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Comparative Study of the Antioxidant Effect of *Sacoglottis gabonensis* Stem Bark Extract, a Nigerian Alcoholic Beverage Additive and Vitamins C and E on the Peroxidative Deterioration of some Common Stored Vegetable Oils in Maiduguri

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Abstract: The study of the protective effects of S*acoglottis gabonensis* stem bark extract on the peroxidative deterioration of some common stored vegetable oils in Maiduguri over a period of time was carried out in comparison with vitamins C and E. Lipid peroxidation was monitored as lipid hydroperoxide carbonyls and lipid aldehydes concentrations on a time course basis over a period of 35 days in the vegetable oil test free and their respective antioxidant treated test samples. The treatment with the bark extract reduced the rate of formation of malondialdehyde and malonaldehyde at a rate that compared favourably with the inhibitions obtained with the antioxidants, vitamins C and E. The mechanism of inhibition of the observed anti-oxidant action of the bark extract appeared to be by inhibition of propagation of lipid peroxidation. Results obtained seemed to have confirmed earlier reports that the bark extract could find ready application as a nutritional antioxidant additive.

Key words: *Sacoglottis gabonensis,* ascorbic acid, d-alpha tocopherol, antioxidants, lipid peroxidation

Introduction

Plants of many species have been reported beneficial in the treatment of some diseases. Some of these reports include that by Ladeji and Okoye (1996) on the antihepatotoxic properties of *Vitex doniana* bark extract, Ladeji *et al.* (1996) on the effect of *doniana* bark extract on blood pressure of rats, by Sugiyama *et al.* (1993) on purpurogalin as an antioxidant protector of mammalian erythrocytes again lysis by peroxyl radicals, by Parasakthy *et al.* (1993) on eugenol as hepatoprotector against CCl_4 and Gilani and Janbaz (1995) on the protective effect of *Cyperus scariosus* extract on acetaminophen and CCl_4 induced hepatotoxicity. These plants have contributed to the antioxidant status of the bodies by exogenous sources fighting to reduce the free radical fluxes that cause toxic diseases.

Scacolottis gabonensis is a tropical rainforest tree found in the tropical rainforest region of West Africa and America (Okoye, 2001). In certain rural communities of Nigeria especially Abia, Akwa Ibom, Rivers, Delta, Edo and Imo States, the stem bark of this tree is commonly used as an additive to palm wine. Palm wine is an indigenous alcoholic beverage tapped/derived from natural fermentation of the sap obtained from either the oil palm tree or raffia palm tree. Freshly harvested unfermented sap is a clear colourless liquid with a sweet sugary taste and no alcohol content but soon becomes milky and increasingly less sugary and more intoxicating (Okoye, 2001). This was reported to be due to suspension of living microorganisms. The *S. gabonensis* bark extract is normally added when the palm wine is fresh.

Among the rural consumers of *S. gabonensis* stem bark extract treated palm wine, claims are unanimous that the extract imparts an ember colour and bitter taste to temper the sugary palm wine. It also increases shelf life of the palm wine, suppresses the foaming and effervescence as well as reducing the intoxicating power of the palm wine. No undesirable effect had been reported in any of the consumers of the bark extract- treated palm wine and it is the popularity that sustained its use that generated the study carried out on *S. gabonensis* stem bark extract.

Ekong and Ejike (1974) reported that the bark extract arrested fall in pH of palm wine during fermentation thus, suggesting antioxidant properties. Okoye and Neal (1988a, b, 1991) reported the *in vivo* effect of the bark extract with aflatoxin B_1, a naturally occurring food contaminant that is carcinogenic. Postulations from these indirect evidences would suggest that the bark extract possess cytoprotetive properties against cytotoxicants. The antioxidant activity of the extract was examined in detail by Maduka (2000) using 2,4-dinitrophenyl hydraxine as the primary experimental oxidant. The extract and its isolate, bergen in were found to inhibit experimental membrane lipid peroxidation *in vivo* at a stage prior to formation of lipid hydroperoxide (Maduka and Okoye, 2002a, b) the first stable intermediate of the lipid peroxidation pathway (Wills, 1987). The extract also effectively inhibited the 2,4-DNPH-induced depletion of

ascorbic acid and vitamin E in the liver, red blood cell and brain as well as the oxidant's superoxide dismutase-depressing effect. *In vitro,* the bark extract and bergen in also acted as antioxidant protectors of mammalian erythrocytes against CCl_4 peroxyl radicals (Maduka *et al*., 2002; Maduka and Okoye, 2000c) by inhibiting free radical propagation. The above report however, did not gives comparative studies with any of the antioxidant vitamins C and E which are used in the assessment of biological antioxidant properties thus, limiting comparativity. The present study has addressed itself to the question of whether the bark extract inhibition compares with the antioxidant effects of vitamins C (ascorbic acid) and E (d-α-tocopherd) as part of its nutritional evaluation.

Materials and Methods

Preparation of *Sacoglottis gabonensis* stem bark extract: Samples of fresh cuttings of *Sacoglottis gabonensis* stem bark were purchased from Ekeapara market, Aba, Abia State, Nigeria wrapped in polythene bag and immediately upon return to Maiduguri refrigerated pending use. They were washed and the inner most layer was scrapped to reveal the dark inner amber coloured layer which was bitten to a fibrous mesh and then stepped in 4% aqueous ethanol solution in 1 liter beaker (1:10 w/v), covered and kept in the dark for three days. After three days, this was filtered through Whatmann filter paper and the filtrate was preserved.

Preparation of stored vegetable oils: Four stored vegetable oils commonly used in Maiduguri Metropolis namely unbranded palm-kernel oil, rap seed oil, palm oil and locally produced groundnut oil commonly called kuli-kuli oil were each used independently for this series of investigations.

Hundred ml of each of the oil samples were put into four clean separately labeled beakers as controls and then left in the open laboratory to normal atmospheric conditions favourable to lipid peroxidation for a period of forty days. To a second set of four beakers separately containing 100 ml of different oil samples was added 100 mg of vitamin C and as vitamin C tests. A third set of four beakers were similarly provided but 100 ml of vitamin E (d-α tocopherol) USP by VARDHMYAN export, Ghafkopar, Mumbai, India) was added into each of the beakers and marked as vitamin E tests respectively. Similarly, to hundred ml of each of the oils samples was added 100 ml of 1:10 w/v *Sacoglottis gabonensis* aqueous stem bark extract prepared in 4% ethanol as described earlier. This set of beakers were marked as *Sacoglottis gabonensis* stem bark extract tests. The three sets of beakers vitamin C, vitamin E and bark extract tests, a total of 12 beakers were then treated as the 1st set of controls and left in the laboratory for 40 days for normal peroxidation to occur under atmospheric conditions. During this period lipid peroxidations were monitored on 6,10, 15, 20, 25, 30 and 35 days in different oil sample tests and their respective controls by lipid hydroperoxide carbonyls and lipid aldehydes. The procedure adopted was described by Maduka (2000) and Maduka and Okoye (2002d). Lipid peroxidation was determined on day 1st only on the control samples. These determinations were performed to study or observe the time course of peroxidation reactions in the controls and their respective antioxidant tests.

Determination of the effects of *Sacoglottis gabonensis* stem bark extract, vitamins C and E on the peroxidative deterioration of stored vegetable oils: The rates of lipid peroxidation were determined in all the stored vegetable oil controls and their respective antioxidant treated tests on the days designated above. The level of malondialdehyde was determined by the method of Hunter *et al.* (1963) as described by Maduka and Okoye (2002d) while malonaldehyde determination was done by the thiobarbituric acid reactivity method of Gutteridge (1984). Results were presented as means±S.D. of triplicate determinations while statistical comparison between the various oil sample controls and their respective tests done by Mann-Whitney 2-pair sample t-test was set at $P<0.05$ (Maduka, 2000; Maduka and Okoye, 2002d).

Results

The protective effect of the antioxidants on the deterioration of repassed oil: The level of lipid hydroperoxide carbonyl (malondialdehyde) formed over the 35 days period for rap-seed oil (Fig. 1) and the course of formation of malonaldehyde over the same period (Fig. 2) indicated that malondialdehyde level increased in the control from day 1st to 35th showing increased lipid peroxidation. This same trend was displayed by the other oil samples treated with antioxidants- vitamins C, E and bark extract even though at different rates (Fig. 1). The three antioxidants protected the oil from deterioration with the antioxidant- with vitamins C and E competing favourably, as shown by significantly reduced levels of malonaldehyde formation with vitamin C displaying the best protection (Fig. 2).

The protective effect of antioxidants on the peroxidative deterioration of unbranded palm kernel vegetable oil: The time course of formation of malondialdehyde in the unbranded vegetable oil over a period of 35 days for the control and antioxidant treated controls indicate that the rate of formation of malondialdehyde was highest in

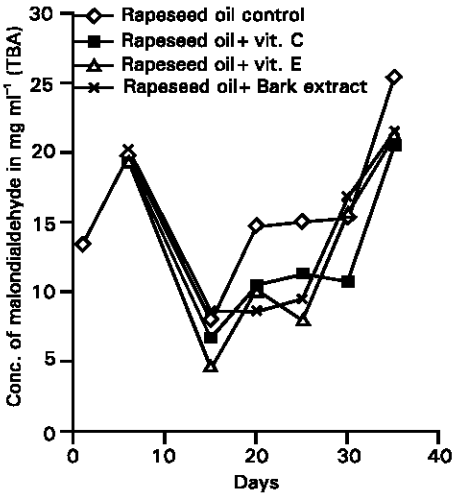

Fig. 1: Profiles of malonaldehyde formation in repassed oil

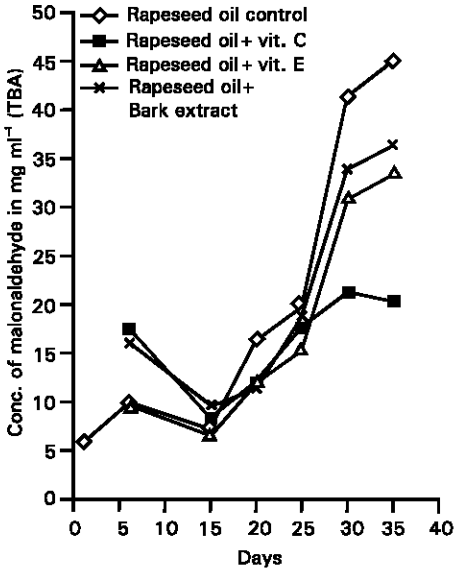

Fig. 2: Profiles of malonaldehyde formation in repassed oil

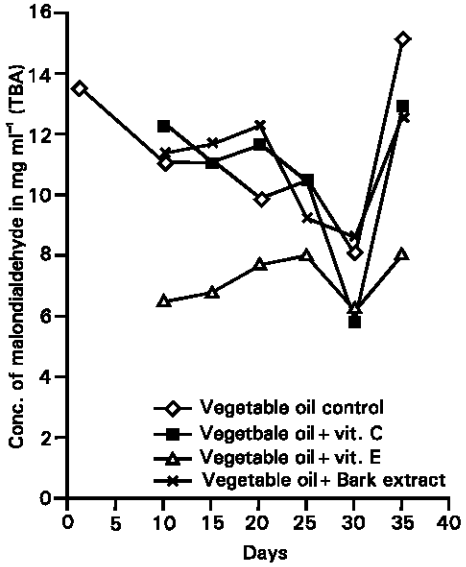

Fig. 3: Profiles of formation of malonaldehyde in vegetable oil

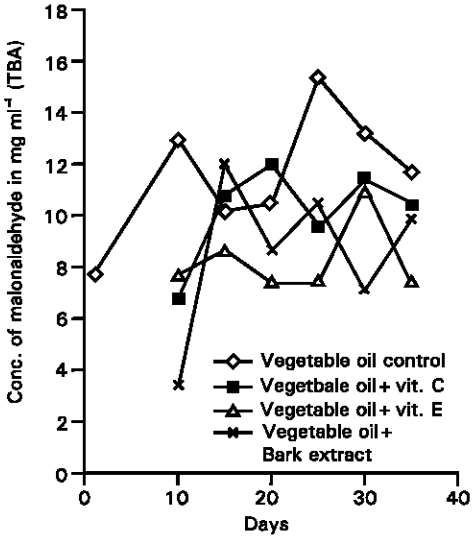

Fig. 4: Profile of formation of malonaldehyde in vegetable oil

control (Fig. 3). The antioxidants considerably reduced the level with vitamin E being the most potent inhibitor followed by the bark extract and lastly vitamin C. The course of formation of malonaldehyde followed essentially the same trend as that observed with malondialdehyde. The malonaldehyde was highest in the control oil sample, vitamin E was the highest inhibitor followed by the bark extract and then vitamin C (Fig. 4). The trend also showed that the three antioxidants stabilized the oil against deterioration.

The protective effect of the antioxidants against the peroxidative deterioration of kuli-kuli: The profile of formation of lipid hydroperoxide carbonyl (MDA) in the locally produced groundnut oil showed a peak on day 20 and an inflection on day 30 at highest level of formation in control (Fig. 5). The level in the three antioxidant treated samples (Fig. 5) showed that lipid peroxidation rate was being considerably inhibited with vitamin E again displaying optimal or maximum inhibition followed by vitamin C and then the bark extract. In malonaldehyde (Fig. 6). Vitamins C, E and the bark extract inhibited peroxidation at rates that compared favourable and significantly ($P<0.05$) compared with the non-treated control.

Effect of three antioxidants on the deterioration of palm oil: The control showed highest level of MDA

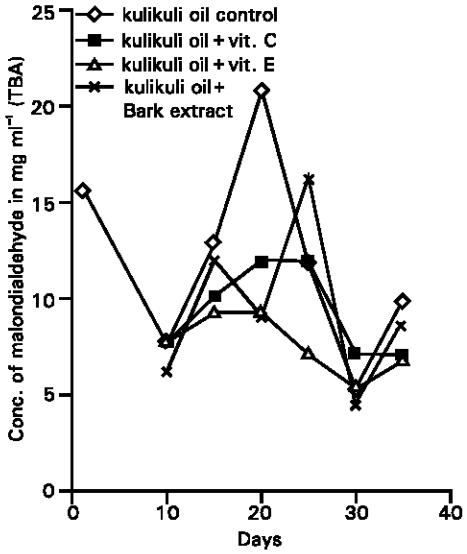

Fig. 5: Profiles of malonaldehyde in kulikuli oil

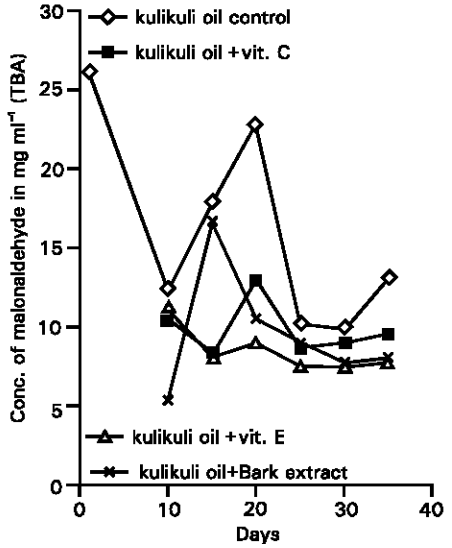

Fig. 6: Profiles of formation of malonaldehyde formation in kulikuli oil

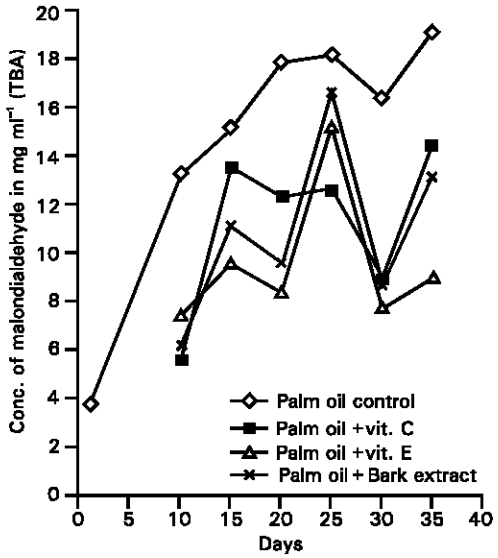

Fig. 7: Profiles of formation of malonaldehyde in palm oil

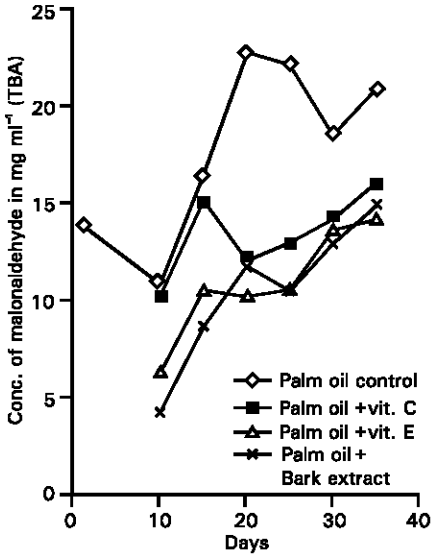

Fig. 8: Profiles of malonaldehyde in palm oil

with a threshold from day 20 to 25, which fell on day 30 and rose steadily (Fig. 7). The three antioxidant treated controls showed peaks on days 10 and 25 with two inflections on days 20 and 30. Overall, three antioxidants inhibited peroxidation significantly ($P<0.05$) compared with the control. The trend of inhibition being vitamin E bark >extract > vitamin C. Fig. 8 shows the profile of malonaldehyde in palm oil samples over the 35 days incubation period. The trend in Fig. 8 follows essentially the same pattern observed in Fig. 7 with the trend of inhibition being vitamin E> bark extract > vitamin C > control palm oil.

Discussion

Maduka and Okoye (2002d) recently reported that the aqueous stem bark extract of *Sacoglottis gabonensis* and its isolate, bergen in protected against lipid peroxidative deterioration of stored vegetable oils by inhibition of free radical propagation over a period of three months. The above study did not report effect of vitamins C (ascorbic acid) and E (d-alpha-tocopherol) two novel antioxidant vitamins of known modes of action used in the assessment of biological antioxidant properties thus, limiting comparativity. The series of investigations being reported has addressed itself to answering the question

arising from the above limitation as to whether the observed antioxidant action of the bark extract can compare favourably with those inhibitions observed with vitamins C and E.

Data have been presented to show that the three antioxidants used in this study namely *Sacoglottis gabonensis* stem bark extract, Vitamins C and E significantly reduced the formation of lipid hydroperoxide the first major product of lipid peroxidation (Wills, 1987) in all the four stored vegetable oils tested compared with their respective antioxidant free controls. This would suggest a possible role for the bark extract as an exogenous antioxidant additive in the preservation of foods and drugs. Lipid hydroperoxides are formed as intermediates after the oxidation of lipid peroxidation pathway in the reaction between isomerized fatty acid free radicals (lipid peroxyl radical) and oxygen. The carbonyl centers of these lipid hdroperoxides combine with thiobarbituric acid to give a complex that absorbs maximally and therefore, quantitatively determined spectrophotometrically as thiobarbituric acid reactive products. The bark extract inhibited peroxidation at rates that compared favourably with the inhibitions by vitamins C and E. The mechanism of action was by inhibition of propagation of lipid propagation, consisted with the earlier reports of Maduka and Okoye (2002d).

There had been earlier reports that the bark extract exerted antioxidant action against 2,4-dinitrophenyl hydrazine induced membrane peroxidation (Maduka and Okoye, 2002a, b) and also spared tissue depletion of natural antioxidant defenses (Maduka and Okoye, 2002e) *in vivo*. This study has shown that the bark extract also has antiperoxidative properties *in vitro*. Earlier postulations of antioxidant actions of the bark extract were based on observations of indirect evidences (Ekong and Ejike, 1974; Okoye and Neal, 1988a, b, 1991). This report used direct reliable indices of lipid peroxidation and seems to have confirmed that the stem bark extract is a good antioxidant. This study also suggests that the antioxidant property of the bark extract may be useful in the assessment of biological antioxidant properties of natural plant products.

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