



INVESTIGATION OF THE EFFECT OF THREE CAMEROONIAN SPICES (*Zingiber officinale*, *Xylopiya parviflora* and *Eugenia caryophyllata*) ON PACKAGING AND SHELF LIFE OF CRUDE SOYBEAN (*Glycine max*) OIL

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ABSTRACT: Lipid peroxidation reduces quality of fats and oils, result in chemical spoilage and produces free radicals which have health implications. This work was set to investigate the effect of three spices (*Zingiber officinale* (ZO), *Xylopiya parviflora* (ZP) and *Eugenia caryophyllata* (EC) packaging and storage on shelf life of crude soybean (*Glycine max*) oil. The spices and soybeans were obtained from Muea market. Crude oil was extracted from soybean. Methanolic extracts of the spices were screened for phytochemical constituents and antioxidant potentials. Qualitative phytochemical tests were used to detect the presence of secondary metabolites, while two quantitative methods; Folin-Ciocalteu and DPPH free radical scavenging assay were used to determine antioxidant potentials of the extracts. Antioxidant effectiveness of the extracts were assessed using crude soybean oil (CSO) as oxidation substrate, under non-accelerated and accelerated storage conditions as well as storage in different types of containers and the quality of the oils were then determined. Results revealed highest amount of extracts from EC (37.97%) and six classes of secondary metabolites (steroids, saponins, alkaloids, cardiac glycosides, phenolics, flavonoids, tannins and triterpenoids) detected to varying degrees in all. Phenols and triterpenoids were detected in all three spices. Total phenolic content of XP, EC and ZO were 9.23, 12.03 and 16.30g of GAE/100g respectively. Their ability to prevent lipid oxidation was comparable to synthetic antioxidants and in the order of ZO, EC, XP. Peroxide values were 47.52±3.20, 12.17±0.46, 21.12±0.27, 15.52±2.39, 25.74±0.15, 31.54±0.25 for control (without preservative), ZO, BHA, EC, BHT and XP respectively. The best storage condition was refrigeration and oil preserved with ZO and stored in dark containers showed the best oil quality indices. This study therefore revealed the extracts as potent antioxidants for stabilization of oils.

KEYWORDS: Lipid peroxidation, Spices, Packaging, Phytochemical, Antioxidant, *Zingiber officinale*, *Xylopiya parviflora*, *Eugenia caryophyllata*.



I. INTRODUCTION

Traditionally in all the cultures of the world, herbs and spices are used as flavoring agents in cookery as well as medicinally to treat various ailments [1]. Spice could be a dried bark, root or vegetable substance of a plant while herb is termed as the part of leafy green plant such as fruit, berries or any herbaceous plant [2]. Herbs and spices contain phytochemicals commonly known as antioxidants such as polyphenols, phenolic acids, tannins, flavonols, isoflavones and curcuminoids [3]. The high proportion of these antioxidants is the reason of various health benefits imparted by the herbs and spices [4]. The free radicals produced as a result of various metabolic processes taking place in the body are one of the important causes of premature aging, cancer, diabetes, dementia and myocardial infarction as they interact with cellular DNA and cause its mutation [5]. Reactive oxygen species (ROS) and nitrogen (RNS) species, as the most frequent pro-oxidants, either originate from normal metabolism or are induced by UV radiation and different pollutants. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by intake of antioxidant substances [6,7]. The antioxidants present in herbs and spices are capable of neutralizing these free radicals by donating required number of electrons to stabilize them. Once the free radicals are stabilized after the acceptance of electrons, they become non-reactive to cellular DNA [8]. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones [9,10]. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants have been found to have genotoxic effect [11,12]. Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous Common Cameroonian spices such as *Zingiber officinale*, *Xylopiya parviflora* and *Eugenia caryophyllata* are the focus in this study in order to bring out their antioxidant profile.

Due to their notable pharmacological effects, some spices are widely used in traditional and modern medicine for preparation of bitter tonics, treatment of digestive disorders, loss of appetite and dyspepsia [13], easing bloating, and as nervous system stimulant. Medicinal substances from plants exhibit antihypertensive [14], antispasmodic [15], antioedematogenic [16], analgetic [17], insecticidal [18], anti-inflammatory [19], antimicrobial [20-22], especially antihelicobacter activity [23], cytoprotective [24] and significant antileukemic activity [25]. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic properties [26-28].

The amounts of soybeans and total vegetable oil crops have been rising for a number of years. World production of soybeans in 2003 was estimated to be 184.49 million MT out of 317.89 million MT total for vegetable oil crops, making soybeans the world's largest oilseed crop, rivaled only by palm oil [29]. The U.S. production of soybean oil was estimated at 8.59 million MT for 2002, of which 7.86 million MT was consumed domestically. During 2002–2003, Brazil produced 4.90 million MT and Argentina 4.12 million MT of soybean oil [30].

Soybeans owe their dominance of the oilseed market to the value of their protein, which is much greater than that of other oilseeds. Of the oilseed meals produced in 2003, 129.58 million MT out of a total of 185.69 million MT was soybean meal [31]. Soybean oil of typical composition performs well as a salad oil, but it is usually hydrogenated for use as a margarine stock or frying oil. Soybean oil's stability to oxidation is limited by its content of linolenic acid. Recent decades have witnessed numerous attempts to manipulate the fatty acid composition of soybean oil to help it compete better in various uses, but the cost of growing, segregating, and testing special varieties and resistance to genetically modified oils have limited the appeal of these altered varieties. The use of natural antioxidants from plants may be very useful for stabilizing soybean oil as well as other vegetable oils [32].

II. METHODOLOGY

2.1 Study design

This was a laboratory based experimental study which was done according to the flow diagram in figure 1.

2.2 Sample collection and preparation

2.2.1 Spices

Fresh plant materials (roots of *Zingiber officinale* (ginger), flowers of *Xylopiya parviflora* (clove) and leaves of *Eugenia caryophyllata*) were bought from marketers at Muea market located in Buea, South West Region of Cameroon. The plants materials were identified at the Department of Botanic and Plant Science, Faculty of Science, University of Buea.

2.2.2 Soybean seeds

Soybean seeds were bought from Mbveh market located in Kumbo, Bui Division, North West Region of Cameroon. The seeds were rinsed with tap water, air dried for an hour and taken to the laboratory for oven drying and further analysis.

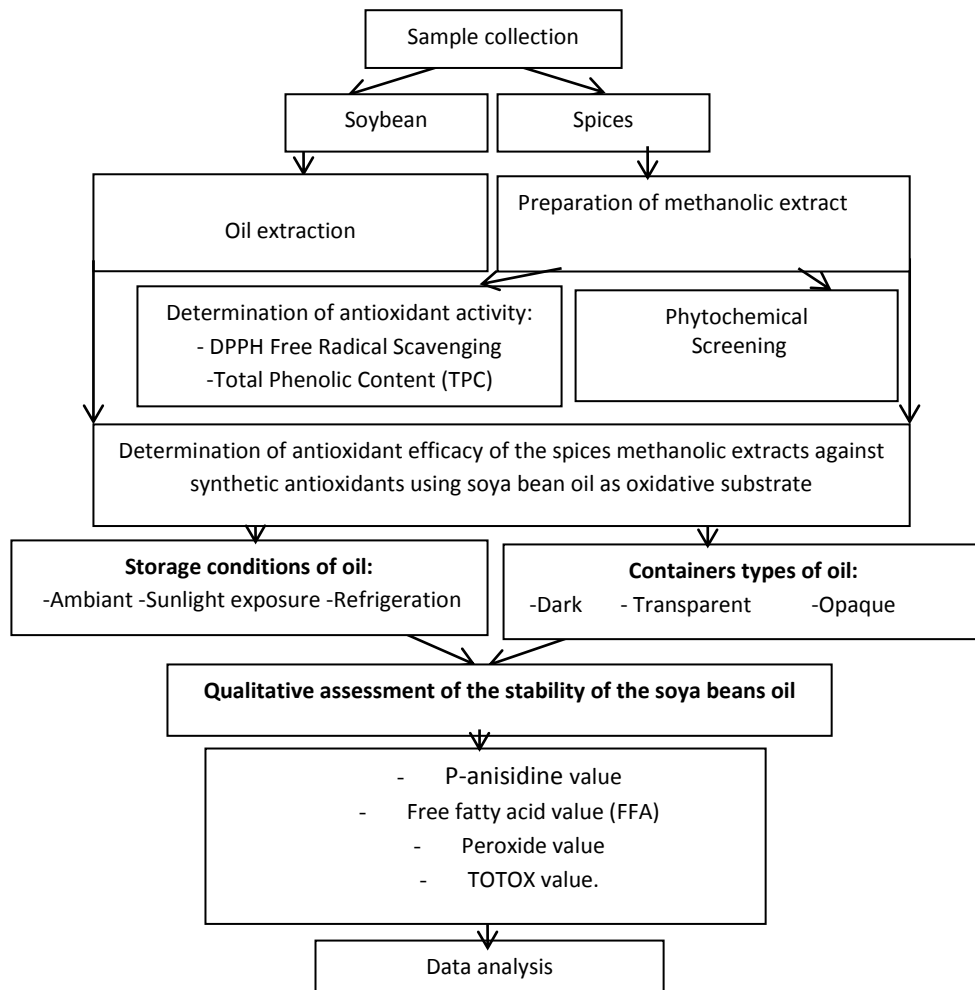


Fig. 1 Work flow chart design

2.3 Preparation of plant extracts

The different spices were rinsed with tap water, oven dried at 45°C for 72 hours and ground to pass through a 1mm aperture sieve. Thirty-seven grams (37.5g) each of *Zingiber officinale*, *Xylopiaparviflora* and *Euglenacaryophyllata* were weighed into 600ml of methanol and allowed to stand at room temperature with regular shaking for 48 hours. The mixture was then filtered and the residue obtained was redissolved in 300ml of methanol and allowed to stand for 48 hours with regular shaking to ensure the complete extraction of phenolic compounds. The filtrate obtained was subjected to rotary evaporation at 45°C under reduced pressure to remove the solvent. Residual solvent was removed by drying in air at room temperature after which the crude extracts were weighed to calculate the yield and stored at 4°C. An aliquot of each crude extract obtained was used for phytochemical tests while the remaining fraction was kept for further studies.

2.4 Extraction of crude Soya bean oil

Soybean seeds were extracted according to the method of Womeni et al [33]. Soybean seeds were oven dried at 50°C for 48 hours and ground to pass through a 1 mm aperture sieve. About 184.2g of soya bean powder was weighed into 500 ml of hexane and allowed to stand at room temperature with regularly shaking for 48 hours. The mixture was filtered and the filtrate obtained was subjected to rotary evaporation at 55°C under reduced pressure for the removal of

the solvent. Crude soya bean oil obtained was weighed to calculate the yield and stored at -18°C prior to further analysis.

2.5 Determination of the antioxidant potential of the methanolic extracts

Both qualitative and quantitative methods were used to determine the antioxidant potential of the methanolic extracts.

1. Quantitative screening of the methanolic extracts of the spices
2. Determination of Total Phenolic Content (TPC)

The total phenolic content of the methanolic extracts was determined using the Folin-Ciocalteu colorimetric method as described by Gao et al. [34]. Twenty microliters ($20\ \mu\text{L}$) of the plant extracts were mixed in a test tube with 0.2 ml of Folin-Ciocalteu reagent and 2 ml of distilled water and incubated at room temperature for 3 min. Following this, 1 ml of 20% sodium carbonate solution was added to the mixture and re-incubated for 2 hours at room temperature. The absorbance of the resulting blue color was measured using a quartz cuvet at 765 nm. Gallic acid was used as standard and total phenolic contents were expressed as gram gallic acid equivalents per 100 g of extracts.

2.5.1.2 Evaluation of the Antioxidant Activity by the DPPH Free Radical Scavenging Assay

The free radical scavenging ability of the methanolic extracts was determined according to the method described by Mensor et al. [35]. One milliliter (1 mL) of 0.3 mM alcoholic solution of DPPH was added to 2.5 ml of the samples in a test tube and shaken to homogenize. The samples were kept at room temperature in the dark and the optical density was measured after 30 min at 517 nm. The optical density of the samples, the control and the blank, were measured in comparison with methanol. One synthetic antioxidant, butylatedhydroxytoluene (BHT) was used as positive control and the antiradical activity (AA) was determined by the following formula:

$$\text{AA}\% = 100 - \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}}$$

Where; Abs = Absorbance

2.5.2 Qualitative phytochemical screening of the methanolic extracts of spices

The presence of major antioxidant secondary metabolite classes, namely; steroids, saponins, alkaloids, cardiac glycosides, phenolics, flavonoids, tannins and triterpenoids was determined using standard phytochemical methods with some modifications [36-39].

2.5.2.1 Test for saponins (Frothing test)

Saponins were tested by dissolving 0.5 g of the crude extract in a test tube containing 3 mL of hot distilled water and then the mixture was shaken vigorously for 1 min and persistent foaming observed indicated the presence of saponins.

2.5.2.2 Test for flavonoids (Cyanidine test)

Crude extract (0.5 g) was dissolved in methanol and 2 mL of concentrated hydrochloric acid added. A spatula full of magnesium turnings was added and the mixture observed for effervescence, then a brick red colouration observed indicated the presence flavonoids.

2.5.2.3 Test for steroids (Lieberman-Burchard test)

About one half gram (0.5 g) of the crude extract was dissolved in dichloromethane to give a dilute solute solution and then 0.5 mL of acetic anhydride added, followed by three drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

2.5.2.4 Test for tannins (Ferric chloride test)

One half gram (0.5 g) of the crude extract was dissolved in 20 mL of boiling distilled water and then boiled for an hour. A few drops of ferric chloride were added and allowed to stand for proper colour development. A blue-black colouration indicated the presence of tannins.

2.5.2.5 Test for Alkaloids (Dragendorff's test)

The sample was dissolved in dichloromethane and then spotted on a thin layer chromatographic plate which was developed in 20 % hexane in ethylacetate. The presence of alkaloids in the developed chromatogram was detected by spraying with freshly prepared Dragendorff's reagent in a fume chamber. A positive reaction on the chromatogram indicated by an orange or darker coloured spot against a yellow background is confirmatory evidence that the plant extract contained alkaloids.

2.5.2.6 Test for cardiac glycosides

To the plant extract (0.5g), 2 mL of glacial acetic acid plus one drop of ferric chloride were added. The set up was underplayed with 1 ml of concentrated sulphuric acid. There was the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer which indicated the presence of cardiac glycosides.

2.5.2.7 Test for phenolics

To 1 mL of the plant extract, one drop of 5% FeCl₃ (w/v) was added. Formation of greenish precipitate indicated the presence of phenolics.

2.5.2.8 Test for Terpenoids (Salkowski test)

Five millilitres (5mL) of the sample was mixed with 2mL of chloroform and 3ml of concentrated H₂SO₄ added carefully to form a layer. A reddish-brown coloration of the inter face formed indicated the presence of terpenoids.

2.6 Evaluation of antioxidant efficacy of extracts using crude soybean as oxidation substrate

The frozen soy oil was melted prior to use for analysis. Antioxidant efficacy of the three plant extracts were determined according to the method described by Iqbal and Bhanger [40] with some modifications. Methanolic extracts of the spices (*Zingiber officinale*, *Xylopiya parviflora* and *Euglena caryophyllata*) were added to 20g of pre-heated crude soybean oil at 50°C for 3 h at concentration of 500 ppm. Synthetic antioxidants (BHT and BHA) were employed at the legal limit of 200 ppm [41] to compare the efficacy of natural antioxidants. The air dried extracts as well as the synthetic antioxidant were mixed with a minimum amount of absolute methanol using a vortex and added to 20g of oil and mixed again for 3 min. The samples were placed in narrow-necked bottles (transparent, translucent and dark) with stoppers and stored under different storage conditions (ambient temperature, refrigeration, and sunlight exposure) for 8 weeks. Some of the samples were heated in an oven at 65°C for 3 days. Control samples were also placed under the same storage conditions. Analyses of oxidation parameters of the crude soybean oil were carried out before and after 8 weeks of storage.

2.7 Measurement of oxidation parameters of crude soybean oil

The quality of soybean oil after addition of natural and synthetic antioxidants was evaluated by measuring the following oxidation parameters; free fatty acid value (FFA), peroxide value (PV) and P-anisidine (AV) value according to the procedure of AOCS [42]. Total oxidation (TOTOX) values were calculated using the equation: $TOTOX = 2PV + AV$ according to Shahidi et al. [43].

2.8 Evaluation of the effect of storage condition on the quality of crude soybean oil

2.8.1 Unaccelerated storage conditions

Antioxidant efficacy of extracts was determined according to the method of Iqbal and Bhanger [40]. Methanolic extracts of spices were added to pre-heated crude soybean oil (at 50°C for 3 hours) at concentration of 500ppm. Synthetic antioxidants (BHT and BHA) were employed at the legal limit of 200ppm [41] to compare the efficacy of natural antioxidants. The dried extracts as well as the synthetic antioxidant were mixed with a minimum amount of absolute methanol using a vortex and added to 25g of oil before mixing again for 5 min. All the samples were placed in narrow-necked bottles of different types; opaque (O), dark brown (D) and transparent (T) containers (without stoppers) and placed under different storage conditions (ambient temperature, refrigeration and sunlight exposure). Control samples were also placed under the same storage conditions and analysis was done after 0 (t₀), 2 and 4 months.

2.8.2 Accelerated storage conditions

Lipid oxidation was accelerated by continuously heating the soybean oil samples (both the control and stabilized oil samples) at 65°C for 24 days while analysing after every 4 days (96 hours) since it is known that heat is one of the factors that accelerate the process of lipid oxidation.



2.9 Statistical Analysis

The data are presented as means ± SEM. The mean values of the control and test groups were compared using Bonferroni tests performed using Graphpad Insat 2000 software. P<0.05 considered to be significant.

III. EXPERIMENTAL RESULTS

3.1. Extract yield of spices and soybean oil

The extract yield of total antioxidative components from the different spices varied from 14.53% to 37.97% as shown in table 1 with the highest amounts of extracts obtained from *Eugenia caryophyllatta* (37.97%) followed by *Xylopiya parviflora* (27.07%) and the lowest amount obtained from *Zingiber officinale* (14.53%). The yield of soybean oil was 20%.

Table 1. Percentage yield of plant extracts and oil yield of soy bean

Plant extracts /soy bean oil	% yield
<i>Zinziber officinale</i>	14.53
<i>Xylopiya parviflora</i>	27.07
<i>Euglena caryophyllatta</i>	37.97
Soy bean oil yield	20.00

3.2. Qualitative Chemical Screening of the Methanolic Extracts of the spices

Table 2 shows the phytochemical profile of *Xylopiya parviflora* (X.P), *Eugenia caryophyllatta* (E.C) and *Zingiber officinale* (Z.O). Qualitative analysis of these plant extracts revealed the presence of at least six classes of secondary metabolites (steroids, saponins, alkaloids, cardiac glycosides, phenolics, flavonoids, tannins and triterpenoids) in all plant extracts, except that of *Zingiber officinale* (Z.O).

Table 2. Phytochemical profile of the plant extracts

Phytochemicals constituent	Plants extract		
	E.C	Z.O	X.P
Steroids	++	-	+
Alkaloids	-	-	-
Cardiac glycosides	+	-	+
Phenolics	++	+	++
Tannins	+	-	++
Flavonoids	-	+	++
Saponins.	+	-	+
Triterpenoids	+	+	+

Where: E.C =*Euglena caryophyllatta*, Z.O= *Zinziber officinale*, X.P= *Xylopiya parviflora*, + = Positive test, - =Negative test.

3.3. Total Phenolic Content (TPC)

Table 3 presents the total phenolic content of *Xylopiya parviflora* (X.P), *Eugenia caryophyllatta* (E.C) and *Zingiber officinale* (Z.O). The amount of phenolics in these plant extracts varied widely and ranged from 9.23 to 16.30 g of gallic acid equivalents (GAE) /100 g of extract. The total phenolic content of *Xylopiya parviflora* (X.P), *Eugenia caryophyllatta* (E.C) and *Zingiber officinale* (Z.O) were 9.23, 12.03 and 16.30g/100 g respectively.



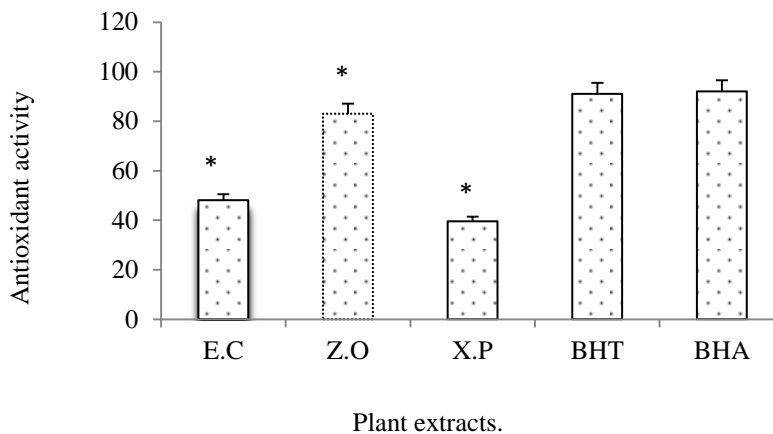
Table 3. Total phenolic content (TPC) of the plant extracts

Spices	TPC (g GAE/100 g of extract)
<i>Zinziber officinale</i>	16.30±0.03
<i>Xylophia parviflora</i>	9.23± 1.02
<i>Euglena caryophyllatta</i>	12.03±0.24

4. 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay

The measurement of the ability of a molecule or substance to scavenge free radicals has become routine in testing the antioxidant property of plant extracts. This has become the fingerprint of primary antioxidants. The DPPH radical has the property to abstract labile hydrogen atoms, and the ability of an antioxidant to stabilize this radical by donating its hydrogen is related to its potential capability to inhibit lipid oxidation [44]. The ability of the tested spices extracts to scavenge the DPPH radical as compared to the synthetic antioxidants BHT and BHA is presented in Figure 2.

Zingiber officinale (ZO) extract exhibited the highest antioxidant activity on DPPH free radicals, similar to that of butylated hydroxy toluene (BHT) and butylated hydroxyanisole (BHA) followed by *Eugenia caryophyllatta* (EC) while the lowest antioxidant activity was observed in the extract of *Xylophia parviflora* (XP).



EC= *Eugenia caryophyllatta*, ZO = *Zinziber officinale*, XP = *Xylophia parviflora*, BHT = Butylatedhydroxytoluene, BHA = Butylatedhydroxyanisole. * = (P<0.05)

Fig. 2 Scavenging effect of spices extract (Z.O, X.P and E.C) and synthetic antioxidants (BHA and BHT) on 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical.

3.5. Antioxidant efficacy of extracts using crude soybean oil as oxidation substrate

Oxidative stability of oils is defined as the resistance to oxidation during processing and storage [45], which can be expressed as the period of time necessary to attain the critical point of oxidation, depending on sensorial changes or a sudden acceleration of the oxidative process [46]. Low-molecular-weight off-flavor compounds are produced during oxidation, and off-flavor compounds make oil less acceptable to consumers or for industrial use as a food ingredient. Furthermore, the oxidation of oil also destroys essential fatty acids and produces toxic compounds and oxidized polymers. Oxidation of oil is very important in terms of nutritional quality and toxicity of edible oils. Storage conditions and oil quality parameters help to provide useful information on antioxidant efficacy. The results of oil quality stored with natural and synthetic antioxidants under different conditions are as follows.

3.5.1 Un-accelerated storage conditions for soybean oil

3.5.1.1 Effect of sunlight exposure

Under direct sunlight exposure there was visible deterioration of the color of the oil and the odor of the oil also changed to rancid by the 4th month. The color changed from greenish yellow to dark yellow. The samples under this study were exposed to sunlight on daily basis for at least 7 hours. However, the fact that the intensity of sunlight for the different days varied could be a limiting factor. In addition, this study extended to the rainy season, so rainfall could also be considered as another limiting factor.



3.5.1.1.1 Free fatty acid value (% FFA)

Acid value defines the predisposition or susceptibility of oil to oxidation. However free fatty acids are unstable and decompose to primary products of oxidation. At the same time, other triglycerides are also converted to free fatty acids. Free fatty acids can also occur in fats and oils as a result of enzymatic hydrolysis by lipases, metal ions acting as free radicals or at an elevation of temperature. However, decrease in free fatty acid value indicates oxidation state of fats and oils. Figure 3 shows the effect of sunlight exposure on the acid value (represented as %oleic acid) of crude soybean oil stored in different containers and treated with different antioxidants. Result reveals a decrease in free fatty acid value of the soybean oil samples with increase in storage time for all container types compared to control. The highest acidity was recorded for oil samples stabilized (preserved) with EC and XP and stored in transparent containers whereas for opaque the highest acidity was recorded for samples stabilized with XP, BHT and ZO for dark brown container. High FFA symbolizes hydrolysis or less oxidation while very low acid value indicates high oxidation. Transparent containers compared to dark brown and opaque containers had lower FFA. A significant increase ($p < 0.05$) in acidity recorded for T2X.P, T2E.C, D2Z.O, O2Z.O and O2BHT compared to control sample (CTR) indicates that decrease in acid value is not only by sunlight exposure but also by temperature catalytic hydrolysis of triglycerides and endogenous and exogenous lipases.

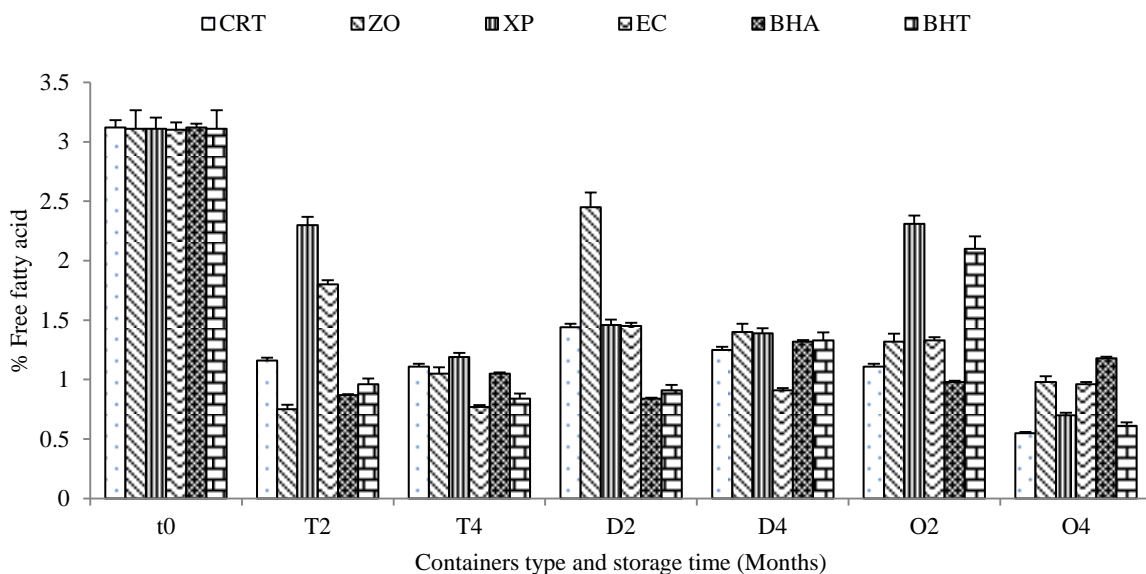
EC= *Eugenia caryophyllata*, ZO = *Zinziber officinale*, XP = *Xylopiya parviflora*, BHT = Butylatedhydroxytoluene, BHA = Butylatedhydroxyanisole, O = Opaque containers, D= Dark containers, T= Transparent containers, t0= Time zero

Fig. 3 Effect of sunlight, container type and storage time on the acid value of crude soybean oil treated with different antioxidants

3.5.1.1.2 Peroxide value

The peroxide value (PV) measures the primary products of lipid oxidation. Figure 4 shows the effect of sunlight on the quality of crude soybean oil treated with different antioxidants in different containers. A significant increase in peroxide value ($p < 0.05$) with storage time and container type was observed for all samples exposed to sunlight. In addition, oil samples without antioxidants (CTR) also showed high peroxide values compared to stabilized (with antioxidant) samples with increase in storage time.

After 2 months of storage, almost all samples under sunlight had peroxide values greater than 10.45meq/kg which is the maximum recommended by CODEX norms for oil quality. This indicates that sunlight is not a good storage condition as such oil will easily become rancid and develop off flavour.



EC= *Eugenia caryophyllata*, ZO = *Zinziber officinale*, XP = *Xylopiya parviflora*, BHT = Butylatedhydroxytoluene, BHA = Butylatedhydroxyanisole, O = Opaque containers, D= Dark containers, T= Transparent containers, t0= Time zero

Fig. 4 Effect of sunlight, container type and storage time on the peroxide value of crude soybean oil treated with different antioxidants

3.5.1.1.3 P-anisidine value

P-anisidine assay is the most widely used method for the measurement of the secondary oxidation products produced in the termination phase of lipid oxidation (i.e aldehydes, ketones, alcohols, etc) which may contribute to off-flavor and rancidity of oils [40]. The P-anisidine value of the control (CTR) and stabilized (with plant extracts and synthetic antioxidants) soybean oil samples inside transparent (T), dark brown (D) and opaque (O) containers under sunlight exposure are shown in figure 5.

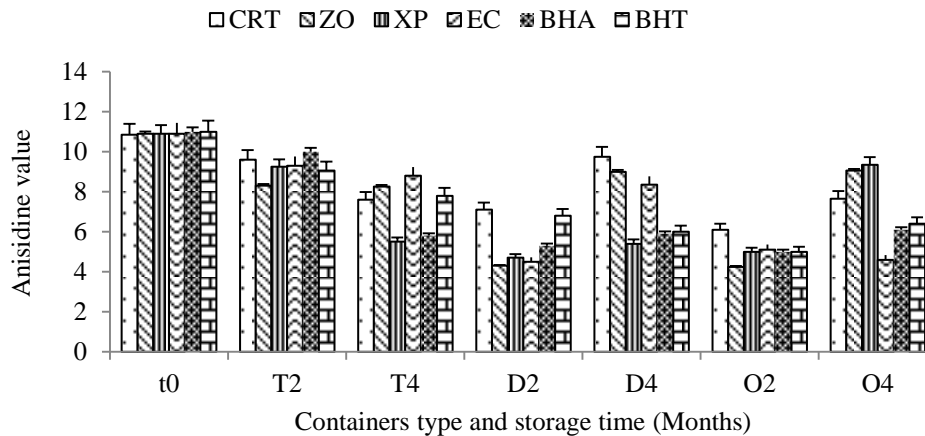


Fig.5 Effect of sunlight, container type and storage time on the P-ANISIDINE value of crude soy bean oil treated with different antioxidants

In comparison with the non-stored oil (t0) a significant decrease in P-anisidine value ($p < 0.05$) was observed which could be due to the formation of more secondary products of oxidation and since these products are volatile; the P-anisidine values of stored samples were less than those of t0 (without storage). For the transparent container, a continuous decrease in P-ANISIDINE value was noted for all the samples with increase in storage time under sunlight exposure whereas with dark brown and opaque containers the P-anisidine values decreased from 0 to 2 month and later increased from 2 to 4 months.

The control samples for all the container types presented with the highest P-ANISIDINE values compared to the stabilized oil samples. This could be explained by the fact that the presence of antioxidants in the stabilized soybean oil samples slowed down the formation of primary and hence secondary products of lipid oxidation. The lower the P-anisidine value of oil, the better it is for consumption.

3.5.1.1.4 TOTOX value.

Figure 6, presents the evolution of the total oxidation (TOTOX) of crude soybean oil during storage in transparent (Figure 6A), dark brown (Figure 6B) and opaque (Figure 6C) glass container and treated with different antioxidants.

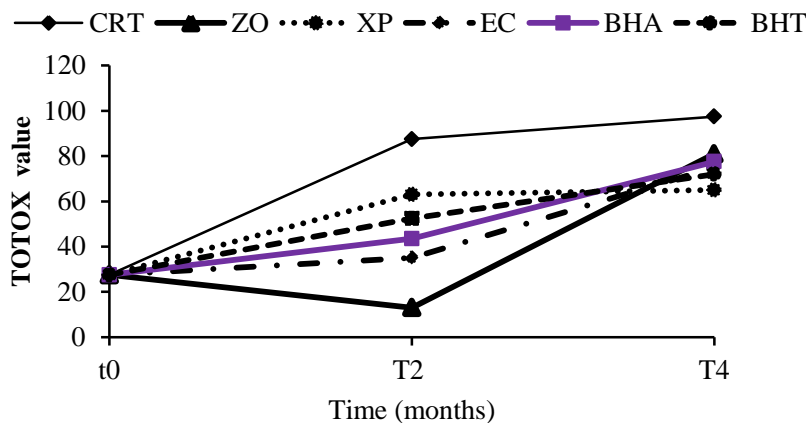


Fig. 6A. Effect of sunlight on the TOTOX value of crude soybean oil treated with different antioxidants and stored in transparent containers

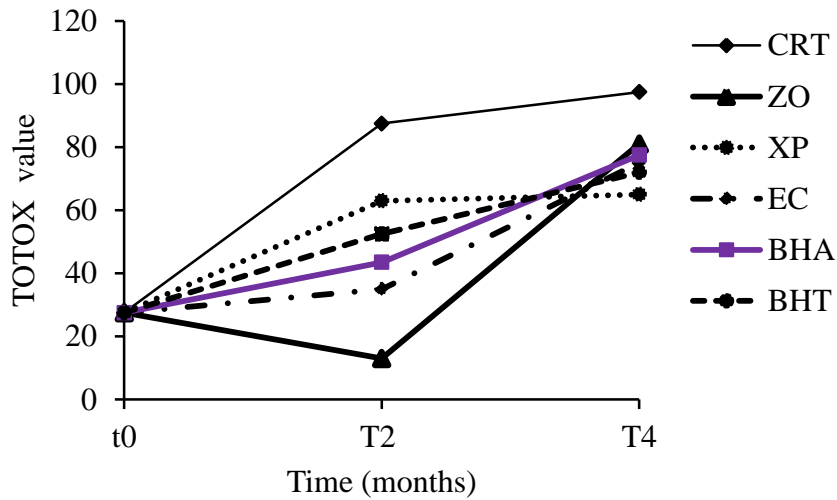


Fig. 6B . Effect of sunlight on the tottox value of crude soybean oil treated with different antioxidants and stored in dark brown containers

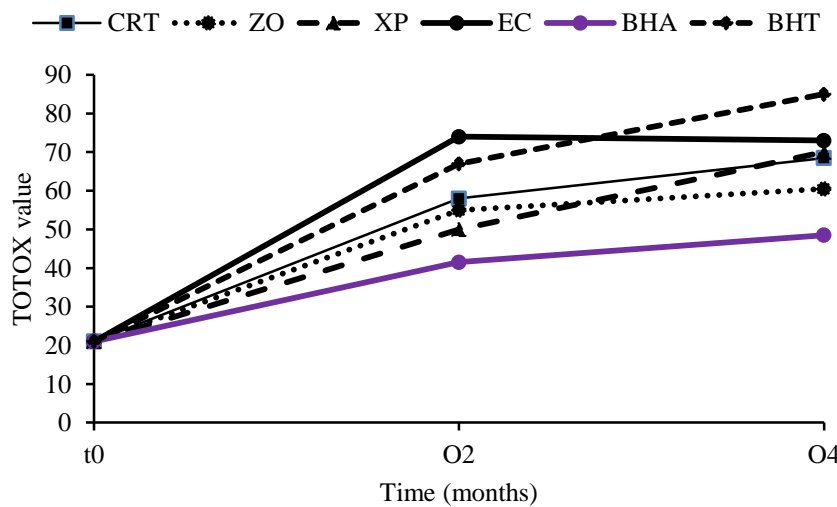


Fig. 6C. Effect of sunlight on the TOTOX value of crude soybean oil treated with different antioxidants and stored in opaque containers.

In general, the TOTOX values of all the oil samples were significantly increasing ($p < 0.05$) in a regular pattern over the 4 month storage time for all the different storage containers. As observed with the acid and peroxide values, the control exhibited the highest total oxidation state. For transparent containers, oil samples containing ZO, EC and BHT (in that order) were much less oxidized compared to the control (CTR); for dark brown containers, samples containing ZO, BHT, EC, and XP (in that order) were less oxidized whereas in opaque containers, samples containing ZO, BHT, BHA were more stabilized. Globally, samples with ZO showed more preservation followed by BHT and EC while samples with XP and BHA were the most oxidized.

3.5.1.2 Effect of ambient temperature

Room temperature varied from 23-28°C and only indirect light exposure was available to the samples under study. There was mild colour change towards the 4th month, which became overtly yellowish towards the end of the 4th month and mild rancid smell was noticed by this time.

3.5.1.2.1 Free fatty acid value (FFA)

FFA values of soybean oil with different sources of antioxidants stored for a period of 4 months at ambient temperature is shown in Figure 7. A significant decrease in acid value ($p < 0.05$) was observed during the storage period. The lowest acid values were observed for the control (CTR) compared with the stabilized samples containing plant extracts (Z.O, E.C and X.P) at 500ppm and synthetic antioxidants (BHA and BHT) at 200ppm for each of the storage container type.

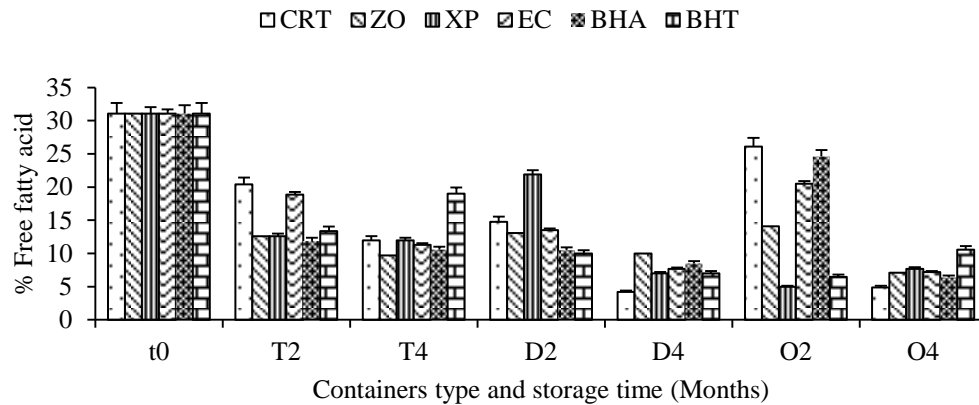


Fig.7 Effect of ambient temperature, container type and storage time on the acid value of crude soybean oil treated with different antioxidants and stored in different containers

3.5.1.2.2 Peroxide value.

A significant increase in peroxide value ($p < 0.05$) with the increase in storage time was observed for all the soy oil samples in the different container types (Figure 8). The control (CTR) crude oil for all the storage containers presented the highest peroxide value in comparison with samples containing preservatives.

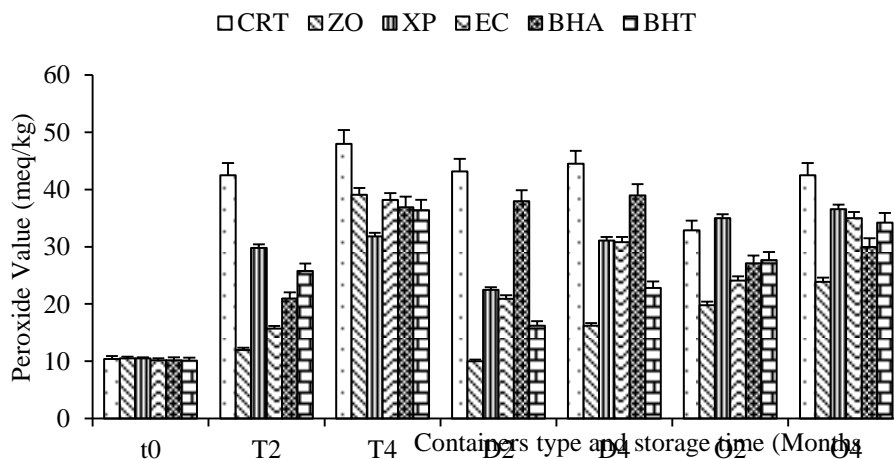


Fig. 8 Effect of ambient temperature, container type and storage time on the peroxide value of crude soy bean oil treated with different antioxidants

3.5.1.2.3 P-anisidine value

P-anisidine values of crude soybean oil during ambient temperature storage in different containers after 0, 2 and 4 months are presented in figure 9. A significant decrease in P-anisidine values were observed with increase in storage time. However control samples showed significantly higher P-anisidine values compared to preserved oil. The initial P-anisidine value (t0) was 11mg/kg and after 2 months storage (control, CTR) presented with significantly higher P-Anisidine values ($P < 0.05$) compared to samples with preservatives (Z.O, X.P, E.C, BHA and BHT) indicating high oxidation state. Samples stored in dark and opaque containers were generally had lower values than those stored in transparent containers.

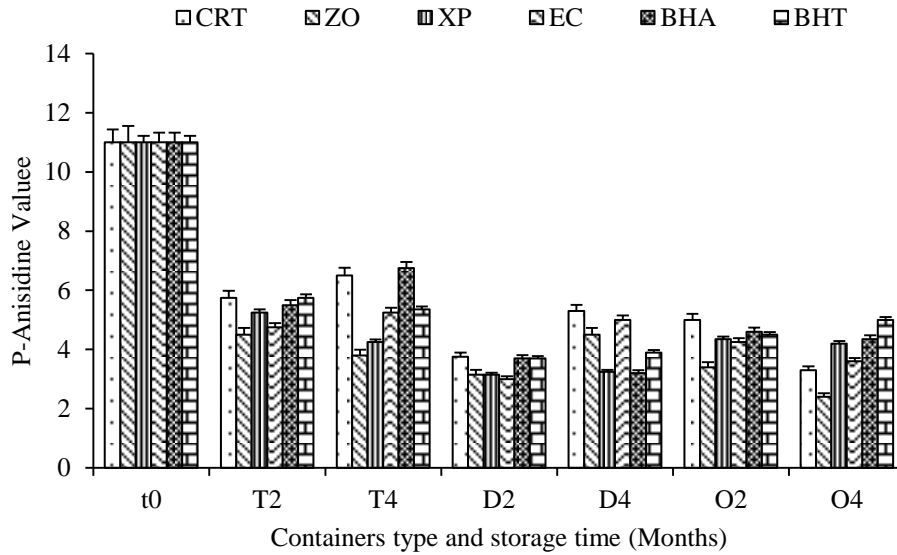


Fig.9 Effect of ambient temperature, container type and storage time on the P-anisidine value of crude soybean oil treated with different antioxidants

3.5.1.2.4 TOTOX value

Figure 10A, 10B and 10C present the total oxidation (TOTOX) of soybean oil samples during a storage period of 4 months at ambient temperature. There was an increase in the total oxidation (TOTOX) values of all the oil samples over the storage period. As observed with peroxide values, the controls exhibited the highest total oxidation.

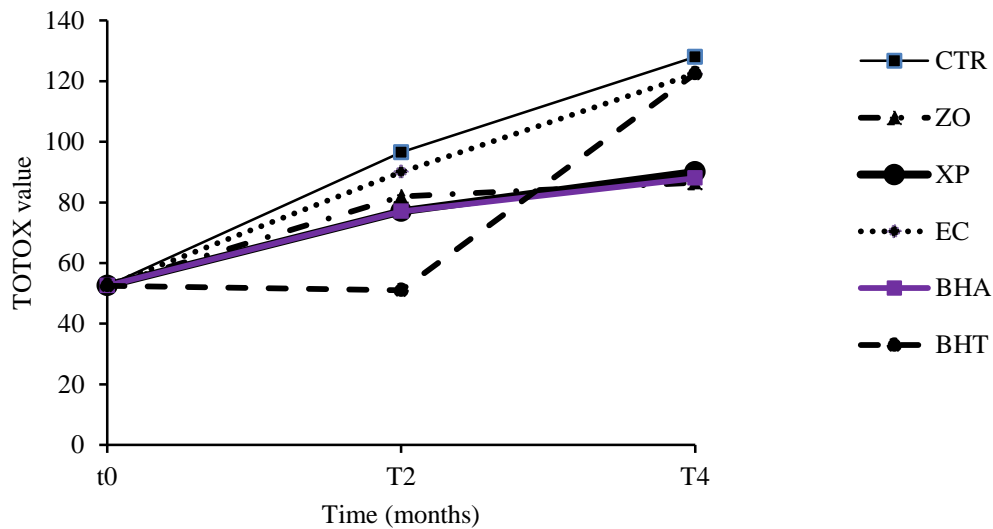


Fig. 10A . Effect of ambient temperature, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in transparent containers.

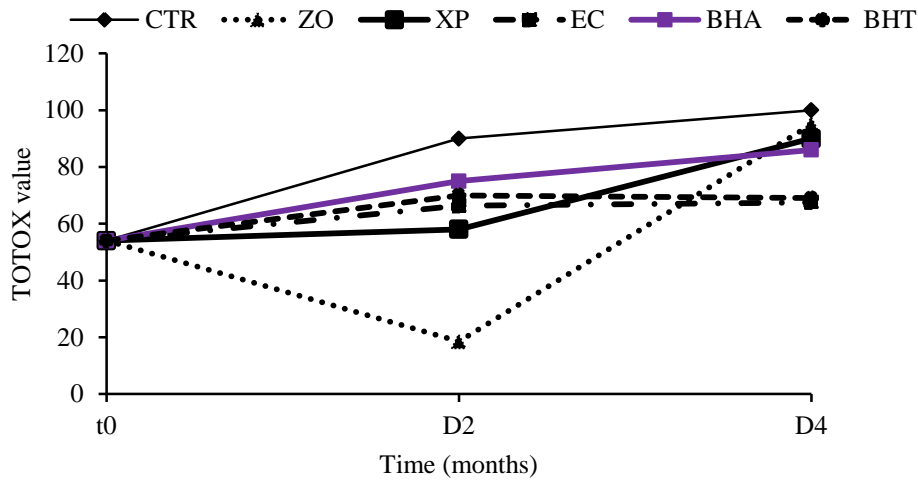


Fig.10B Effect of ambient temperature, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in a dark brown containers

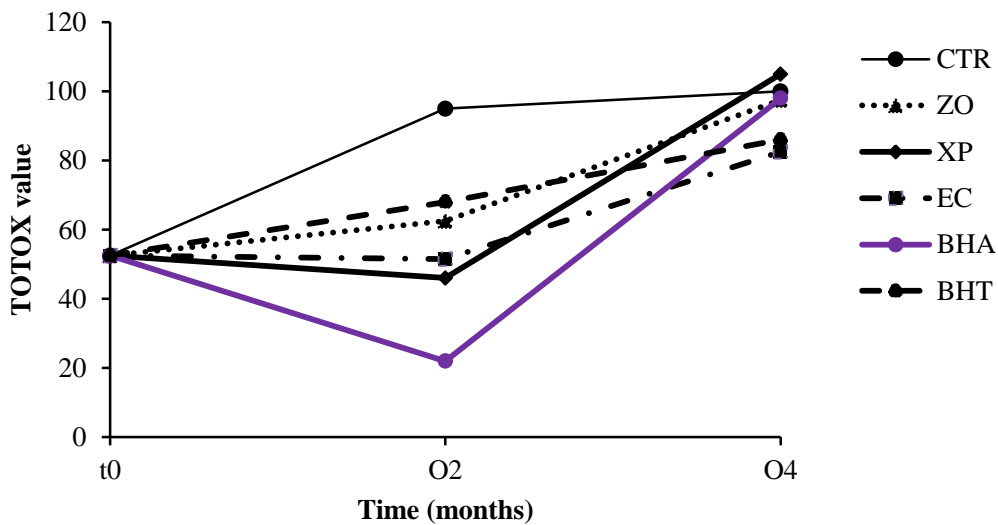


Fig. 10C Effect of ambient temperature, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in a opaque containers

3.5.1.3 Effect of refrigeration
3.5.1.3.1 Free fatty acid value.

The changes in free fatty acid values expressed as % oleic acid during refrigerated storage of soybean oil in different containers at 4°C for a period of 4 months are shown in figure 11. A significant decrease in acid value ($p < 0.05$) was observed in all samples compared to the initial acid value (t0).

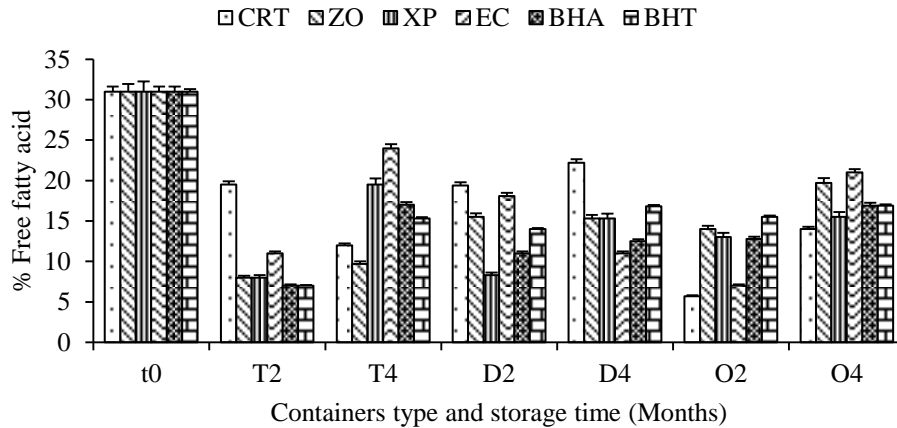


Fig.11. Effect of refrigeration, container type and storage time on the acid value of crude soybean oil treated with different antioxidants

3.5.1.3.2 Peroxide value

A significant increase in peroxide value ($p < 0.05$) with the increase in storage time was observed for all the samples in the different containers. Figure 12 shows the effect of refrigeration, container type and storage time on the peroxide value of crude soybean oil treated with different antioxidants and stored in different containers.

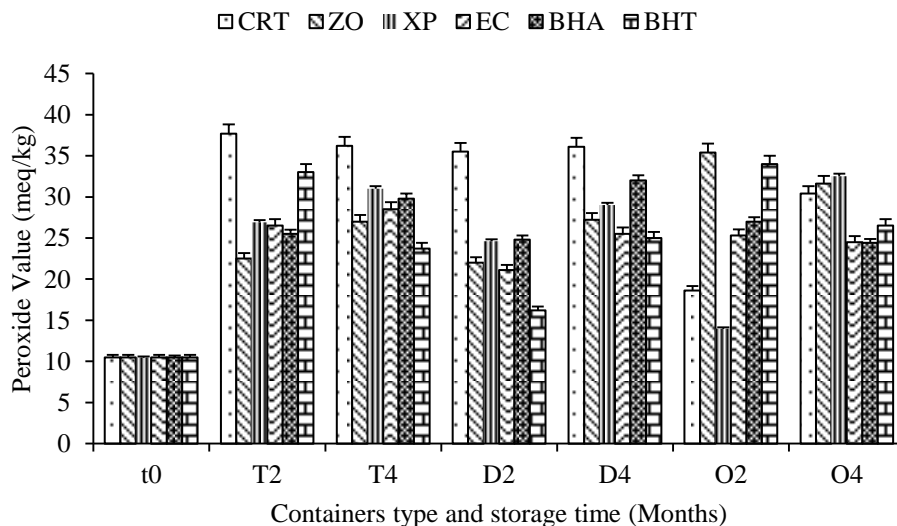


Fig.12 Effect of refrigeration, container type and storage time on the peroxide value of crude soy bean oil treated with different antioxidants

Soybean oil without antioxidant (control (CTR)), exhibited the highest peroxide value for all container types compared to those containing preservatives during the complete storage period. The lowest peroxide value was observed with ZO, BHA and BHT samples.

3.5.1.3.3 P-anisidine value

P-anisidine value assay is the most widely used method for the measurement of secondary oxidation products, which may contribute to the off-flavour in oxidized oils [40]. The changes in P-anisidine value of stabilized and control soybean oil samples are presented in Figure 13. A significant increase in P-anisidine value ($p < 0.05$) was observed for

soybean oil samples stored under this condition with increase in storage time. It is noted that at all stages of analyses during oil storage, control samples exhibited the highest P-anisidine value, while the values in soy oil treated with synthetic or natural antioxidants were lower. P-anisidine values of oil samples containing plant extracts (ZO, EC and XP) as a source of natural antioxidants were close to those of BHT and BHA during the storage period.

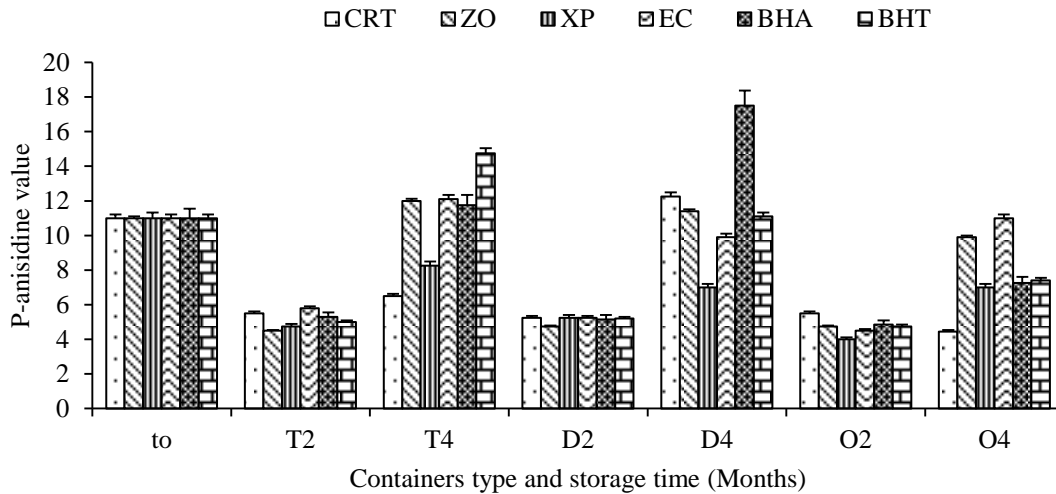


Fig.13. Effect of refrigeration, container type and storage time on the P-ANISIDINE value of crude soy bean oil treated with different antioxidants

3.5.1.3.4 TOTOX value

Figure 14 (A, B, and C) presents the evolution of total oxidation of soybean oil samples during a 4 month storage period under refrigeration conditions in different containers. Significant increases TOTOX values ($p < 0.05$) of all the samples were observed over the storage time.

As observed with peroxide values, the control (CTR) samples exhibited the highest total oxidation (TOTOX) values. Soybean oil samples containing were much less oxidized than the control (CTR) samples. It was also observed that the TOTOX values of soybean oil samples supplemented with plant extracts were not far from those of BHT and BHA.

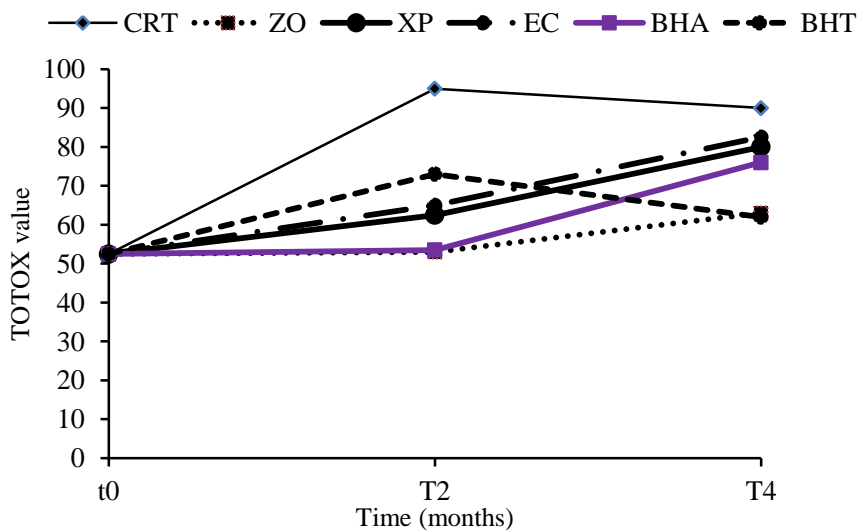


Fig. 14A. Effect of refrigeration, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in transparent containers

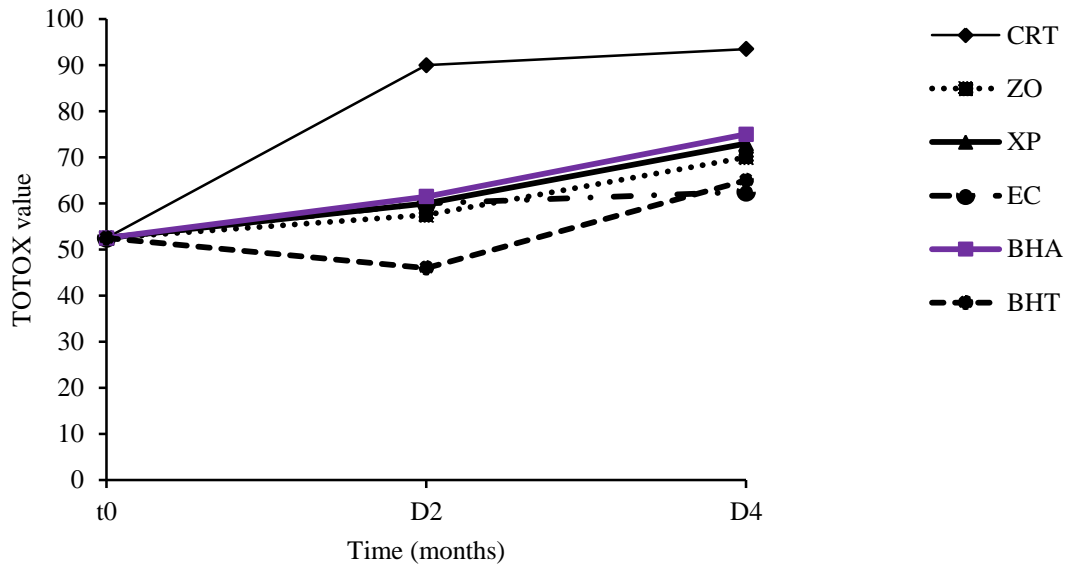


Fig. 14 B. Effect of refrigeration, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in a dark brown container.

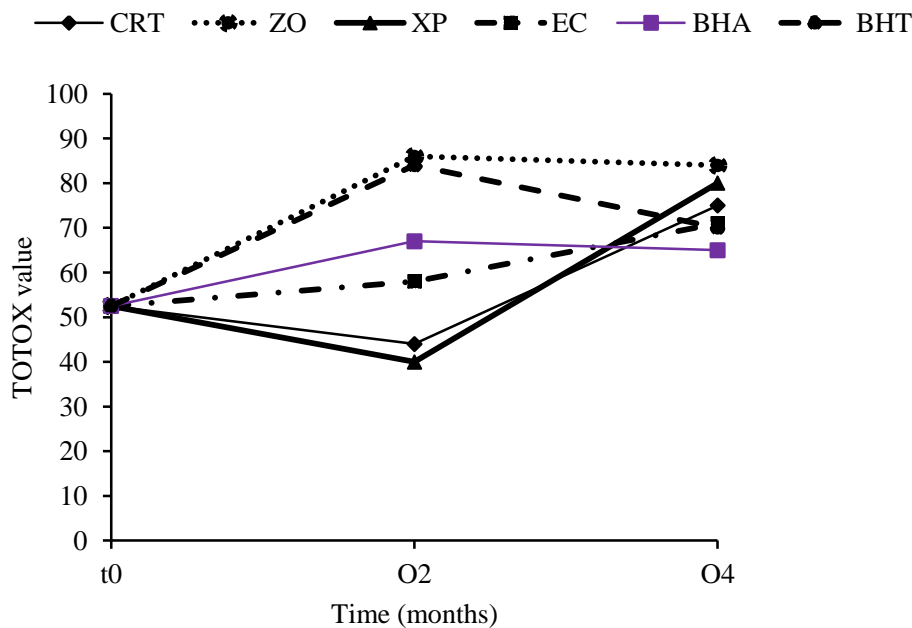


Fig.14C. Effect of refrigeration, container type and storage time on the TOTOX value of crude soybean oil treated with different antioxidants and stored in opaque containers

3.5.2 Accelerated storage conditions

Data of oil quality parameters stored under accelerated conditions are as follows.

Variation in acid value of the control and stabilized soybean oil samples over an incubation period of 24 days at 65 °C is shown in Figure 15. A progressive significant decrease in FFA value ($p < 0.05$) was observed during the storage period. The lowest acid values were observed in the control (CTR) compared with the stabilized samples containing plant extracts (ZO, EC and XP) and synthetic antioxidants (BHA and BHT).

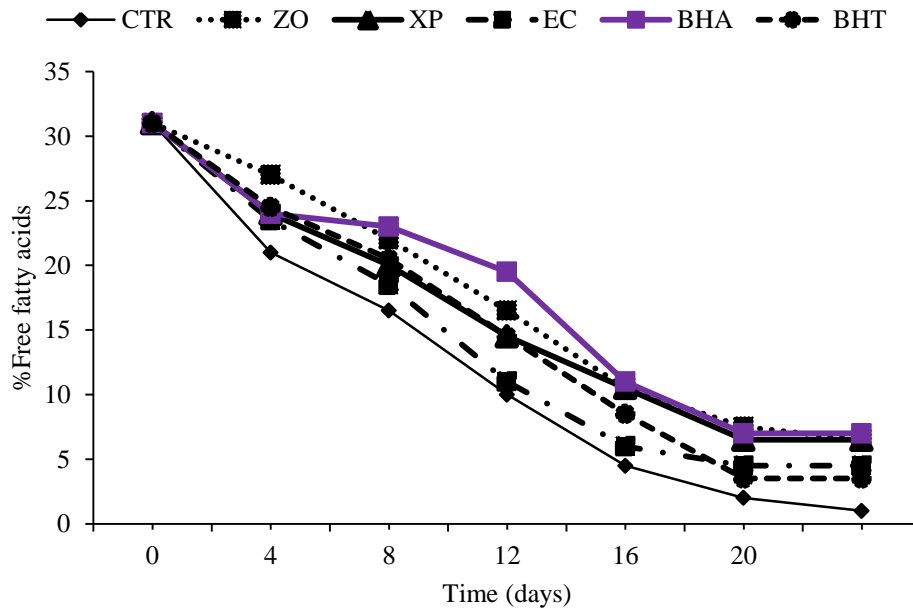


Fig. 15 Effect of heating time on the acid value of crude soybean oil treated with different antioxidants

The peroxide value of stabilized oils and control crude soya bean oil samples over a storage period of 24 days at 65°C is shown in Figure 16. A significant increase in peroxide value ($p < 0.05$) was observed in all the samples up to the 12th day and thereafter decreased except that of ZO and BHA which decreased after 16 and 20 days respectively. The control (CTR) presented the highest value of peroxide showing that this sample was more oxidized. The lowest values of peroxide were observed in samples stored with BHA, EC, XP and BHT.

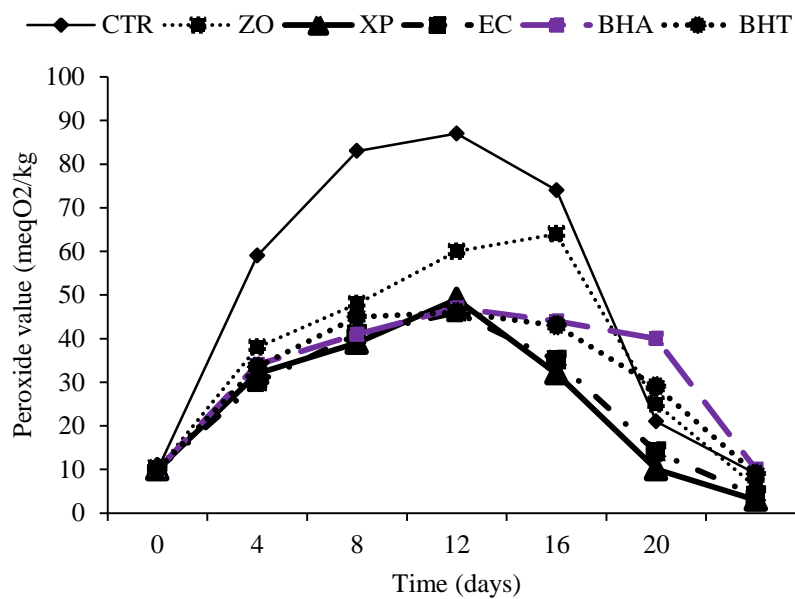


Fig. 16. Effect of heating time on the peroxide value of crude soybean oil treated with different antioxidants

A significant increase in P-anisidine value ($p < 0.05$) was noted for all the samples with increasing heating time. It was observed that control (CTR) samples exhibited higher P-anisidine values throughout the 24 day period while the P-anisidine values in samples supplemented with synthetic or natural antioxidants were lower.

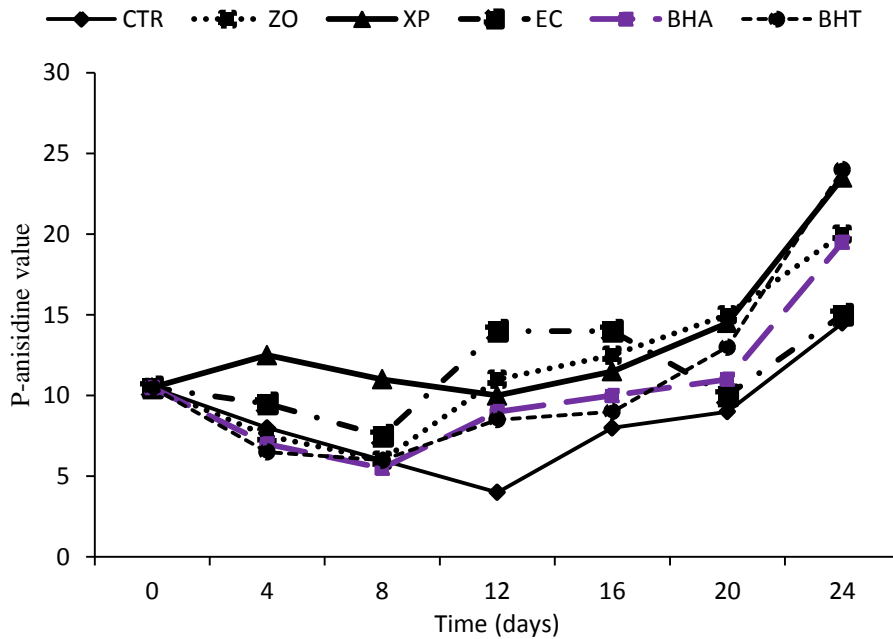


Fig.17 Effect of heating time on P-anisidine value of crude soybean oil treated with different antioxidants

The TOTOX value value of stabilized oils and control crude soya bean oil samples over a storage period of 24 days at 65°C is shown in Figure 18. A significant increase in TOTOX value ($p < 0.05$) was observed in all the samples up to the 12th day and thereafter decreased except that of ZO which decreased after 16 days. The control (CTR) presented the highest value of TOTOX showing that this sample was more oxidized due to the lack of Preservative. The lowest values of TOTOX were observed in samples stored with BHA, EC, XP and BHT.

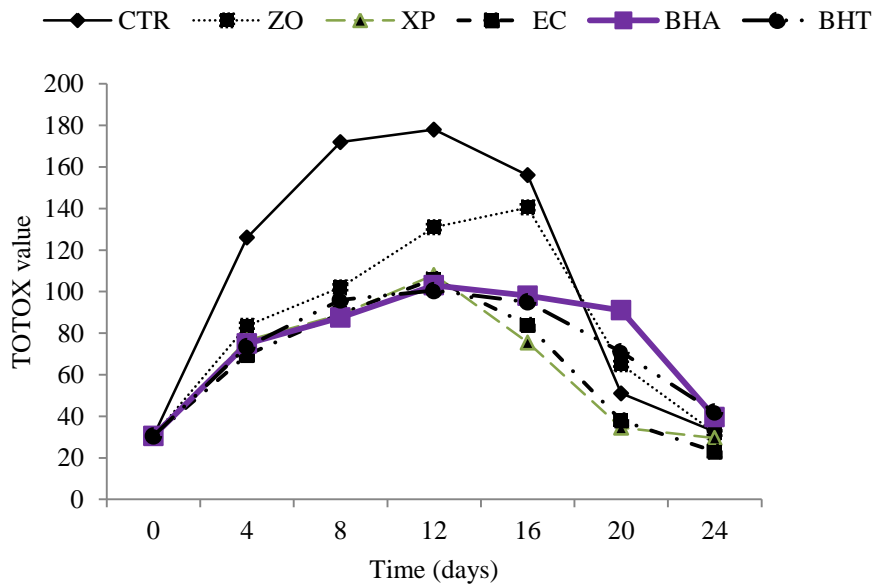


Fig.18 Effect of heating time on TOTOX value of crude soybean oil treated with different antioxidants



IV. DISCUSSION

Vegetable oils form a good part of human diet, providing concentrated sources of energy and essential nutrients. Lipid peroxidation which results from the reaction between unsaturated fatty acids and molecular oxygen has been identified to be the main cause of stability problems of these oils. This lack of stability is also a severe problem for the oil and fat industry as retarding lipid oxidation does not only extend product's shelf life but also reduces raw material waste, nutrient losses, and widens the range of lipids that can be used in specific products. Thus, control of lipid oxidation would allow food processors to use more available, less costly and more nutritionally favorable oils for product formulations.

This problem (lipid oxidation) has been overcome by the use of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), etc. but recent reports have revealed that these compounds may be implicated in many health risks, including mutagenesis and carcinogenesis [47]. It is well accepted that plants are the richest source of antioxidants among which spices are the most prominent because they contain a wide array of antioxidants including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. which render them or their antioxidant components good preservative agents in food [48]. These spices also contain polyphenolic compounds which have been proven to have antioxidant activity. In view of the beneficial effects and the vital role that natural antioxidants can play in human health, there is currently a greater demand for their isolation from plants. Hence the ability of extracts from *Zingiber officinale*, *Euglena caryophyllata* and *Xylopiya parviflora* to stabilize crude soybean oil in comparison with synthetic antioxidants BHA and BHT were investigated in this study.

Phenolic compounds are a much diversified group of phytochemicals that are widely distributed in plants such as fruits, vegetables, tea, olive oil, tobacco and so on. Nowadays, there is a growing interest in substances exhibiting antioxidant properties, which are supplied to human organisms as food components or as specific preventive pharmaceuticals. Consequently, antioxidants have become an essential part of food preservation technology and contemporary health care. It is well known that plants which possess antioxidative and pharmacological properties are related to the presence of phenolic compounds, especially phenolic acids and flavonoids.

The amount of methanol extractable components from *Eugenia caryophyllata* (37.97%) and *Zingiber officinale* (14.53%) are higher than values of 23.9% and 9.2% respectively reported in literature by Voukeng [49] and 32.75%, 29.5% and 8.25% respectively reported by Womeni et al. for the same plant extracts [33]. The yield of soybean oil (20%) in this study is in agreement with the classification of [50] where soya beans are classified as oleaginous with an oil yield less than 35%. The difference in the yield of extracts from the different plant materials might be attributed to the availability of different extractable components, defined by the chemical composition of the plant, nature of the soil and agro-climatic conditions [51]. Among other parameters, the effectiveness of the extracting solvent to dissolve endogenous compounds might also be a contributing factor.

The differences in composition of the secondary metabolites observed might be attributed to the environmental variations, age of the plant, extraction process, nature of the extraction solvent and part of the plants used [52]. Although the detection of such metabolites does not automatically predict the antioxidant activity of a plant extract, it has clearly been demonstrated that several compounds belonging to the investigated classes of metabolites showed antioxidant activities [53]. Phenols and triterpenoids were detected in the three plant extracts. The presence of phenols in almost all the spices could be explained by the fact that they are mostly distributed in plants due to their important function in defense mechanisms [54]. Osabor [55] also reported the presence of such phytochemicals in *Zingiber officinale* while Mujeeb et al. reported the presence of alkaloids, glycosides, steroids, carbohydrates, terpenoids, tannins and phenolic agents in dichloromethane extract of *Euglena caryophyllata* and *Aegle marmelos* Correa (Rutaceae) [56]. The TPC values obtained in these extracts were found to be similar to the values of 8.96, 12.35 and 17.72g reported for ZO, EC and XP respectively [33].

The antioxidant activity of EC and XP were significantly ($P < 0.05$) low compared to BHA and BHT while that of ZO was similar. It was observed that extracts with greater amounts of total phenolics also exhibited higher antioxidant activity while the extract of *Xylopiya parviflora* (XP) which showed the lowest total phenolics exhibited the lowest antioxidant activity. These results are in line with the findings of Zahra et al. [57], Naima et al [58] and Velioglu et al [59], who reported that extracts with higher total phenolic content also showed strong antioxidant activity against linoleic acid peroxidation. So, the antioxidant activity of these extracts might be attributed to the presence of phenols. Moreover, the phytochemical screening showed the presence of triterpenes and phenolics in all of the spices extracts

which have been proven to have good antioxidant properties [53]. These compounds might have contributed to the antioxidant properties observed in these spices.

Oil quality parameters (FFA, PV, P-anisidine and Totox values) under different storage conditions of sunlight exposure, ambient temperature and refrigeration as well as different storage containers presented interesting results. Free fatty acids (FFA) measures fatty acid changes that occur in oils. They are pro-oxidants and contribute to the decreased shelf life of oil [60]. In the early stages of oil storage, free fatty acids are produced by oxidative breakdown, but in later stages, hydrolysis of the fat causes the increase in free fatty acids [61]. FFA produced by hydrolysis is too small to affect the quality of food, while FFA formed by oxidation adversely affects the product [62]. Detection of increases in FFA by titration does not measure oil quality because it does not differentiate between acids formed by oxidation and those formed by hydrolysis. Under sunlight exposure, transparent containers compared to dark brown and opaque containers recorded lower FFA values indicating that dark brown and opaque containers preserved oil better. This could be explained by the fact that transparent containers allow sunlight to pass through promoting photo-oxidation.

The gradual decrease in acid value of soybean oil observed under ambient temperature might generally be attributed to the conversion of free fatty acids to the primary products of lipid oxidation caused by oxidation reactions during the storage period. These results are in accordance with those of Womeni et al [33] who reported that methanolic extracts and powders of some Cameroonian spices were able to limit the transformation of free fatty acid to other products of lipid oxidation of soybean oil during an accelerated storage. Higher acid values obtained for the control compared to oils stabilized with synthetic and plant extracts indicate that triglyceride hydrolysis can still occur on such samples since the ambient temperature was close to optimal conditions of ester bond hydrolysis by lipases.

The initial % oleic acid value (t_0) of the control under refrigeration storage was seen to be 31.22 which decreased significantly during the first 2 months of storage and thereafter started increasing. This increase may be brought about by the presence of active lipase in the soybean seeds which upon grinding are activated and begins to hydrolyse triglycerides into free fatty acids, diglycerides and monoglycerides and the lipase eventually will decompose all the triglycerides present over a period of several months even under refrigeration. Similar changes were reported by Aslam et al, Sarojini and Bhavani, and Semwal and Arya [63-65]. Although hydrolytic changes may not be predominant, free fatty acid formation was found to increase with increase in time of storage.

Oxidation of oils often take place when oil, oxygen, and catalysts are in contact. Both concentration and type of oxygen affect the oxidation of oils. Oxygen in oil is dependent on its partial pressure in the headspace of the oil [66], when the partial pressure of oxygen in the headspace is high, a higher amount of oxygen is dissolved in the oil, which leads to increase oxidation of the oil [67]. Incorporation of different preservatives (ZO, EC, XP, BHA and BHT) reduced oxidation and improved sensory stability of soybean oil under sunlight exposure as observed in this study and as reported by Madhujith and Sivakanthan [68] and Kozłowska and Gruczyrska [69]. The increase in peroxide value observed could be attributed to the formation of hydroperoxides which are the principal markers of primary oxidation of lipids. This increase in peroxide value variations can also arise from different factors such as the degree of unsaturation of the fatty acids present in the oil and the content of metals or other compounds that may catalyze the oxidation processes [70]. These hydroperoxides are converted to other secondary oxidation products when their concentration reaches a certain level [45]. Other factors that could affect PV are differences in conditions and climate of area where the process of refinement takes place. Moreover, storage time also has an effect on the quality of oil.

The lowest peroxide value under ambient temperature storage was obtained with ZO under dark container storage which indicated that dark brown containers do not allow light to pass through them. Lower peroxide values in crude soybean oil containing natural antioxidant in comparison with the control indicated the highest antioxidant activity of this extract in preventing lipid oxidation. This activity could be attributed to the detected phenolic and triterpenoids antioxidant compounds. Many other authors have proven the same activity with other plants methanolic extracts in sunflower oils [71]. The observed increase in peroxide value as storage time increases could be due to the conversion of free fatty acids to hydroperoxides during the storage period. However, the slow rise in peroxide value of the treated oils compared to control, clearly indicated a good antioxidant activity of the extracts and synthetic antioxidants. This increase in peroxide value might be due to the fact that antioxidants remain effective over a specific period of time, and with the passage of time their effectiveness decreases until they finally become ineffective [72].

The significant increase in peroxide value under refrigeration temperature with increase in storage time could be attributed to the formation of hydroperoxides, which are the principal markers of primary lipid oxidation. Lower peroxide values in crude soybean oil containing natural antioxidants in comparison with the control indicated the

highest antioxidant activity of this extract in retarding peroxide formation during storage. This activity could be attributed to the detected phenolic antioxidant compounds through their ability to stop free radicals by donating their hydrogen atoms or electrons. Many other authors have proven the same activity with other plants methanolic extracts in soybean and sunflower oils [33, 73, 71].

Total oxidation (TOTOX) value, calculated from twice the peroxide value plus the acid value, is another useful indicator of measuring the onset of progressive deterioration of vegetable oils and provides information regarding the progression of the formation of primary (mainly hydroperoxides) and secondary oxidation products (aldehydes, ketones, alcohols, etc) bi-products [74, 75]. After 4 months of sunlight exposure, the oils already had TOTOX values greater than 40 according to CODEX norms except for those packaged in dark containers with ZO, BHA, BHT and EC as preservatives. However, increase in Totox value could also be due to the presence of polyunsaturated fatty acids (PUFA) and mono unsaturated fatty acids (MUFA) [76].

Peroxide and acid values analyses together gives a more complete picture of total oxidation than each test separately [77]. Since peroxide value measures hydroperoxides (which increase and decrease) and acid value measures free fatty acids, the totox value usually rises continually during the course of lipid oxidation [78]. Soybean oil containing BHT, BHA and plant extracts (ZO, EC and XP) stored under ambient temperature were much less oxidized than the controls (CTR) and totox values of soybean oil supplemented with plant extracts were not far from those of BHT and BHA, meaning that the plant extracts significantly limited the oxidation of soybean oil during storage. Antioxidant compounds detected in the plant extracts from phytochemical screening could be responsible for their observed protective effect. These results are in accordance with those of [79] who have proven that extracts of Kenaf and Roselle (at 1500ppm) significantly limited the total oxidation of sunflower oil during an accelerated storage.

The significant decrease in P-anisidine value ($p < 0.05$) at the end of the storage period could be due to the formation of more secondary products of oxidation and since these products are volatile; the P-anisidine values of stored samples were less than those of t_0 . This could be explained by the fact that antioxidant activities of plant extracts reduced with time. A saturated lipid matrix and the existence of water in the lipid matrix can accelerate polyphenol oxidation which leads to decrease in antioxidant activity [76] (Yashi and Anil, 2015). The significant ($p < 0.05$) decrease in P-anisidine values under ambient temperature can be explained by the fact that at ambient temperature, less oxidation occurs and initial malonaldehyde production is low.

The closeness of P-anisidine values of oils s containing plant extracts (ZO, EC and XP) as a source of natural antioxidants to those of BHT and BHA during refrigeration showed low formation of malonaldehyde in these oils which is the main compound detected by this method. These results confirm the efficiency of the natural antioxidant in limiting the formation of secondary oxidation products in soybean oil at approximately the same level as BHT and BHA especially under low temperature of refrigeration. The antioxidant compounds found in these extracts might also be responsible for the inhibition of malonaldehyde formation. Similar results were obtained by Iqbal and Bhanger [40, 73] in sunflower oil supplemented with garlic and pomegranate methanolic extracts, respectively.

The significant decrease in acid value ($p < 0.05$) of oils as shown in figure 20 might generally be attributed to the conversion of free fatty acids to the primary products of lipid oxidation caused by oxidation reactions during the storage period. The slow decrease of the acid value in stabilized soybean oils compared to the control might be the consequence of the protective action of these antioxidants on the unsaturated fatty acids of the oil. Womeni et al. [33] have shown that methanolic extracts and powders of some Cameroonian spices were able to limit the transformation of free fatty acid to other products of lipid oxidation of soybean oil during an accelerated storage at 65 °C for 24 days. The significant increase in peroxide value ($p < 0.05$) of the oil was due to the formation of hydroperoxides which are the primary products of lipid oxidation. The lowest values of peroxide were observed in BHA, E.C, X.P and BHT, showing good stability of the oils. The significant decrease in peroxide value from day 12 forward might be explained by a volatilization of antioxidant compounds. In fact, it might be hypothesized that after volatilization of antioxidant compounds [80], the oils oxidize more rapidly and when the hydroperoxide content is very high, the primary oxidation products which are hydroperoxides break down in secondary oxidation products which can be volatiles or non-volatiles [81]. It is known that the alteration of fatty acid double bond is due to their attack by free radicals.



The significant increase in P-anisidine value ($p < 0.05$) confirms the efficiency of the natural antioxidant in limiting the formation of secondary products of lipid oxidation in soybean oil at almost the same level as BHT. The antioxidants found in this extract might also be responsible for the inhibition of malonaldehyde formation. Similar results were obtained by Iqbal and Bhangar [40, 73] in sunflower oil supplemented with garlic and pomegranate methanolic extracts, respectively, during accelerated storage at 65 °C for 24 days.

V. CONCLUSION

The plant crude extracts are rich in phenolic content and secondary metabolites. The plants possess antioxidant activities similar to those of BHA and BHT. The best spices in preventing lipid oxidation was *Zingiber officinale* (ZO) followed by *Eugenia caryophyllata* (EC) and then *Xylopiya parviflora* (XP). Extracts with high total phenolic content also possessed high antioxidant activity. Effectiveness of the extracts in slowing down the process of lipid oxidation depended on the storage container and condition with the best storage container and condition being dark and refrigeration respectively. The spices might therefore be explored as viable sources of potent antioxidants for the protection of vegetable oils from oxidation. This study showed that extracts with high total phenolic content also possess high antioxidant activities. The spices should be used in the preservation of oils and oils for long term storage should be stored in dark containers.

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