8	3.2
28	1.8	5.3	5.1	4.9
42	1.9	7.1	5.9	6.3
56	2.5	17.2	10.3	18.1
70	4.2	24.9	17.7	32.7
84	5.7	54.7	41.3	62.0

**Table 2d. Influence of light intensities on peroxide values of *G. max* oil**

Raw oil				
Days	In the dark	Fluorescent light	Daylight	Direct sunlight
0	2.00	2.00	2.00	2.00
14	2.90	2.80	2.40	3.50
28	4.08	9.00	6.00	8.70
42	5.00	13.67	10.00	13.55
56	10.00	29.00	24.00	44.00
70	17.00	66.00	39.00	71.00
84	22.00	112.00	79.00	134.00
Degummed oil				
0	3.20	3.20	3.20	3.20
14	3.50	4.800	4.09	4.30
28	3.91	12.00	6.89	10.40
42	6.00	41.23	18.43	22.76
56	12.30	73.00	54.11	66.98
70	17.80	125.21	83.00	110.43
84	27.51	133.45	123.71	178.21

**Table 2e. Influence of light intensities on peroxide values of *D. edulis* oil**

Days	Raw oil				
	In the dark	Fluorescent light	Daylight	Direct sunlight	
0	4.00	4.00	4.00	4.00	
14	4.80	5.400	4.86	8.20	
28	7.91	10.46	8.37	18.69	
42	9.23	31.00	25.10	37.00	
56	12.88	72.00	45.00	66.00	
70	19.00	110.00	78.00	79.00	
84	23.42	130.00	115.00	151.00	
		Degummed oil			
0	5.40	5.40	5.40	5.40	
14	6.01	6.21	5.90	10.21	
28	8.00	15.34	11.60	28.59	
42	12.34	47.77	27.23	51.34	
56	17.00	89.00	53.68	95.00	
70	25.00	126.00	94.40	127.00	
84	37.00	132.00	123.74	184.00	

## 4. Conclusion

All the oils investigated in this study demonstrated relative good stability when stored in the dark at room temperature for three months of study. However, stability decreases as they were exposed to light of higher intensities due to loss of antioxidant's activity during the degumming process. Even though the degumming process reduce the antioxidants capacity of the oils, it suffices to mention that the process has led to an improved physico-chemical and biodiesel properties of non-conventional vegetable oils compared with that of the raw oils thus making them potential raw materials for different domestic and industrial applications.

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