Inhibition of Singlet Oxidation of Vegetable Oils by Ethanolic Extract of Apple Fruits

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Abstract
Samples of stripped and oven dried soya bean oil (SBO), ground seed oil (GNO) and melon seed oil (MSO) were oxidized in the presence of methylene blue dye as a sensitizer with and without added apple fruit extract. Samples of the unoxidized and the oxidized oils were analyzed for their peroxide values free fatty acid values and iodine values to access the inhibitory effect of the apple fruit extract on the oxidative deterioration of the oil samples studied. The results obtained show increases in the peroxide and free fatty acid values of the oxidized oil samples relative to those of the unoxidized samples as indicated by percent increases of the parameters. On the other hand, decreases in values were observed for the iodine values of the oxidized oil samples relative to the unoxidized oil samples. Percent increases in the peroxide values of the oxidized oil samples: - SBO: 378.3% (without extract), 94.2% (with extract); GNO 304.7%, (without extract) 26.67% (with extract); MSO 248.6% (without extract) 83.3% (with extract). Percent increase in the free fatty acid values for SBO: 140.6% (without extract) 91.5% (with extract), GNO 126.7% (without extract), 51.7% (with extract), MSO: 109.6% (without extract), 58.1% (with extract). Percent decrease in the iodine values for the oxidized oils: SBO: 6.74% (without extract) 3.99% (with extract), GNO: 12.19% (without extract), 6.81% (with extract); MSO: 27.63% (without extract) 21.71% (with extract).

***Key words**: Vegetable oils, grape fruit extract, sensitizer, photo-irradiation, quality parameters.*

Introduction

Oils are very useful natural products on account of their nutritional value and numerous industrial applications (Clark and Shivik, 2002; Calmasur, *et al*., 2008). However, their utility is limited by their easily of spoilage due to rancidity. As a result, oil on their own, oil containing foods and other oil-containing products usually have limited shelf-lives.

On exposure to light, oxygen, heat or moisture oils and oils containing substances undergo deterioration (rancidity). Rancidity in oils is either due to the hydrolysis of the constituent triacylglycerols (TAGs), producing free fatty acids and glycerol (hydrolysis rancidity) or a reaction between various reactive oxygen species (ROS) and the multiple bonds in the acyl chains of the constituent TAGS (Shahidi and Zhong,2010; Kamal-Eldin and Pokorny ,2005).

The oxidative deterioration of oils and oil-containing substances may give rise to the following: -

(i) A reduction in the nutritive value of the oil/food via the destruction of vitamins and essential fatty acids.

(ii) The introduction of new substances to the oil/ food that will alter the composition thereby affecting the odor, colour, taste and texture of the oil.

(iii) Some of the new substances may be injurious to human cells/ tissues. Some specific diseases have been traced to the consumption of deteriorated oils either in experimental animals, or humans (Calder, 2013; Cameron-Smith, *et al*., 2015).

(iv) Industrially, deterioration of oils via oxidation limits the application of oils/oil containing substances/products.

In a bid to extent the shelf-life of oils/oil containing substances, and maximally utilize oils various steps are adopted to limit the unwanted deterioration of oils. Some of these includes:

(i) Minimizing the exposure of oil / oil containing substances to conditions that favour deterioration.

(ii) The stripping of oils (refining) in order to remove possible chromophoric impurities.

(iii) The addition of natural / synthetic antioxidants to the oil in order to delay the onset of oxidative deterioration.

Both natural and synthetic antioxidants have been used to improve the shelf lives of edible oils. Vegetable oils such as palm oil and olive oil contain natural antioxidants that account for their extended shelf lives compared to other edible oils.
The use of natural products, especially plant extracts as antioxidants has the added advantage of being biodegradable and can be metabolized by mammalian cells (Rammohan, 2016).

The antioxidants are usually added to the oil during processing. Some authors have used vegetable extracts to retard lipid oxidation in either model systems or edible oils.
Yalcin, *et al*, (2011) used apple pomace extracts and some agricultural wastes (potato and orange peels) as antioxidant to stabilize sunflower oil. The results suggest that the extracts studied can be used as alternative to synthetic antioxidants. Yu, *et al*., (2013) showed that apple phenolics exhibited strong protective capacity on phospholipids. The authors suggested that ethanolic extracts of apple can be used to enhance the oxidative stability of meat products during storage. Sultana, *et al*, (2008) used methanolic extracts of apple peels and other agricultural wastes as antioxidants to stabilize corn oil.

Proteggente, *et al*, (2002) studied the antioxidant activity of some regularly consumed fruits and vegetables such as berries, grapes, onions and apples. The authors noted that the extracts contained phenolic compounds and vitamin C that were responsible for their antioxidant activity. The study also showed that the antioxidant content of the extracts correlated with their antioxidant activities. Bellion, *et al*, (2010) examined the effect of apple fruit extract on the oxidative damage of DNA in CaCO-2 cells. The study revealed that the presence of the extract effectively diminished DNA damage and reactive oxygen species level after twenty-four hours' incubation. The active constituents in the extracts were quercetins.
Bail *et al*., (2013) studied the antioxidant activity and high performance liquid chromatography (HPLC) analysis of polyphenols rich extract from industrial pomace. From the analysis it was observed that industrial pomace was rich in phenols and exhibited potent antioxidant activity.

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Huber and Rupasinghe, (2009) studied the phenolic profile and antioxidant properties of ethanolic extract of apple skin as a natural antioxidant. The extracts were found to effectively inhibit the oxidation of polyunsaturated fatty acids in model systems. The authors suggested that the extracts can be considered as a potential source of natural food antioxidants.

Experimental

Materials and Reagents

Ethyl acetate, triphenyl phosphine, hexane, iodine crystals, dichloromethane sodium thiosulphate, potassium iodide, methylene blue and silica gel.

Sample Collection

Raw seeds of soya beans, melon and ground nuts were obtained from local suppliers at the Mile 3 market in Port Harcourt, Rivers State, Nigeria. Also green apple fruits were obtained from local suppliers in Port Harcourt, Rivers State, Nigeria.

Methods

Oil Extraction

The seeds were cleaned, milled and oil was extracted from the crushed seeds using n-hexane as solvent in a Soxhlet apparatus. All the extracts were desolventized to obtained the oils. The oils were stripped and dried in a hot air oven at 120 ^{o}C, stored in a freezer until used.

Extraction of antioxidant

The green apple fruits were sun-dried, crushed and extracted with ethanol. 10 g of the crushed fruit were soaked 250 ml of ethanol for 72 hours with intermittent shaking. The supernatural was carefully removed and desolventized to obtain the extract.

Irradiation

2.0 g of each oil sample was dissolved in 100ml of 10% methanol in dichloromethane. The mixture was transferred into an impinger and 5 g of methylene blue was added as a sensitizer. The impinger was immersed in an ice-water bath and oxygen gas was gently bubbled in the set-up was externally irradiated with a 200-watt flood light (Fekarurhobo, *et al*., 2013). Control samples were made up and stored in a dark cupboard. The irradiation was similarly repeated for all three oil samples containing 1.0 g of the apple extract. The progress of the irradiation was monitored by thin layer chromatography (TLC) and chemical analysis by reaction with potassium iodide/ starch solution/sodium thiosulphate solution (Angaye, *et al*, 2013).

Analysis of the oxidation mixtures

The peroxide, iodine and free fatty acid values of the oxidation reaction mixtures with and without added apple extracts were determined according to the American Oil Chemist Society Official methods (AOCS, 2004).

Results

Chemical analysis of the oxidation reaction mixtures by reaction with potassium iodide/ starch solution/ sodium thiosulphate solution showed the presence of peroxides (Angaye,S.S.*et al*.,2013) indicating that oxidation has taken place in the course of the irradiation process.

The results of the analyses of the oxidation reaction mixtures are displayed in Table 1 and Figures 1. The peroxide values and free fatty acid values increased for all the irradiated reaction mixtures.

Table 1: Peroxide, free fatty acid and iodine values unoxidized/oxidized oil samples / percentage (%) increases/quenching of the oil samples.

Sample	Parameter	SBO	%INCREASE /DECREASE	GNO	% INCREASE /DECREASE	MSO	% INCREASE /DECREASE
PV	Fresh Oil	3.094		4.89		7.2	
	Oxidized without extract	15.8	378.3%	19.8	304.7%	25.1	248.6%
	Oxidized with extract	5.8	94.2%	6.0	26.6%	13.2	83.3%
FFA	Fresh Oil	0.59		0.56		0.62	
	Oxidized without extract	1.42	140.6%	1.27	126.7%	1.3	109.6%
	oxidized with extract	1.13	91.5%	0.85	51.7%	0.98	58.1%
IV	Fresh Oil	73.02		79.9470		116.5	
	Oxidized without extract	68.1	6.47%	70.2	12.19%	84.3	27.67%
	Oxidized with extract	70.1	3.99%	74.3	6.81%	91.2	21.71%

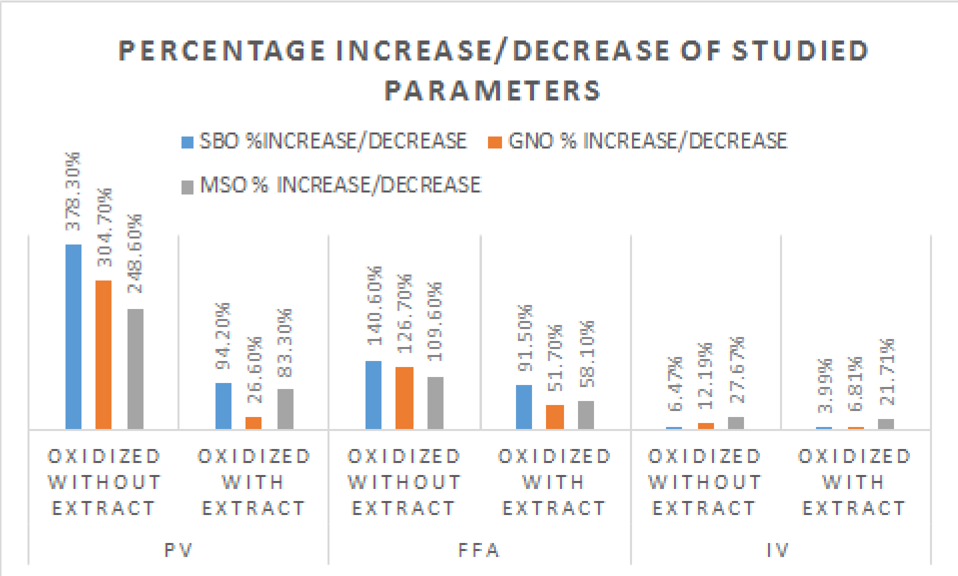

Figure 1: Bar Chart showing increase/decrease of the oil parameters.

The results show that the peroxide values of the reaction mixtures without added grape fruit extract increased by 378.3% (SBO), 304.7 % (GNO) and 248.6% (MSO). On the other hand, the percent changes in peroxide values of the reaction mixtures with added extract 94.2% (SBO), 26.6% (GNO) and 83.3% (MSO). The percent changes in free fatty values follow the same pattern. The percent increases of oil samples without added grapes extracts were as follows: 140.6% (SBO), 126.7% (GNO) and 109.6%(MSO) and the percent increases with added grape fruit extract were 91.5% (SBO), 51.7% (GNO) and 58.1%(MSO) the free fatty acid values of the oils with added grape fruit extract. The percent increases in free fatty acid values were more for the samples without added extract.

A decrease in the iodine values were observed for all the oil samples studied. The percent decreases in iodine values of the oil samples without added grape fruit extract: 6.7% (SBO), 12.19 % (GNO) and 27.63% (MSO). The percent decreases in iodine values with added grape fruit extract were 3.99% (SBO), 6.81% (GNO) and 21.71% (MSO). The percent decreases were more for the samples without added grape fruit extracts.

Discussion

The peroxide values of the oxidized oils increased in comparison to the unoxidized oils. The increases were more with the oils samples without added apple extracts. The same trend was observed for the free fatty acid values. The results indicate that the presence of the extract has inhibited both oxidative and hydrolytic rancidity in all the oil samples under study. As noted earlier, the iodine value of given oil sample indicates the level of unsaturation of the oil. Sensitized oxidation affects the unsaturated carbon linkages in the acyl chains of the constituent TAGs and the decreases in the iodine values of the oxidized oils are in agreement with this. Also the observation that the decrease was more in the oil samples without the extract further confirms the observation that the presence of the extract slows down the oxidative deterioration of the oil samples.

Apple fruits are known to contain polyphenols which exhibit antioxidant properties. The results of the present study are agreement with the observations of Sekhon-Loodu *et al*., (2013). These authors isolated polyphenols from frozen and dried apples which showed antioxidant properties. The extracts when added to omega-3 enriched fished oils stabilized the oils by delaying onset of deterioration. The study also showed that the flavonol-rich fractions inhibited fish oil oxidation by 40-62% at a total phenolic concentration of 200 micro gm /ml.

The results are also in agreement with the observations of Yalcin, *et al*., (2011). These authors added ethanol extracts of potato and orange peels and apple pomace to sunflower oil with a view of improving the oils shelf-life. The results of the study showed that all the extracts exhibited various levels of stabilization to the oil. The peroxide and free fatty acid values were lower than control samples while the decrease in iodine values was also observed. It was the view of the authors that these extracts can function as natural antioxidants agents for the inhibition of oxidative deterioration in vegetable oils and oil containing food products.

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