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EFFECT OF SOME PLANT EXTRACTS ON THE STABILISATION OF RUBBER SEED OIL

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ABSTRACT

In this study, the effects of extracts of orange and potato peels, as natural antioxidants, on the stability of rubber seed oil were studied and compared with the effects of the synthetic antioxidant butylated hydroxyl toluene (BHT). The antioxidants of orange and potato peels were extracted using methanol as solvent, and the total content of phenolic and flavonoids compounds present were determined using the Folin–Ciocalteu and aluminium chloride respectively. Free radicals scavenging of the extracts were also determined using 1,1-diphenyl-2-picrylhydraxyl (DPPH). The effects of different concentrations of extracts in delaying oxidative rancidity in rubber seed oil were studied through calculating the peroxide, iodine and free fatty acids values of the oil periodically and these effects were then compared with those of the synthetic antioxidant, BHT. Results obtained showed that, compared with synthetic antioxidants, high concentrations of extracts of orange and potato peels significantly increased rubber seed oil stability. Also, the extracts of potato and orange peels contained 34.72µgTAE/g and 55.90µgTAE/g and 28.04µgQE/g and 33.704µgQE/g of total phenolics and total flavonoids contents respectively. The capacity for DPPH free radicals to scavenge 50% of the potato and orange peels extracts, $lC_{50,}$ was given in concentrations terms as 1.74 and 0.75 in mg/ml respectively. These results compared with that of the control and synthetic antioxidants justify orange and potato peels extracts as natural potential antioxidants.

Keywords: Antioxidants, orange peel, potato peel, rubber seed oil, waste

INTRODUCTION

Rubber seeds oil (RSO) contains saturated acids (palmitic and stearic) and unsaturated acids (oleic, linoleic and linllenic) with the unsaturated acids having about 77.4% of the acid composition [1]. Rubber seed oil has high free fatty acid content [2]. Due to the high content of unsaturated fatty acids in rubber seed oil, the stabilization of this oil against oxidation has

attracted attention. Oil oxidation is a free radical chain process leading to the deterioration of oil and lipid containing materials. Studies have shown that antioxidants terminate these chain reactions by probably removing free radical intermediates, or by being oxidized themselves [3]. In foods, these reactions can lead to rancidity, loss of nutritional value from the distribution of vitamins and essential fatty acids and the possible formation of toxic compounds and coloured products [4].

In order to overcome the stability problems of oil and fats which do not only deteriorate the quality of fats and fatty foods and bring about chemical spoilage, but also produces free radicals and reactive oxygen species (ROS) which are reportedly associated with carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases [5,6], synthetic antioxidants, such as butylated hydroxyanisone (BHA), butylated hydroxytoluene (BHT) and ter-butyl hydroquinone (TBHQ) are being used as foods and oils additives. Recent reports revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis [7, 8]. To avoid this health risks, natural antioxidants from plants origin are being given serious attention. Thus, interest in natural antioxidant, especially of plants origin, has greatly increased in recent years [9]. Rubber seed oil was selected for this research work as little or no work has been carried out on its oxidative stability studies.

The main aim of this research study is to investigate the effect of extracts from orange and potato peels as antioxidant in stabilizing rubber seed oil.

MATERIALS AND METHODS

Potato peels (*Solanum tuberosum*), were obtained from a local potato chip producer at Oba Market, Benin City, Nigeria; and orange peels were obtained from oranges bought from the same market. The oranges were peeled manually. Chemicals including BHT, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, methanol, chloroform, glacial acetic acid, potassium iodide, sodium thiosulphate, potassium hydroxide, folinciocalteu reagent were bought from a chemical shop. All chemicals used were analytical grade.

Rubber seeds oil was produced from Rubber Research Institute of Nigeria (RRIN) and was free from any synthetic antioxidants.

SAMPLE PREPARATION

Potato peels and orange peels were washed and sun dried for two weeks and ground to fine powder in a mill. The powders were filtered through a sieve with 90 μm aperture. 10 g each of

the waste in separate experiment were turned into 100 ml of methanol in an electric shaker at room temperature for six hours followed by filtration through Whatman No 1 filter paper. The residues were re-extracted under the same conditions. The combined filtrates were evaporated in a rotary evaporator below 40 °C. The concentrates were placed in a water bath below 40 °C to drive off the remaining methanol. The extract obtained after complete evaporation of methanol were weighted to determine the extract yield and stored at until further use.

DETERMINATION OF TOTAL PHENOLICS CONTENT

The total phenolics content were determined colorimetrically using Folin-Ciocalteu reagent according to the method described by Ebrahimzadeh et al. [10]. About 0.5 ml of the extract samples were mixed with Folin-Ciocalteu reagent (5 ml with distilled water by rate 1:10) for 5 min and 4 ml aqueous solution of 1M sodium trioxocarbonate (IV) were added. The mixture was allowed to stand for 15 min and the polyphenols were determined by a UV spectrophotometer at 765 nm. The standard curve was prepared by 0, 0.25, 2.5, 25 and 250 µg/ml solutions of tannic acid in methanol: water (50:50 v/v).

TOTAL FLAVONOIDS CONTENT

Colorimetric aluminium chloride method was used for flavonoids determination according to the methods described by Clabro et al. [11] and Ebrahimzadeh et al., [10]. 0.5 ml solution of each extracts was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of potassium acetate and 2.8 ml distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve, which was prepared using quercetin solutions at concentrations (31.25, 62.5, 125 and 250) µg/ml in ethanol.

SCAVENGING EFFECT ON DPPH RADICALS

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometer assay uses stable radical DPPH as a reagent [12]. Ali-quots (50 µl) of various concentrations of the extract in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After 30 min incubation at room temperature the absorption was read against a blank at 517 nm. Inhibition of free radical of DPPH in percentage terms (I%) was calculated in the following way:

$$1\% = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100 \quad (1)$$

where A blank is the absorption of the control reaction (containing all reagents except the sample) and A sample indicates the absorption of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract. All tests were done in triplicate.

Sample Preparation for Oxidative Stability Determination

Methanol extracts of orange and potato peels at three concentrations (200, 1000, and 2000 ppm) and the synthetic antioxidant BHT at the concentration of 200 ppm were added to antioxidant free rubber seed oil in clean glass containers (100ml) and thoroughly mixed for a few minutes for the antioxidants to be completely dispersed in the oil .A control sample was also prepared by putting same volume of the oil free- antioxidants in 100ml glass container. The oil samples were then kept at room temperature for 36 days and their peroxide value free fatty acid values and iodine values were measured on the same day and at 12 days interval (12, 24 and 36 days) measured by AOCS official method [13].

STATISTICAL ANALYSIS

Means of all the data were taken. Line graphs were used to plot calibration curves, coefficient of determination which is the value that indicate how well data fit statistical model was determined

RESULTS AND DISCUSSION

Table 1 shows the extraction yields of potato and orange peels. The yield of the orange peels is higher than that of the potato peels.

Table 1: Extraction yield of fruit peels using methanol solvent

Plant	Extraction yield (%)
Potato	12.45
Orange	15.97

Table 2: Total phenolic (TPC) and total flavonoid content (TFC) of the methanolic extracts

Plant	**TPC (µgTAE/g)**	**TFC (µgQE/g)**
Potato peels extract	34.72	28.04
Orange peels extract	55.90	33.70

The phenolics and flavonoids contents of potato and orange peels extracts are presented in table 2. It can be seen that they both have antioxidants potentials though in different amounts. Cuvelieret al., [14] shows a direct relationship between antioxidant activity and the amount of total flavonoids or total phenolcs. These results are in agreement with that of Hegazy and Ibrahim, [15], but different from that of Azadeh et al., [4] and Sima and Esmaeil, [16]. The difference may be as a result of using different drying and extraction methods.

Table 3: DPPH free radical scavenging capacity

Plant	**DPPH , IC_{50} (mg/ml)**
Potato peels extract	0.75
Orange peels extract	1.74

Table 3 shows the result of the free radical scavenging capacity of both extracts using DPPH. It was found that the radical scavenging capacity of both extracts increased with increasing concentration. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. From the result therefore in Table 3, the concentrations of the extracts which 50% of DPPH free radical scavenges are 1.74mg/ml for orange peels and 0.75mg/ml for potato peels..

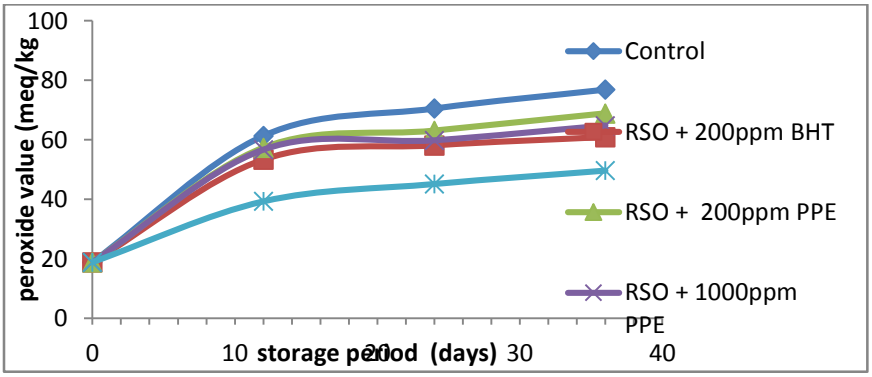

Figure 1: Effect of potato peel extracts (PPE) on peroxides values in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

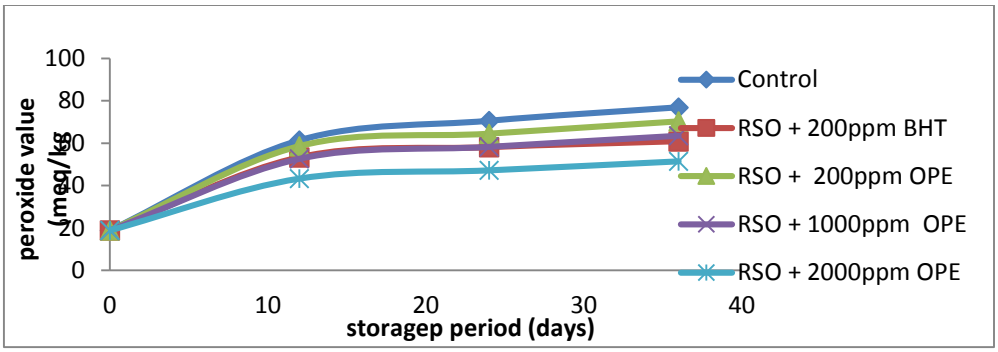

Figure 2: Effect of orange peel extracts (OPE) on peroxides values in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

Figures 1 and 2 show the variation of synthetic antioxidant, BHT and extracts from potato and orange peels on peroxide values of rubber seed oil (RSO) stored for 36 days. From the figures it is observed that peroxide value increases as days of storage of oil increases, both for BHT and extracts of these peels. RSO to which no antioxidant was added served as the control. This increase in the peroxide value can be attributed to the formation of hydroperoxides, which are the initial products of oxidation. Addition of the peels extracts to rubber seed oil made the oil more resistant to oxidation, and this rise in resistance caused a reduction in the gradient of the increase in the peroxide value. Samples having 2000 ppm of the extracts were recorded to have the least effect, followed by the ones with 200 ppm of BHT. It was found that 200 ppm samples have the greatest effects. Samples of oils containing the BHT and extracts from these peels evaluated for free fatty acid values show similar results to that of peroxide values as shown in Figure 3 and 4. Iodine values of sample of oils to which BHT and peels extract of potato and orange were added show that iodine value decreases as days of storage of oil increases as presented in Figure 5 and 6. Samples having 2000ppm of the extracts decreased the least followed by samples containing 200 ppm BHT. Oil sample with 200 ppm of the extracts decreased the most.

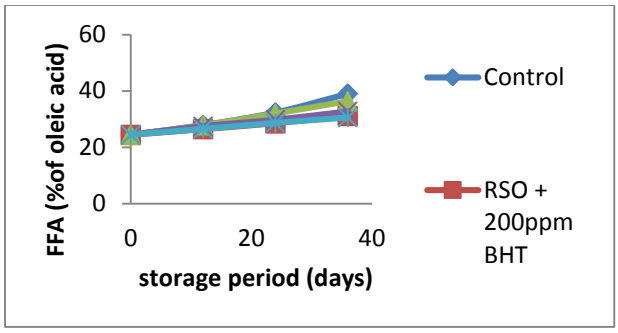

Figure 3: Effect of potato peel extracts (PPE) on free fatty acids in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

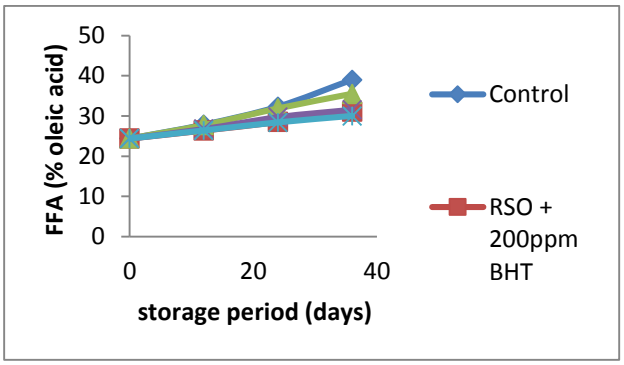

Figure 4: Effect of orange peel extracts (OPE) on free fatty acids in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

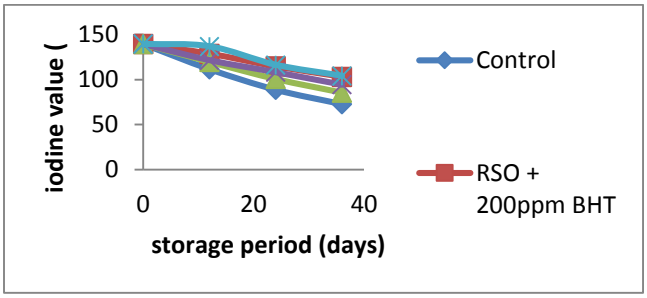

Figure 5: Effect of potato peel extracts (PPE) on iodine values in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

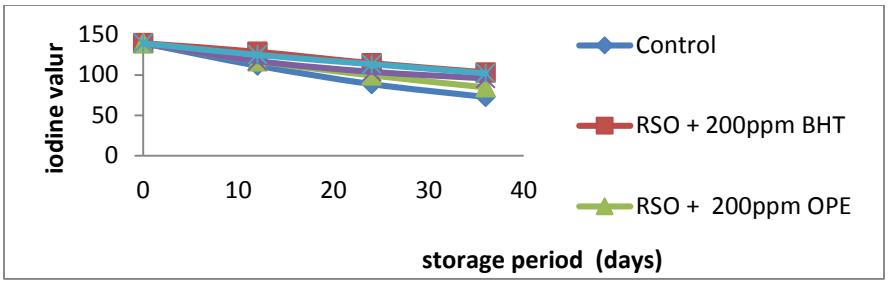

Figure 6: Effect of orange peel extracts (OPE) on iodine values in comparison with control and BHT in rubber seeds oil stored at room temperature for 36 days

CONCLUSION

It has been shown that potato and orange peels extracts can be considered as an antioxidant for vegetable oils in general and for rubber seeds oil in particular. Methanol extracts of these peels showed antioxidant potency comparatively when added to rubber seeds oil. The protective effects that the orange and potato peels extracts have on RSO were comparable with that of the widely used synthetic antioxidant BHT. The extracts showed the capacity to scavenge for free radicals. Total phenolics and total flavonoids contents in the extracts made them exhibit antioxidants potency by delaying or resisting oxidation of vegetable oils. Thus, methanol extracts of orange and potato peels could be prepared and added to the commercial vegetable oils especially rubber seeds oil as natural antioxidant and suitable alternative for some synthetic antioxidants.

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