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THE DEVELOPMENT OF NATURAL ANTIOXIDANT ACTIVE *EHURU* POLYSULFONE FILM AND ITS EFFECT IN LIPID OIL STABILIZATION

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ABSTRACT: *A natural antioxidative Monodora myristica (Ehuru) active film package for lipid food preservation was produced by cast method. 0 - 5% w/w ehuru antioxidant extract (EAE) was incorporated into PSF film. Into another PSF resin was incorporated 5% alpha tocopherol (AT) and another, a blend of (EAE)/ α-tocopherol (AT). Tetrahydrofuran (THF) and N-methylpyrrolidinone (NMP), were used as solution solvent, in ratio 3:1. The radical scavenging abilities of the developed films were analyzed and compared to the pure PSF (control) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution. Finally, the ability of the films to stabilize peanut oil against oxidation were tested by measuring some rancidity analysis of the actively packaged peanut oil. The result showed that the radical scavenging properties of the active films increase with concentration of EAE infused inside the film increased from (0% - 5%), and 5% EAE incorporation created a compact film without any optical noticeable pore. This was confirmed by the permeation rate of oxygen across the film which decreased from (0.1079-0.0277) m^3/s as concentration EAE increased. Generally, results showed that natural antioxidant derived from Ehuru seeds (EAE) exhibited the best delay of lipid hydrolyses by exhibiting the shortest oxidation time. It was also deduced that concentration between 2.5 and 5% could comfortably be used to produce the active package because their effect on oxidation of lipid oil are not significantly different from each other 7.85± 1.13 and 7.27± 1.69 respectively. Results obtained from the oxidative analysis confirmed the effectiveness of the active film of natural antioxidant derived from Ehuru seeds (EAE) in slowing down lipid hydrolysis and increasing the oxidative stability of peanut oil, having the smallest PV of 7.35±2.00 and the smallest P-AnV of 18.12± 1.57*

KEYWORDS: development, natural antioxidant, active ehuru polysulfone film, lipid, oil, stabilization

INTRODUCTION

Food spoilage through oxidation is characterized by alterations of nutritional and sensory characteristics of food, such as production of off-flavours and off-odours as well as undesirable changes in texture and colour and also chemical changes (production of radicals) that can harm human. These food losses due to oxidation constitute a great economic challenge for the food Industry and consumers alike. Studies has shown that antioxidant packages ameliorate the quality and safety of packaged foods and efficiency in preserving. As a result, new packaging technologies have been studied in order to provide good quality, safer food products which stays longer before spoilage (Gherardi *et al*., 2016; Otoni *et al*., 2016; Siripatrawan & Vitchayakitti, 2016).

Among these technologies is active packaging, where the packaging material interacts with the packaged food in a desirable way, overcoming the passive role of just containment and protecting food products from the outside environment (Ahmed *et al*., 2017). Package with natural antioxidant properties has now taken the attention of food packaging technology research. This is due to the fact that the main problems affecting quality and safety are oxidation and microbial contamination. Active polymer package systems are special food polymer package with significant quantity of 'active' constituents that provide beneficial preserving roles to food products. The process is called "active packaging" because the package does not only serve as a passive container but functions as antioxidant.

This new direction of research came into place because of the negative pro-oxidant action reported in preserving food through straight introduction of natural antioxidants into bulk food. On the other hand, synthetic preservatives are shown to be deleterious to human health. Such adverse effects include the carcinogenic effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Tátraaljai *et al*., 2013; Samsudin *et al*., 2017). These Synthetic antioxidant agents have the tendency to leach into contents of the package under high temperature or long term storage reason being that these additives are not covalently bound to polymer network. Polyphenols has been used as alternative to the synthetic antioxidants in food contact materials.

In most foods, the surface growth of peroxides as a result of oxidation is the major cause of lipid food spoilage. Since oxidation is a chain reaction process, an extremely proactive approach must be taken to prevent or limit the initiation step. This implies that if the surface peroxides can be eliminated as soon as they are formed then the process of oxidation would be greatly slowed down, and shelf-life of food would be extended. This approach would eliminate the need for further addition of antioxidants in the bulk of the food. Foods with unsaturated oils, like vegetable oil, processed meat and nuts are mostly prone to spoilage as a result of oxidation. The need for antioxidants should also be extended to food products low lipid level like cereal components. Apart from nutritional and sensory problems that oxidation leaves food to, it also produces free radicals and reactive oxygen species (ROS) in the food. ROS have been reported to be carcinogenic, causing other health problems like resistance to drugs, cardiovascular motility, and chemotherapy response (Das Sarma *et al*., 2010; Huang & Wong, 2013).

Synthetic (BHA, BHT) and natural antioxidants (e.g. ascorbic acid, tocopherols) are added directly to foods as primary antioxidants, which donate hydrogen atoms to quench peroxyl radicals before they can further react with unsaturated lipids. Phenolic antioxidants are stable due to the fact that they form a radical with very low reactivity, due to delocalization of their unpaired electron on the aromatic ring, with this they exhibit no further potential to react with lipids after hydrogen abstraction unlike their oxidation radicals. Even though Lipid foods has some quantity of inherent defence system (antioxidant) that resists oxidative damage due to reactive oxygen species (ROS). However, supplementing this natural defence mechanism with external antioxidants offers a better protection against the risk of certain oxidative deterioration (Tian *et al.*, 2013).

It has been established that there is great antioxidant potential and health benefits contained in natural products like green tea, rosemary, oregano, spices like Ehuru, herbs, clove, blueberries, barley husk, and other plants.(Pereira *et al*., 2010; Colon & Nerin, 2012). Many researchers have been recounted on the infusion of tocopherol (Vitamin E) and ascorbic acid (Vitamin C) as active agents into a polymer (Siró *et al*., 2007; Gemili *et al*., 2010; Noronha *et al*., 2014) Other works on

leaf spices with antioxidant qualities such as green tea extracts *(Camellia sineensis L.)* had also been reported by Colon & Nerin (2012) and López-deDicastillo *et al*. (2011), while works on Rosemary (*Rosmarinus officinalis L*.) had been reported by Sánchez-Escalante *et al.* (2001); extracts of oregano also known as *"thyme"* (*Origanum vulgare L.)* was reported by Camo *et al*. (2011) and Liu *et al*. (2016); mint *(Mentha spicata L.)* and extracts of sage (*Salvia officinalis L*.) as active agents introduced into polymeric packaging for preservation have all been reported. Similar researches dwelt on the infusion spices from roots of plants such as Cucumen (*turmeric*) and Ginger *(Zingiber officinale Rosc.)* into polymer food packaging materials (Gemili *et al*., 2010).
However, till date, no work on the use of *ehuru* seed spices has been reported as active agent included in polymer film packaging material. Interestingly, *Ehuru* (*Monodora myristica*) extracts has been reported by researchers to exhibit a potent antioxidant activity and also effectiveness for achieving high sensory scores and lowering lipid oxidation (George & Osioma, 2011; Akinwunmi & Oyedapo, 2013; Okonkwo & Ogu, 2014).

Therefore, there is need to investigate its performance as active additive in polymer packaging for stabilization of peanut oil. This work was aimed at developing natural antioxidant active *ehuru* polysulfone film as well as studying its effect in the stabilization of lipid oil.

MATERIALS AND METHODS

Materials

All chemicals used for this study were of analytical grade: α-Tocopherol of 98% purity and 2,2-Diphenyl-1-picrylhydrazyl (DPPH), anhydrous sodium carbonate, sodium thiosulphate, chloroform, PSF resin, THF, NMP, methanol and all other reagents were all purchased from Sigma Aldrich, South Africa. *Ehuru* seeds were gotten from Eke-Ukwu Market, Owerri, Imo State, Nigeria.

Preparation of *Ehuru* Extract

Extract was prepared by method according to (Gurnani, Gupta *et al*., 2016) which was modified. *Ehuru* seeds were oven-dried to constant weight. Laboratory Mill 3100 (Falling Number, Huddinge, Sweden) was used to crush them to fine powder and sieved to size <0.8µm. Using a Soxhlet apparatus over an oil bath set at 70 ^{o}C. One hundred grams of the seed powder was extracted using 500ml of 70% ethanol. The extracts obtained was centrifuged and the supernatant was concentrated using a rotary evaporator (Rotavapor R-200, BÜCHI Laboratory Equipment, Flawil, Switzerland) at 70^{O}C. The concentrate was subsequently dried in an oven to the extract. The crude extract (EAE) was stored in refrigerator till further needed.

Preparation of Active packaging material

Three sets of packages were developed. A set containing 0-5% Ehuru Antioxidant Extract (EAE) compositions, 5% α-tocopherol (AT) and 5% blend of AT and (EAE).
Tetrahydrofuran (THF) and *N*-methylpyrrolidinone (NMP) were used as solution solvent, in ratio 3:1. PSF (10g) was dissolved in the NMP/THF mixture at room temperature; the mixture was vigorously mixed in mixer until complete dissolution. The aftermath was combined with the different proportion of EAE and AT, PSF (control). The films were produced by casting on a glass plate using a casting blade. The casting solution was kept at room temperature for at least 2 h before casting in order to remove air bubbles. The newly cast active film was immediately immersed in distilled water coagulation bath within 10s and peeled off the glass plate. The residue solvent mixtures in the film were removed by immersing the film in distilled water for 24 h.

Radical scavenging (antioxidant) activity of the Active film

Oxidation protection effect of the active film was determined using the method as described by Jouki *et al*. (2014). Two millilitres of methanol was mixed with 0.1g of pieced active polymer films which was vigorously vortexed for 3 minutes while allowed to stand at room temperature for 3h followed by centrifugation at 2300 rpm for 10 minutes. The supernatant obtained was analyzed for DPPH radical scavenging activity. An aliquot of methanol extract (1ml) was mixed with 2ml of 0.1mM DPPH in methanol. The mixture was vigorously vortexed for 1 min and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517nm using a UV- VIS spectrophotometer. The methanol was used as control and was mixed with 0.12mM DPPH. DPPH radical scavenging activity was calculated as follows (Singh & Ragini, 2004):

$$\%\, Radical\ scavenging\ activity = \left(\frac{A_{reference} - A_{sample}}{A_{reference}}\right) \times 100 \quad \ldots\ldots\ldots\ldots \quad \text{Equation 1}$$

Where A_{sample} is the absorbance of sample and $A_{reference}$ is the absorbance of the DPPH solution without the sample film.

Active package test on lipid food.

Determination of oxidation rate of packaged peanut oil

The oxidation of processed peanut oil packaged in the produced active films was monitored for accumulation of peroxides. The package 5cm x 5cm was prepared using a heat sealer (Thermal impulse sealer, mode PE5- 260) filled with 10cm^2 peanut oil, heat sealed and stored in the dark at room temperature for 9 weeks. 4 packages (sachets) where prepared one for the control film and 3 from each active film all labelled for identification. The oxidation process was monitored by syringe withdrawing of samples at intervals of one week and determining the peroxide value (PV) and anisidine value (AV). Peroxide value measures the primary oxidation products while the AV measures the secondary oxidation products therefore it points out the secondary stage of oxidation.

Peroxide value (PV)

The peroxide value (meq O_2/kg oil sample) was determined according to the official AOAC 965.33 iodometric method with some modification as described by Zuta & Simpson (2007). Five grams of sample was weighed into 500 ml conical flasks and 60 ml of 3:2 (v/v) acetic acid/chloroform mixture were introduced into the sample and swirled to dissolve the sample. Aliquots of 1ml of 2% KI solution were added to the samples and the reaction mixtures were left to stand for 1 min at room temperature (about 28^{o}C) with occasional shaking. 30mls of distilled water was added to the sample and the resulting reaction mixtures were each titrated with 0.1 N standardized sodium thiosulphate to a pale yellow colour, then 2 ml of starch indicator were added and the titration was continued to a colourless end-point. A blank titration was carried out using a sample containing all the above reagents except oil sample. Triplicate measurements of peroxide values of each treatment concentration were carried out initially at weekly intervals up to the 9th week and the PV was calculated as:

$$PV = (S - B) \times \frac{N}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Equation 2}$$

Where S and B are the volumes of titrant for the sample and blank, respectively; and N is the normality of sodium thiosulphate solution. M is mass of sample in g

P - Anisidine Value (p-AV)

Anisidine value measures the amount of carbonyl compounds, aldehydes (principally 2 alkanals and 2, 4-dienals) in the oil sample. P-AV was determined using the CDR food laboratory analyser, working principle followed AOCS standard method; CD 18 – 90. 2mls of P- anisidine reagent in

cuvette was incubated for 10minutes in the incubation cell. P- anisidine analysis was selected while the blank reading of the reagent was taken by inserting the cuvette containing the reagent in the reading cell. 10um of the oil sample was introduced into the cuvette containing the blank and shaken to ensure thorough mixture then the p-anisidine value was read by inserting the cuvette containing the mixture in the reading cell. The aldehyde derived from the secondary oxidation of the fat matrix reacts with the p-anisidine determining the variation in the absorbance measured at 366nm. This test has an enhanced sensitivity for unsaturated aldehyde, especially 2, 4-dienals, but does not measure the ketonic secondary products of oxidation. The lower the p- anisidine value the better the oil analysed.

Statistical analysis

One-way analyses of variance were carried out. The SPSS computer program (SPSS Inc., Chicago, IL, USA) was used. Differences in pairs of mean values were evaluated by the Tukey test for a confidence interval of 95%. Data are represented as mean and standard deviation.

RESULTS AND DISCUSSION

Table 1: Tensile stress of the active films

Property	0.0%EAE	1.0%EAE	2.5%EAE	5.0%EAE	5.0%AT	5.0% EAE/AT
Tensile stress (mPa)	8.132±0.31^{a}	7.739±0.71^{b}	7.538±0.25^{c}	7.426±0.34^{d}	5.636±0.22^{f}	6.405±0.54^{e}
Elongation at Break (%)	2.002±0.72^{d}	2.353±0.11^{c}	2.673±0.33^{b}	3.221±0.09^{a}	1.702±0.55^{f}	1.751±0.23^{e}

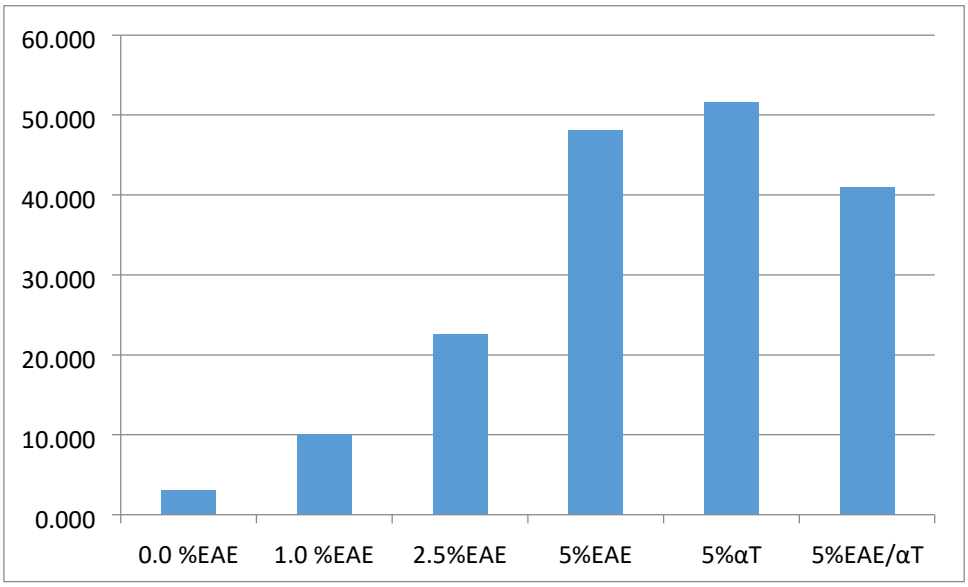

Figure 1: Antioxidative strength of the active films

3.1 Antioxidative capacity of the active film

The results shown in figure 1 show the results of antioxidant activity (DPPH) for the active polysulfone package. The result shows that antioxidant performance increases with the percentage of EAE incorporated. The antioxidative level of the 1% EAE (pure sample) read 3.1% while that of the 5% EAE improved to 51.59 %. When a blend of AT with EAE was incorporated the antioxidative performance reduced to 40.90%. DPPH is a free radical itself and the exposure of antioxidative active samples to DPPH was used to measure the antioxidant performance or the capacity at which it can act as a scavenger of free radicals.

Table 2: Permeability Properties of the Active Films

Description	Film Flow rate [mL(STP)cmcm^{-2}s ^{1}(cmHg)$^{-1}$] dV/dt (cm^3/s)	Permeability of film
1.0%EAE	0.0463^{c}	7.502x10^{-7c}
2.5%EAE	0.0393^{d}	2.244x10^{-7e}
5.0%EAE	0.0277^{e}	3.431x10^{-7d}
5.0% EAE/AT	0.1079^{b}	2.1228x10^{-6b}
5.0%AT	0.2209^{a}	3.5791x10^{-6a}
LSD	**0.0022**	**0.0066**

Table 2 shows the barrier properties of the active films. Oxygen gas was used as gas permeant and the transmission pressure was 90cmHg while the operating temperature was 25ºC. The permeation rate of neat PSF was recorded to be 0.1256cm/s. The rate of permeation of the gas in the active films was shown to be reducing significantly as concentration of EAE incorporated. The rate of permeation of the gas in the active films was shown to be reducing significantly as concentration of EAE incorporated in the film increased. On combination of EAE with AT a film of lesser barrier to the gas was produced, hence more porous with volumetric flow rate of 0.1079 cm^3/s and subsequent permeability of $2.1228x10^{-6}$[mL(STP)cmcm^{-2}S^{-1}(cmHg)$^{-1}$],d in the film increased. On combining of EAE with AT produced a film of lesser barrier to the gas, hence more porous with volumetric flow rate of 0.1079 cm^3/s and subsequent permeability of $2.1228x10^{-6}$[mL(STP)cmcm^{-2}S^{1}(cmHg)$^{-1}$], while only AT incorporated active film was shown to have the least barrier to gas, having flow rate of $0.2209cm^3/s$ and highest permeability of $3.5791x10^{6}$[mL(STP)cmcm^{-2}S^{-1}(cmHg)$^{-1}$]. Increasing the thickness of AT film did not impede the permeability rate, this was shown when AT film with double the thickness of 5%EAE produced far less barrier and highly permeable film compared to the 5%EAE film. (18.22 meq/kg) before a decrease, and this decrease could be as a result of the decomposition of the unstable peroxide to secondary products. This is in agreement with the report given by (Pereira De Abreu *et al*., 2011) who reported that when the maximum level of PV was reached the value decreases as a result of lower substrate availability and instability of peroxide molecules which easily decomposes. This decrease of produced peroxides towards the last stage of oxidation was also observed by Jongjareonrak et *al*. (2008). The maximum level of PV for AT and EAE/AT blend were both on the 7th and 5th week of storage (12.70±0.63, 12.83±0.84) meq/kg respectively. This show that they both stored more than the control sample. However EAE packaged oil recorded.

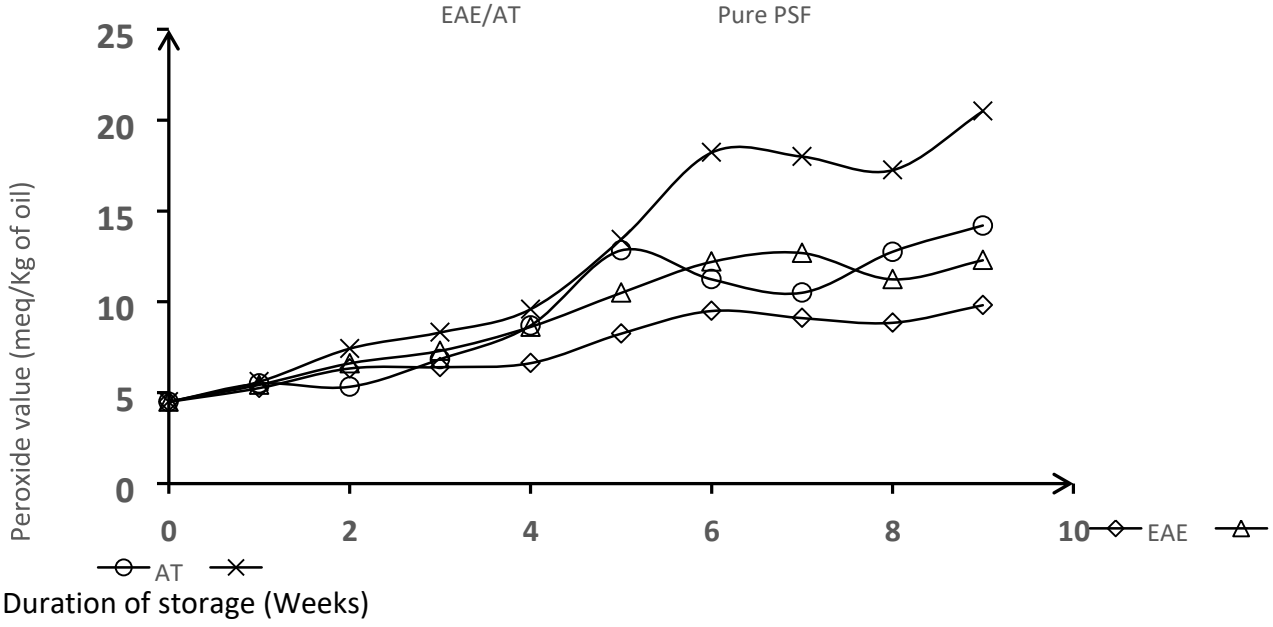

Figure 2: Peroxide value (PV) of stored Peanut oil packaged with film containing different Antioxidants

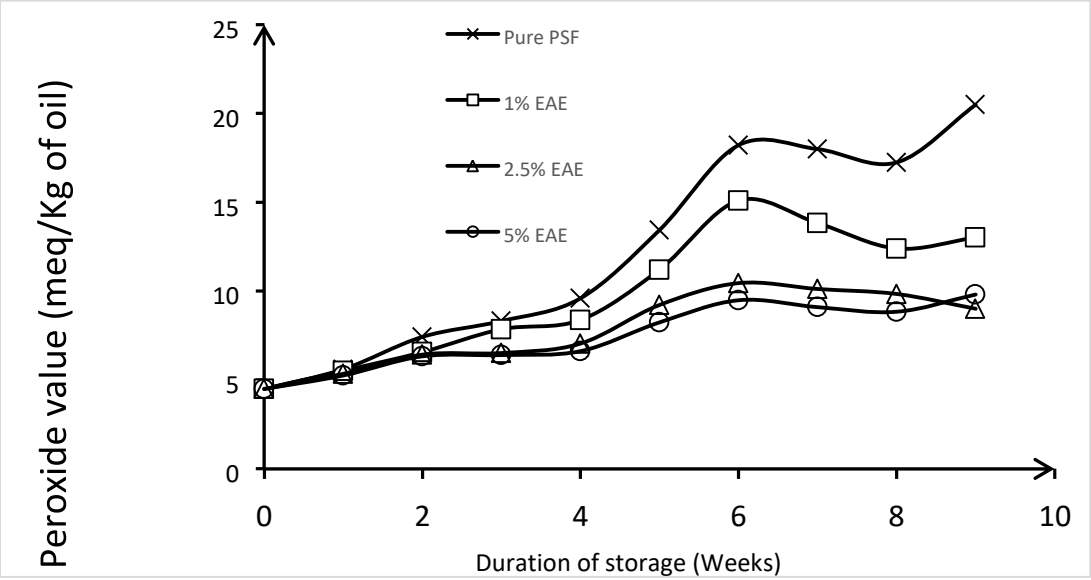

Figure 3: PV of stored peanut oil packaged with active film of varying concentrations of EAE

Maximum PV value at the 6th week of storage before a decrease. It was also observed to have a constant or PV values that are not significantly different at ($p< 0.05$) during 2nd to 4th week of storage. This shows that the EAE was able to halt oxidation within this period and this led to the lowest value of PV being recorded at the end of 9th week of storage indicating that the antioxidant EAE could have scavenged the initial peroxyl radical (peroxides) produced thereby inhibiting the radical induced oxidation. (Chain-breaking activity of H-atom transfer) (Bentayeb *et al*., 2007). Baschieri *et al*. (2017) also agreed that the scavenging activity could be as a result of the high polyphenol content shown by the presence γ-terpinene, α-monoterpene components present in the EAE which possesses a significant antioxidant activity could be responsible for slowing down the autoxidation of methyl linoleate by oxidation mechanism. The terpene causes a faster oxidative chain-termination due to the generation of hydroperoxyl radicals (Foti and Ingold, 2003).

The forestalled radicals up to the 4th week of storage made peanut oil packaged with EAE contained film to record the lowest maximum PV level (9.5±0.65 meq/kg) at the 6th week of storage and also at the end of storage (9.82±1.50meq/kg). Result showed that there was no significant difference between the PV value of peanut oil stored with EAE/AT and AT films. EAE incorporated film recorded the lowest PV at the end of storage duration. The PV in the sample packaged with control film (without antioxidant) was significantly higher ($P<0.05$) than all the other samples with antioxidants. This showed the fact that antioxidants act by inhibiting the mechanism of lipid auto-oxidation of free radicals. The ability could be as a result the phenolic structure within the molecular structure of the EAE. Phenolic substances act as free radical acceptors and able to hinder rate of oxidation of the peanut oil. The peroxide value measures the rate of lipid oxidation and indicates the quantity of oxidised substances. Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odourless and relatively unstable compounds. They decompose to form volatile aromatic compounds, which are perceived as off-flavours and as a warning that food is no longer edible. They are monitored by the liberation iodine on reaction with potassium iodide. (Breu *et al*., 2011) However reported that their decay leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to rancid taste and off-flavours. During early stage of oxidation the PV tends to increase, increases further when the rate of production is greater than the rate of decomposition.

Figure 2 shows the peroxide values over storage time of peanut oil packaged with different concentration of EAE. The result showed that the PV of all peanut oil samples where increasing with time of storage. The PV of the control film (0% EAE film) over time was significantly increasing than all rest of the peanut oil samples packaged with film containing EAE irrespective of their concentrations. This is because the film has no antioxidative property in it. Generally, this figure 2 shows that as the concentration of EAE incorporated in the film increases the PV of the packaged oil reduces. 2.5% EAE recorded the least PV with value 9.01 meq/kg compared to the control (0% EAE) which recorded the highest PV of 20.50 meq/kg. The result also shows that the peroxide value of 0% EAE and 1% increases significantly ($p \leq 0.05$) from the first week of storage to the 6th week of storage, with value (18.00±0.35 and 15.00 ±0.30) respectively. For the 2.5% EAE, there was no significant difference between the peroxide values recorded in the 2nd and the 3rd day of storage. The highest PV recorded before a decrease for 0 and 1% EAE was recorded in the 6th week of storage, with lower PV (10.45±0.02). Meanwhile for the 5% EAE there were no significant difference between PV's recorded between the 2nd and 4th week of storage with values (6.34±0.00, 6.41±0.00 and 6.63±0.00) respectively. The suppressed oxidation brought about by stagnant PV between the 2nd and 4th week of storage led to the lowest PV of 9.5±0.04 at the 6th week of storage.

Figure 3 show the result of p-anisidine value of peanut oil packaged without and with antioxidant compounds. The result showed that p-AV of all the samples excluding the EAE packaged samples was significantly increasing ($P<0.05$) with storage time. The control (no antioxidant) was more susceptible to oxidation than packaged oil samples. This is indicated by its higher rate of production of p-AV especially between the 4th and 5th week of storage (23.45±0.25 and 40.27±0.44) and between the 8th and 9th week (43.25±0.30 and 49.37±0.24) respectively. This led to highest p-AV (46.50) at the end of storage period. However for the EAE packaged sample showed no significant difference ($p<0.05$) 13.31, 14.63 between the third and fourth week of storage and between the 6th and 7th week (24.21, 24.25). This shows that within these period oxidation was highly suppressed to almost becoming constant.

At the end of 9th week duration of storage, oxidation of peanut oil packaged in film containing EAE gave the lowest p-AV of 30.45 from oil of other antioxidative packs. This shows that EAE incorporated in the film (pack) had the capacity to suppress the rate of oxidation of the peanut oil in contact with it. All the three samples with the antioxidant packs had significant increase of p-AV between 7th and 8th week of storage indicating reaction of panisidine with produced aldehydes. Hydroperoxides are unstable at storage therefore, they decompose to give secondary oxidation products that are *p*-anisidine-reactive (Moigradean *et al*., 2012).

No significant ($P<0.05$) difference was observed between oil in AT and blend of EAE/AT. Comparing EAE the blend (EAE/AT) shows that AT reduced the effectiveness of EAE which could be as a result of their incompatibility. AT being hydrophobic while EAE is hydrophilic. Figure 2 illustrates the effect of concentration of EAE active film on PV of packaged peanut oil. The result showed that the PV of all peanut oil samples where increasing with time of storage. The PV of the control film (0% EAE film) over time was highly significant than all rest of the peanut oil samples packaged with film containing EAE irrespective of their concentrations. This is because the film has no antioxidative property in it. The PV of peanut oil samples stored with package of 1% EAE also show significant PV over time showing that EAE incorporated has no significant antioxidative effect on the package. It has no value was significantly increasing with time of storage. The PV of peanut oil samples stored with package of 2.5% and 5% EAE had a significantly reduced PV and

these values from day one of storage to the end of storage is not significantly different from each other. The rate of increase of both samples where significantly minimal compared to the 1% EAE and the control. At the end of storage time (9 week), 5% EAE recorded the least PV with value 7.82 meq/kg while 0% EAE recorded the highest PV of 20.50 meq/kg. Thus 5% EAE PSF film proved to poses greater antioxidative property and therefore superior in hindering oxidation rate of peanut oil.

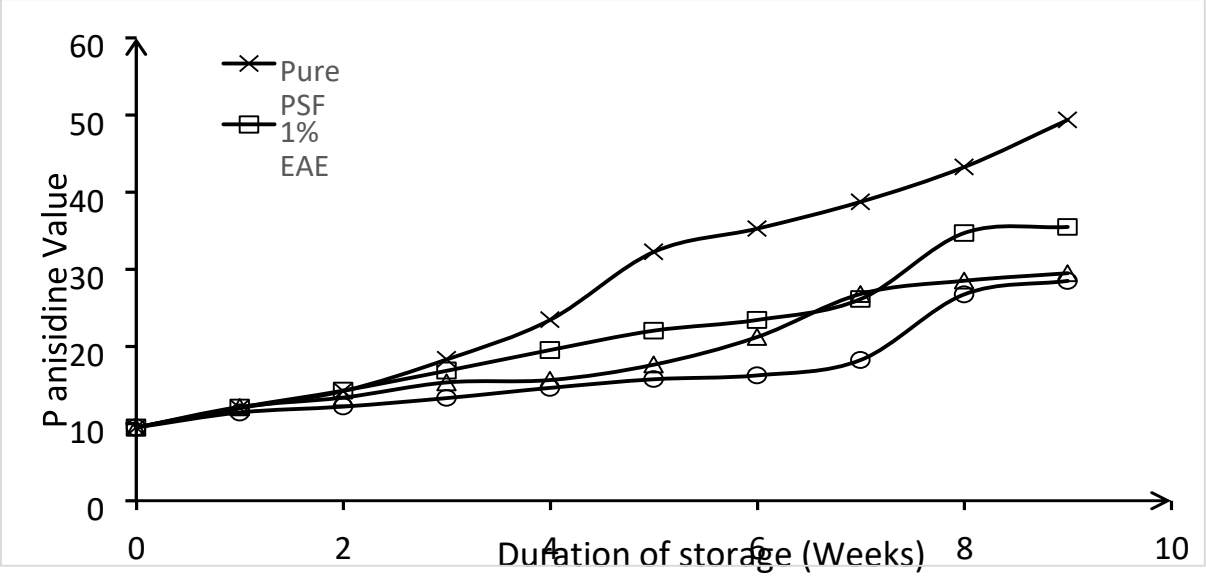

Fig 4 : P-anisidine value of stored peanut oil packaged with film containing different concentrations of EAE

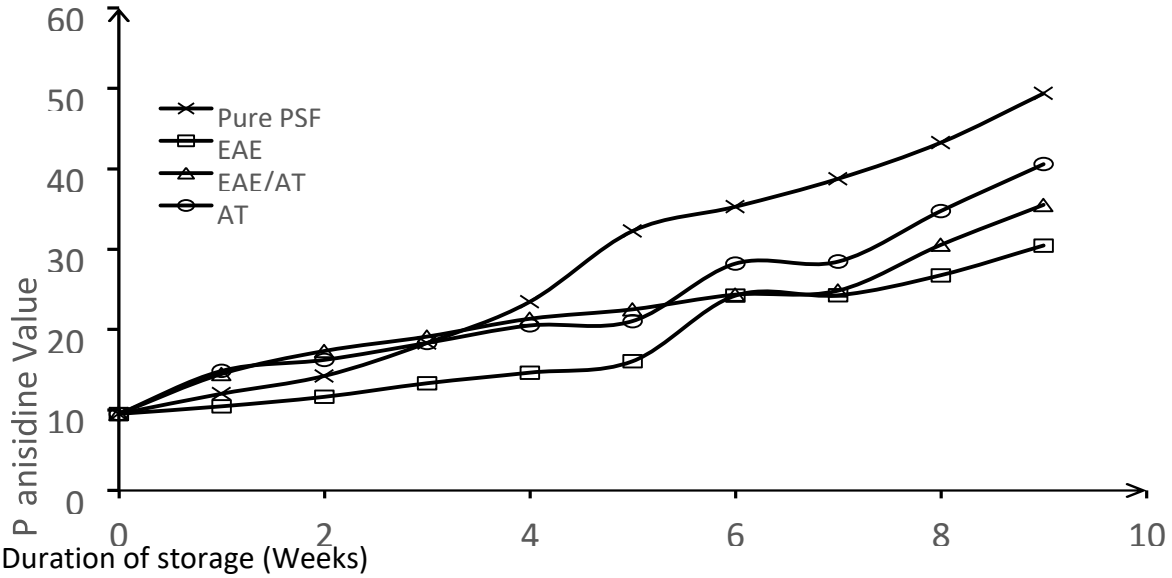

Figure 5: P-anisidine value of stored peanut oil packaged with film containing different antioxidants

Figure 3 gives the result of p-anisidine (p-AV) value of stored Peanut oil packaged with film containing different Antioxidants (Peanut oil packaged with EAE, Peanut oil packaged with AT and peanut oil packaged with equal blend of AT/EAE).The result showed that p-AV for all the samples

increased significantly (P<0.05) with storage time the difference was in rate of increase. The control (pure PSF) was more susceptible to oxidation than the three oil samples with antioxidant packs. This is indicated by its higher rate of production of p-AV especially between the 4th and 7th week of storage (23.45 and 40.27) respectively. This led to higher p- AV (46.50) at the end of storage period. The oxidation of peanut oil packaged in film containing EAE was significantly lower than all other samples as indicated by its lowest rate of p-AV production from the beginning of storage till the 6th week of storage (9.5 to 16.25), and eventually lowest p-AV at the end of storage. This shows that EAE incorporated in the film (pack) had the capacity to suppress the rate of oxidation of the peanut oil in contact with it. All the three samples with the antioxidant packs had significant increase of p-AV between 6 and 7th week of storage indicating reaction of p-anisidine with produced aldehydes. Hydroperoxides are unstable at storage therefore, they decompose to give secondary oxidation products that are *p*-anisidine-reactive (Moigradean *et al*., 2012)

No significant difference (P<0.05) was observed between oil in AT and EAE/AT. Comparing EAE with its bend with AT (EAE/AT) shows that AT reduced the effectiveness of EAE which could be as a result of their incompatibility. EAE in table 5 gave the least PV. This showed that it is the most efficient antioxidant that could be used to produce the active film while Table 5 revealed that either 2.5 or 5% EAE will adequately serve to produce the active film since they gave the least PV and their effect is not significantly different from each other.

Table 4: Effect of different antioxidant on the P-anisidine value of actively packaged peanut oil.

SAMPLES	PV(meq O_2/ kg oil) of peanut oil	P anisidine value of peanut oil
CONTROL	20.29± 0.90^{a}	27.64± 1.05^{a}
EAE	7.35± 2.00^{c}	18.12± 1.57^{bc}
EAE/AT	8.8± 1.07^{b}	21.94± 1.54^{b}
AT	9.24± 3.55^{b}	23.24± 1.06^{b}
LSD	**1.78**	**3.23**

Values are the means of duplicate determinations. a, b.... means with the same superscript in the same column are not significantly different at p< 0.05. CON = control, EAE = *ehuru* antioxidant extract, EAE/AT = combination of *ehuru* antioxidant extract and α-tocopherol, AT = α-tocopherol, P-AnV = P- anisidine value, PV= Peroxide value

Table 5: Effect of concentration on the PV and P-AnVof actively packaged peanut oil.

SAMPLES	PV(meq O_2/ kg oil) of	P -AnVpeanut oil
0%EAE	12.29± 0.90^{a}	27.64± 1.05^{a}
1% EAE	9.84± 1.74^{b}	21.61± 1.64^{b}
2.5%EAE	7.85± 1.13^{c}	18.84± 1.24^{bc}
5%EAE	7.27± 1.69^{c}	16.65± 1.30^{c}
LSD	**1.85**	**3.6**

Values are the means of duplicate determinations, a,b.... means with the same superscript inthe same column are not significantly different at p< 0.05. CON = control, EAE = ehuru antioxidant extract, EAE/AT = combination of ehuru antioxidant extract andα- tocopherol, AT = α- tocopherol, P-AnV= P- anisidine value, PV= Peroxide value

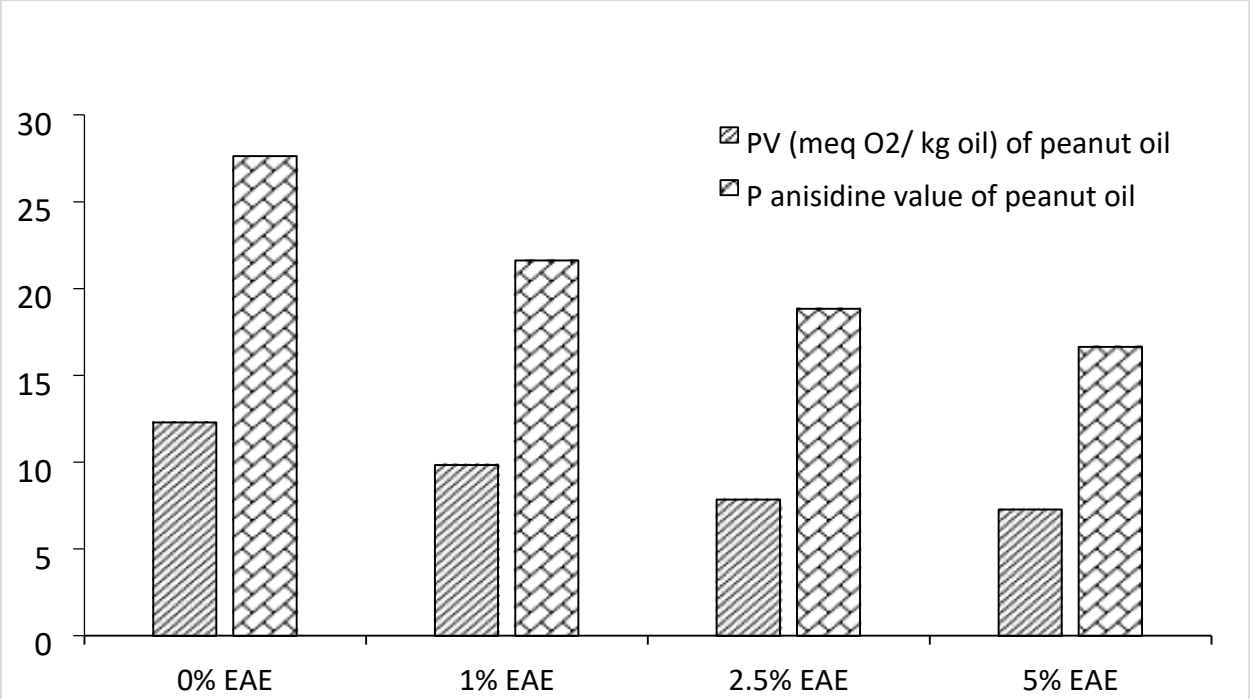

Figure 6: Effect of concentration of EAE on the oxidation of actively packaged peanut oil

CONCLUSION

The study has shown that active film can be produced using *ehuru* spice extract. The produced *ehuru* natural antioxidant active film showed to be more effective in retarding lipid oxidation of peanut oil than the established natural antioxidant (α-tocopherol). The incorporation of EAE active compound. The film barrier was increasing as concentration of EAE increases. 5% EAE produced the highest gas barrier which also reduced the inflow of oxygen that serve as initiator to lipid oxidation. Incorporation of EAE active component in the PSF film presented a potential antioxidant ability which enhanced the scavenging ability of the film. Therefore, these films may be an alternative for lipid food preservation and shelf life extension since they retard oxidation of the peanut oil.

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