European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 4; Issue 6; June 2016; Page No. 30-33

African pear seed extract potential to inhibit oxidation of soybean oil

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Abstract
The present study evaluates the ability of African pear seed extract (APSE) to inhibit oxidation of soybean oil (SBO). Commercial SBO stripped of its phenolic fraction, was spiked with 50, 100 and 200 µg/g of APSE (samples SB50, SB100 and SB200 respectively), and stored in an oven set at 60°C for 5 days. At 1-day intervals samples were withdrawn from the oven and evaluated for degree of oxidation by measurement of peroxide value (PV) and extinction coefficients k_{232} and k_{270}. SBO stripped of its phenolic fraction was also subjected to oxidation as described above, and served as control. Results from this study indicate that increases in PV ranged between 5.93-49.36 meq/kg, 5.93-51.75 meq/kg and 5.93-59.50 meq/kg for SB50, SB100 and SB200 respectively, and were less than the increase (5.93-60 meq/kg) observed for the control; suggesting that APSE was effective in inhibiting the formation of hyroperoxides at all the concentrations (50-100µg/g) studied. However, B50 was the most stable sample throughout the entire 5 days of storage, followed by B100, and finally by B200 whose oxidative stability after day 4 was similar to that of the control. Similar trend was observed for the changes in K_{232} and K_{270}, with 50µg/g being the most effective APSE concentration in inhibiting the formation of conjugated dienes and trienes, followed by 100µg/g, and finally by 200µg/g. The above findings suggest that APSE is a potential source of natural antioxidants which could be added to food products that are high in fat, to help inhibit lipid oxidation.

Keywords: Soybean oil; African pear; Phenolic compounds; Antioxidant; Lipid oxidation

1. Introduction
The African pear (*Darcryodes edulis*), popularly known as ''ube'' in south eastern Nigeria is an indigenous fruit tree of tropical Africa. At maturity stage the skin of the fruit is dark blue or violet, whereas the edible pulp is pale to light green. When in season (may-June) the fruit pulp constitutes an important and much cherished delicacy. In Nigeria, African pear fruit is normally consumed in its fresh form (unprocessed) or after tenderizing through soaking in hot water or roasting, and the seed is typically discarded after consumption of the fruit pulp obviously due to lack of information on its possible usefulness. While literature abound on the physiochemical and nutritional compositions of the edible fruit pulp of Africa pear [1, 2], there is little information on the usefulness of the seed. Extracts from many plants and seeds have been reported to have varying degrees of antioxidant activity in fats and oils [3]. Likewise there are several potentially useful fruit seeds which may have active components inherent in them but have been left unstudied. The seed of Dacryodes edulis is among such seeds.

Soybean oil on the other hand is a common frying oil in many Nigerian households. The oil is highly unsaturated, its major unsaturated fatty acids being oleic acid (19–30%), linoleic acid (44–62%), and linolenic acid (4–11%) [4]. As a result, the oil is prone to rapid oxidation which leads to formation of peroxides among other products and development of rancid and off flavours [5]. Generally, the rate of oxidation depends on factors including the degree of unsaturation of oil, its temperature and the presence of antioxidants. Consequently, oxidative stability of soybean oil can be improved by the addition of antioxidant which can effectively inhibit its oxidation. Phenolic compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an unpaired electron to form stable radical intermediates [6]. In fact, several seeds and have been investigated for phenolic compounds in search of safe sources of natural antioxidants [7]. The present work investigates the usefulness of African pear seed by assessing the ability of its phenolic compounds to inhibit lipid oxidation.

2. Materials and Method
2.1 Source of samples
Soybean oil and African pear, from which African pear seed was obtained, were purchased from a grocery store in Abakaliki, Ebonyi State. The pulp of the African pear was removed to expose its seed which was size-reduced manually and sun dried to a constant weight. The seed coast was removed and the seed was milled using a blender.

2.2 Extraction of phenolic compounds
Total phenolic compounds were isolated from pear seed according to the method reported by Gutfinger [8] with modification. Precisely, 2g of the pear seed were accurately weighed into a centrifuge tube, and dissolved in 20ml of methanol. The mixture was placed in a shaker at room temperature for 40min, then centrifuged at 4000rpm for 20min; after which the supernatant was carefully collected for spectrophotometric evaluation of total phenolic compounds.

2.3 Stripping and Spiking of SBO
Commercial soybean oil (SBO) was stripped of its phenolic fraction by mixing with methanol in a separatory funnel at oil to solvent ratio of 1:10 (w/v), and agitating manually for about 5min. The methanolic fraction (which contained phenolic compounds) was carefully removed, and the SBO layer (stripped oil) was condensed in a rotary evaporator at 45 °C.

Appropriate amounts of the phenolic compounds obtained in section 2.2, were then added to 30g of the stripped oil, to obtain soybean oil samples with final concentrations of 50, 100 and 200 microliter of phenolic compounds per gram of oil (µg/g).

2.4 Spectrophotometric Evaluation

The method reported by Gutfinger was used [8]. Exactly 0.1ml of pear seed extract was measured into 10-ml volumetric flask, then 5ml of distilled water followed by exactly 0.5ml Folin-Ciocalteau reagent were added. The flask was inverted (about 4 times) and allowed to sit for 3 min. At the end of 3 min, exactly 1ml of Na_2CO_3 was added and the solution was diluted to volume with distilled water, and then stored in the dark for 1 hour. After 1 hour the absorbance of the solution at $\lambda = 725$ was measured using reagent solution (prepared in the same manner as the sample) as blank. Concentrations of total phenolic compounds were calculated from calibrated curve of gallic acid standard, in the range 10-100µg/ml ($r^2 = 0.9978$). Results are expressed as microgram of gallic acid equivalence per gram of oil sample (µg/g).

2.5 Antioxidant activity

To evaluate the antioxidant activity of extract from African pear seed, SBO samples spiked at 50, 100 and 200 µg/g (section 2.3) were placed in a 50ml-beaker and stored in an oven set at 60°C, according to the Schaal oven test method [9]. At 24hr intervals, samples were withdrawn from the oven and analysed for peroxide value and extinction coefficients K_{232} and K_{270}, for a total of 120hrs (5 days).

Peroxide value was analysed according to AOCS official method Cd 8b-90 [10], while absorbance at $\lambda = 232$nm (for conjugated dienoic compounds) and $\lambda = 270$nm (for conjugated trienoic compounds) were analysed using the method described by Kamal-Eldin and Pokorny [11]. Exactly 0.05g of oil sample was weighed into a 10ml tube. Five milliliters of isooctane was added to dissolve the sample and the absorbance at λ=232 and 270nm were recorded for each sample using a UV/vis spectrophotometer (Spectronic 2D, USA). A blank determination in an equal volume of iso-octane was also performed. All reagents were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.6 Statistical Analysis

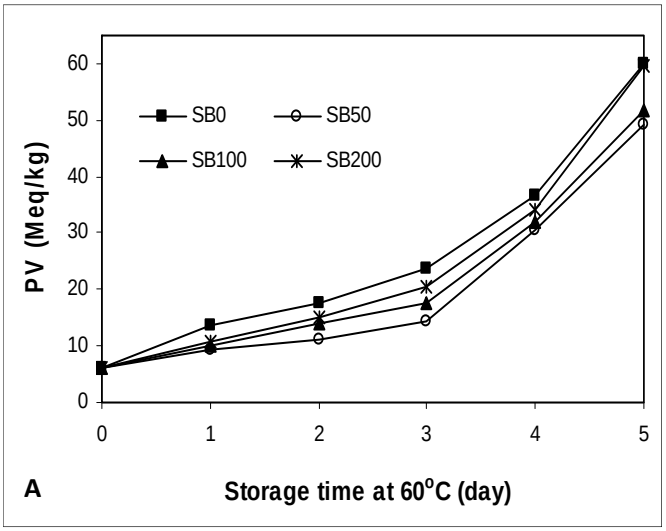
Regression analysis was carried out using Microsoft Excel 2007, to compare the rates of changes occurring in PV, K_{232} and K_{270} of the spiked and unspiked (control) samples during storage at 60°C.

3. Result and Discussion

The initial concentration of total phenolic compounds found in the African pear seed extract (APSE) used in this study was 33.30 mg/g (expressed as gallic acid equivalence (GAE) per gram of dried pear seed) as determined using the Folin–Ciocalteu method which measures the reduction of the Folin-Ciocalteu reagent by phenolic compounds, with the formation of a blue complex which is measured at 725nm. This amount of phenolic compounds is higher than values reported for extracts from potato peel (2.91mg GAE/g) [12], Ocimun basilicum, Apium graveolens and Lepidium sativum seeds (51-92mg GAE/100g) [13] and Okra (Abelmoschus esculentus) seed (142.48mg GAE/100g) [14]. Thus, the seed of African pear can be considered significantly high in phenolic compounds.

Figure 1 depicts the changes occurring in stripped soybean oil with added APSE in the range of 50-200 µg/g, during storage at 60°C. Progressive increment in PV, K232 and K270 were observed in both the spiked and control samples (fig. 1). Good linear relationships were also observed between storage time and changes in these parameters, with correlation coefficients (R^2) ranging from 0.8245-0.8968, 0.7138-0.8993 and 0.9253-0.9815 for PV, K232 and K270 respectively (table 1). Throughout the entire storage period, sample spiked at 50µg/g level (SB50) recorded lower PVs than samples spiked at 100 and 200µg/g levels (SB100 and SB200); and sample spiked at 100 µg/g recorded PVs lower than sample spiked at 200µg/g (fig. 1a). SB200 was the least stable among the spiked samples but on the other hand, more stable than the control (SB0) from the beginning of storage till the fourth month. After the fourth month, SB200 appeared to become unstable, recording PV (59.50 meq/kg) similar to that of the control (60 meq/kg), and suggesting that at 200ppm, APSE lost its inhibitory activity within 4 months of storage (fig. 1a).

Regression analysis (Table 1) indicates that there was faster rate of formation of peroxides in the control (9.86 meq/kg per day) than in the spiked samples (8.12, 8.54 and 9.83 meq/kg per day for SB50, SB100 and SB200 respectively). The rates of peroxide formation observed for the spiked samples indicate that 50µg/g of APSE was more effective in inhibiting the formation of peroxides than 100 and 200µg/g. This inverse relationship is contrary to expectation, and may be attributed to antagonistic effect. Natural phenolic compounds are typically mixtures of free phenolic acids, esters and phenolic glycosides as well as flavonols and polyphenols [6]. Therefore it is possible that depending on their concentrations, these compounds may act synergistically (increasing the antioxidant activity of the extract) or antagonistically (decreasing the antioxidant effectiveness of the extract) [15]. There is reported that the antioxidant activity of α-tocopherol decreased when its concentration exceeded 200ppm [16]. The reduction in antioxidant power observed for APSE at 100 and 200µg/g levels suggests that there is an optimum concentration, beyond which the APSE becomes less effective as antioxidant. This finding emphasises the need to further evaluate the antioxidant activity of APSE at concentrations below 50µg/g.



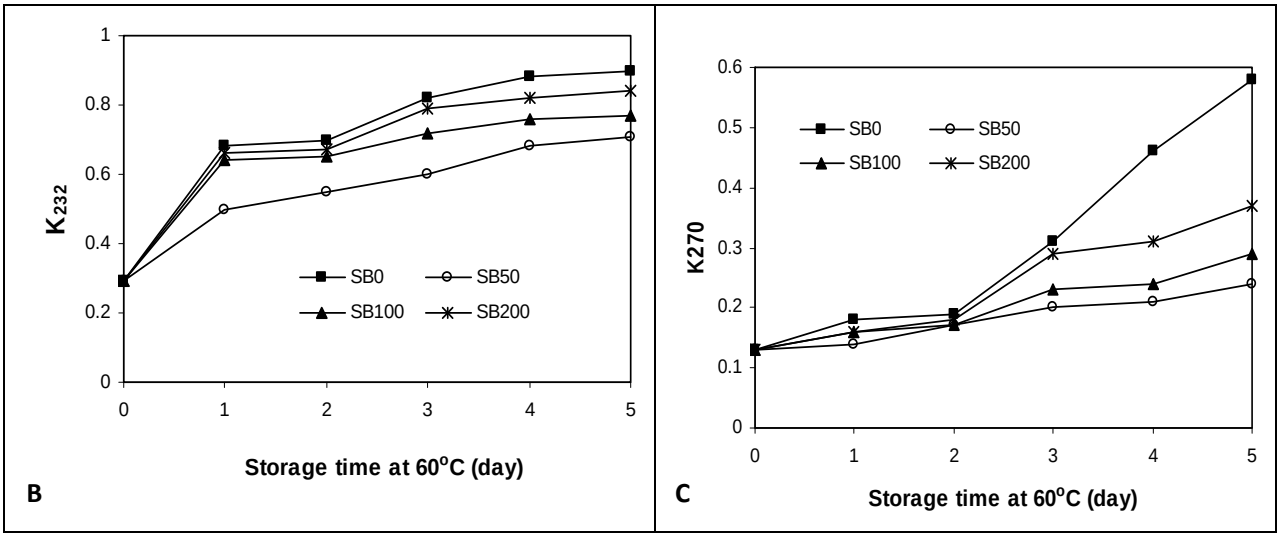


Fig 1: Effect of storage time at 60°C on peroxide value (A), K232 (B) and K270 (C) of spiked and control samples.

Similar trends of increment were also observed for extinction coefficients at 232nm (K232) and 270nm (K270); with the samples containing 50-200ppm APSE recording lower values of K232 and K270 than the control. Measurement of absorbance at 232 and 270nm, quantified by K_{232} and K_{270} has been used as an effective technique for monitoring oxidative stability at a later stage

Table 1: Regression model and correlation between storage time and oxidation indices

Oxidation Index	Sample	Regression: y = ax + b (y = oxidation index; x = storage time; a = slope; b = intercept). R^2 = correlation coefficient.		
		Slope (a)	Intercept (b)	R^2
PV	SB0	9.86	8.25	0.8968
	SB50	8.12	8.36	0.8245
	SB100	8.54	8.02	0.8654
	SB200	9.83	10.11	0.8613
K_{232}	SB0	0.108	0.334	0.7979
	SB50	0.077	0.286	0.8993
	SB100	0.081	0.355	0.7138
	SB200	0.096	0.343	0.7639
K_{270}	SB0	0.092	0.013	0.9253
	SB50	0.023	0.103	0.9815
	SB100	0.031	0.093	0.9639
	SB200	0.050	0.064	0.9537

of oxidation [17]. Lipids containing methylene interrupted dienes or polyene show a shift in their double bond position during oxidation. The resulting conjugated dienes exhibit intense absorption at 232nm; similarly, conjugated trienes absorb at 270nm. The higher the levels of conjugated dienes and trienes, the lower the oxidative stability of a given oil [18]. The changes in K232 and K270 (figs. 1b and 1c) indicate that 50ppm was also the most effective APSE concentration for the inhibition of the formation of conjugated dienes and trienes, and is in line with the inhibition of the formation of hydroperoxides observed figure 1a. While APSE concentration of 200ppm appeared to be ineffective in inhibiting the formation of hydroperoxide (fig. 1a), the same concentration was clearly effective in inhibiting the formation of conjugated dienes and trienes throughout the entire storage period. At 200ppm level the rates of formation of conjugated dienes and trienes was 0.096 and 0.050 per day (SB200) versus 0.108 and 0.092 per day, for the control (SB0) (table 1, slope).

4. Conclusions

Changes in PV, K232 and K270 of spiked samples compared to those of the control, indicate that APSE was effective in inhibiting the formation of primary (hydroperoxides) and secondary (conjugate dienes and trienes) products of oxidation; and thus improved the oxidative stability of soybean oil. However, the ability of APSE to enhance the oxidative stability of soybean oil decreased with increasing concentration in the range (50-200ppm) studied, suggesting that there is an optimum concentration above which APSE becomes less effective as antioxidant. Soybean oil containing 200ppm of extract (SB200) recorded similar peroxide value as the control after the fourth day of storage, indicating that at 200ppm level, APSE lost its ability to prevent the formation of peroxides within 4 days at 60°C. Paradoxically, APSE concentration of 50ppm (SB50) was found to be the most effective concentration for inhibition of the formation of peroxides, conjugated dienes and conjugated trienes. This highlights the need for further work on the antioxidant activity

of this extract at concentrations below 50ppm. Findings from the present work suggest that APSE is a potential source of natural antioxidants which could be added to food products that are high in fat, to help inhibit lipid oxidation.

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