

Garcinia kola Extract (kolaviron) Delays Accelerated Oxidation of Linoleic Acid

EBOH AS^{1*}, Ogbomade RS², Daminola AUF⁵, Okuroemi HO³ and Arhoghro EM⁴

^{1,4}Biochemistry Department, Niger Delta University, Bayelsa State, Nigeria

^{2,3}Science Foundation Department, College of Health Technology, Bayelsa State, Nigeria

⁵Department of Human Anatomy, Niger Delta University, Bayelsa State, Nigeria.

*Corresponding Author: EBOH AS, Biochemistry Department, Niger Delta University, Bayelsa State, Nigeria.

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Abstract

Accelerated lipid peroxidation inhibitory effects of *Garcinia kola* extract (kolaviron) on linoleic acid was experimented. Linoleic acid was oxidized at 60°C in the dark for 14 days in the absence and presence of *Garcinia kola* extract (kolaviron) 0.025%, 0.05% and 0.1% as compared with α -tocopherol. The peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), and p-anisidine value were determined. *Garcinia kola* extract (kolaviron) significantly ($p < 0.05$) reduced the accelerated linoleic acid peroxidation and it was concentration dependence. *Garcinia kola* extract (kolaviron) reduced the formation of lipid peroxide and aldehyde products as showed by the lower values of PV, TBARS and p-anisidine values as compared to its control counterpart. These results indicated that *Garcinia kola* extract (kolaviron) could be useful in preventing oxidative damages of food oils.

Introduction

One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals (Hras et al., 2000). The presence of high amounts of polyunsaturated fatty acids such as linoleic and linolenic acids in oils and fats make them more susceptible to oxidation. The problem is further exacerbated if the oil is exposed to factors such as oxygen, light, high temperatures or trace metals, mainly transition metals such as Fe and Cu (Sikwese and Doudu, 2007).

Antioxidants are major compounds that protect the quality of oils and fats by retarding their oxidation (Abdalla et al., 2007). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are widely used to prevent the oxidation of oils and fats and extend

the shelf-life of lipid-containing foods. In recent years, their use in foods has suffered severe criticism such as their carcinogenicity and toxicity (Padmashree et al., 2007). These have led to an increasing interest in the search for naturally occurring antioxidants. Phenolic compounds from plant sources may act as antioxidants by scavenging lipid radicals. It is believed that phenols may act as both radical scavengers and metal chelators (Sikwese and Doudu, 2007). Suspected health risk of synthetic antioxidants and a low thermal stability of natural antioxidants like tocopherol have encouraged the interest for searching alternative natural antioxidants (Madsen and Bertelsen, 1995).

Garcinia kola belongs to the family of plants called Guttiferae and the genus Garcinia. The seed, commonly known, as 'bitter kola' is eaten by many and it is culturally acceptable in Nigeria. Extracts of the seed have been employed in the African herbal medicine for the treatment of ailments such as laryngitis, liver diseases, cough and hoarseness of voice (Iwu, 1993). Kolaviron (KV) is an extract from the seeds of Garcinia kola, containing a complex mixture of biflavonoids and polyphenols namely Garcinia biflavonoid 1 (GB1), Garcinia biflavonoid 2 (GB2) and Kolaflavanone (Iwu, 1985). Many studies have confirmed the antioxidative, anti-lipid peroxidation, chemoprevention in colon carcinogenesis and anti-inflammatory effects of kolaviron in chemically-induced toxicity, animal models of diseases and in cell culture (Abarikwu et al., 2012, Adedara et al., 2013, Farombi et al., 2013 and Eboh et al 2016).

In view of these considerations, the functional health effect of garcinia kola extract (kolaviron) in protecting against linoleic acid oxidation would be of current interest.

Materials and Methods

Materials

Linoleic acid, potassium iodide (KI), potassium dichromate ($K_2Cr_2O_7$), sodium thiosulphate ($Na_2S_2O_3$), p-Anisidine, thiobarbituric acid (TBA), trichloroacetic acid (TCA), tocopherol, were purchased from Sigma Co. (St Louis, USA). All the other chemicals used were of analytical grade.

Extraction of kolaviron

Garcinia kola seeds purchased from a local market in Yenagoa, Nigeria, were certified at the Department of Botany, Niger Delta University, Nigeria. Peeled seeds were sliced, pulverized with an electric blender and dried at 40°C in a drying oven. Powdered seeds were extracted with light petroleum ether (boiling point 40–60°C) in a soxhlet for 24h. The defatted dried marc was repacked and extracted with acetone. The extract was concentrated and diluted twice its volume with water and extracted with ethylacetate (6 x 300 mL). The concentrated ethylacetate yielded kolaviron as a golden yellow solid (Iwu, 1985).

Oxidation of Linoleic acid

Linoleic acid was exposed to accelerated oxidation similar to the method used by Abdalla and Roozen (1999). Linoleic acid (20g) containing 0% 0.025%, 0.05% and 0.1% of garcinia kola extract (kolaviron) were mixed in screwcapped glass bottles covered externally with aluminum foil and incubated at 60°C in darkness for

14 days. Initial 6-h incubation was done without closing the cap of the bottles in order to remove the ethanol added to kolaviron and tocopherol 0.1%.

Peroxide Value (PV)

Peroxide value of linoleic acid stored under accelerated oxidation conditions was determined by the iodometric determination method according to the AOAC (1995) methods.

Thiobarbituric Acid-reactive Substances Assay (TBARS)

The assay was conducted according to the method of Madsen et al. (1998). One gram of the oil sample was dissolved in 3.5mL of cyclohexane and 4.5mL of TCA-TBA mixture (7.5% TCA and 0.34% TBA) subsequently. Resultant mixture was shaken for 5 min and centrifuged at 2780x g for 15 min. The TCA-TBA phase was removed and heated in a boiling water bath for 10 min. Absorbance was recorded at 532 nm and the antioxidant capability was expressed as equivalent mmol of malonaldehyde per kg.tetraethoxypropane was used as standard curve.

Determination of p-anisidine value

p-Anisidine value of oil was analyzed according to the method of AOCS Recommended Practice (AOCS, 1990). The weight of linoleic oil (100 mg) was dissolved in 25 ml of isooctane and measured at 350 nm using UV-visible spectrophotometer. This solution (2.5 ml) was mixed with 0.5 ml of 0.5% (w/v) p-anisidine in acetic acid for 10 min. The absorbance was read at 350 nm. The p-anisidine value was calculated.

Statistical analysis

The data obtained were carefully subjected to statistical analysis using ANOVA with the aid of computer software package, SPSS version 12

Discussion

Oxidation of unsaturated fatty acids in biological membranes leads to formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the doublebonds in unsaturated lipids and eventual destruction of membrane lipids, which produce breakdown products such as malonaldehyde and 4-hydroxyalkanal. The extract of garcinia kola successfully inhibited the oxidation of linoleic acid.

Oxidation time (days)	Control	Tocopherol 0.1%	Kolaviron 0.025 %	Kolaviron 0.05 %	Kolaviron 0.1 %
5	123.5 ± 0.09 ^a	101.3 ± 0.14 ^b	110.0 ± 0.18 ^b	107.1 ± 0.01 ^b	104.8 ± 0.28 ^b
10	165.1 ± 0.23 ^a	121.7 ± 0.27 ^b	145.5 ± 0.08 ^b	118.6 ± 0.16 ^b	126.9 ± 0.13 ^b
14	213.9 ± 0.07 ^a	148.4 ± 0.11 ^b	189.3 ± 0.05 ^b	158.3 ± 0.21 ^b	147.7 ± 0.15 ^b

Table 1: Peroxide value (meq/kg) of linoleic acid in various concentration of garcinia kola extract (kolaviron) during accelerated oxidation at 60°C for 14 days. Values are mean ± SD (n = 3).

Values followed by different letters across the row are significantly different (p < 0.05).

Oxidation time (days)	Control	Tocopherol 0.1%	Kolaviron 0.025 %	Kolaviron 0.05 %	Kolaviron 0.1 %
5	15.2 ± 0.26 ^a	10.5 ± 0.16 ^b	12.8 ± 0.08 ^b	10.09 ± 0.20 ^b	9.8 ± 0.04 ^b
10	19.4 ± 0.15 ^a	14.2 ± 0.23 ^b	14.3 ± 0.31 ^b	12.7 ± 0.18 ^b	12.9 ± 0.07 ^b
14	24.26 ± 0.21 ^a	17.8 ± 0.17 ^b	18.1 ± 0.11 ^b	14.7 ± 0.12 ^b	14.5 ± 0.12 ^b

Table 2: Thiobarbituric acid reactive species (TBARS) value mmolmalondialdehyde/kg of linoleic acid in various concentration of garcinia kola extract (kolaviron) during accelerated oxidation at 60°C for 14 days. Values are mean ± SD (n = 3).

Values followed by different letters across the row are significantly different (p < 0.05).

Oxidation time (days)	Control	Tocopherol 0.1%	Kolaviron 0.025 %	Kolaviron 0.05 %	Kolaviron 0.1 %
5	6.8 ± 0.06 ^a	4.2 ± 0.07 ^b	5.8 ± 0.09 ^b	5.2 ± 0.12 ^b	4.9 ± 0.24 ^b
10	20.5 ± 0.11 ^a	10.5 ± 0.09 ^b	15.3 ± 0.17 ^b	13.4 ± 0.14 ^b	12.2 ± 0.18 ^b
14	31.6 ± 0.03 ^a	15.7 ± 0.27 ^b	21.2 ± 0.14 ^b	16.7 ± 0.19 ^b	17.4 ± 0.21 ^b

Table 3: p-Anisidine value of linoleic acid in various concentration of garcinia kola extract (kolaviron) during accelerated oxidation at 60°C for 14 days. Values are mean ± SD (n = 3).

Values followed by different letters across the row are significantly different (p < 0.05).

Peroxide value is a method of monitoring the primary stage of lipid oxidation and measuring the peroxides and hydroperoxides concentration. Changes in PVs are as shown in Table 1. PV values of control increases according to the increment in the number of days linoleic acid is exposed. The decrease in the values of peroxide is due to the presence of garcinia kola extract at different concentration obtained findings were confirmed by the results of Kishk and Elsheshetawy, 2013. This decrease could be due to the antioxidant properties of garcinia kola extract.

Thiobarbituric Acid-reactive Substances (TBARS) Aldehydes, especially malonaldehyde, the breakdown products of oxidized fatty acids (lipid peroxy radical), result in off-flavours (rancid flavour)

in oxidized oils that can be quantified through their reaction with TBA. The TBARS formation inhibitory effects of garcinia kola were significantly (p < 0.05) higher than their control counterparts as shown in Table 2. The inhibition effect of garcinia kola extract on the TBARS formation was also dose-dependent and its 0.1% level effect was compatible to the inhibition effects of alpha tocopherol.

Similarly, secondary lipid peroxidation product of linoleic acid was determined by examining p-anisidine value. Determination of p-anisidine value was based on color intensity of the reaction between p-anisidine and aldehydes. The formation of p-anisidine values during oxidation of linoleic acid is shown in Table 3. The p-anisidine values of linoleic acid without containing garcinia kola extracts were

significantly higher ($p < 0.05$) than those of linoleic acid containing garcinia kola extract after 14-day storage. There was significant difference of p-anisidine values between linoleic acid without garcinia kola extract and 0.025%, 0.05% and 0.1% garcinia kola extract.

Conclusion

In this work, the efficacy of garcinia kola extract kolaviron, on the oxidation stability of linoleic acid was studied throughout the 14 days at 60°C. The results obtained from the primary and secondary oxidation analysis could confirm the antioxidant efficiency of the extract.

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