An Evaluation of the Level of Synthetic Phenolic Antioxidants in Virgin Palm Oil

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***Abstract*—Palm oil contains important dietary nutrients and it is important to humans since it contains antioxidants, which are mediators of various diseases especially of the cardiovascular system. Virgin palm oil is pressed from the oil palm fruit and stored in vessels prior to usage. During storage, virgin palm oil undergoes various oxidation processes making it rancid. This reduces its shelf life and causes it to change in colour and composition giving off an offensive odour. It is possible for small and large-scale oil palm plantation owners to preserve the virgin palm oil by some natural means or artificially with the use of synthetic phenolic antioxidants. Synthetic phenolic antioxidants are added to food for preservation and to prolong its shelf life. In this study, virgin palm oil was analysed for synthetic phenolic antioxidants by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) coupled with a Ultraviolet /Visible (UV-Vis) detector. A mixture of solvents, hexane, and acetonitrile was used for the extraction method. Virgin palm oil was analysed qualitatively and quantitatively to determine the concentration of Butylatedhydroxytoluene (BHT), Butylatedhydroxyanisole (BHA), Propyl Gallate (PG) and 2-ethylhexyl 4-methoxycinnamate (EEMC). PG was detected below the detection of the method. A new extraction method was proposed to validate the experimental procedure**.

***Index Terms*—virgin palm oil, RP-HPLC, UV-Vis detector, synthetic phenolic antioxidants**

I. Introduction

Edible oils are needed for cooking and for most industrial processes. They are also an active ingredient in most processed foods. Edible oils could be extracted from plants, seeds, nuts, or fruits. Irrespective of the method of extraction, they are extensively used in food and non-food productions and a feedstock for the production of biodiesel. There is a growing desire for edible oils due to an increasing world population and improved diets. Elaeisguineensis is commonly known as African oil palm. It consists of pinnate-leaved palms with dense clusters flowers and bright red fruit that produce rich quality red palm oil [1]. It is aborigine to West Africa (Madagascar and Nigeria) [2], [3], and some parts of Asia [4] and widely propagated in the tropical regions of these countries. Virgin palm oil (Elaeisguineensis) is edible oil used in several culinary dishes in improving the diets of the populace. In addition, it contains tocotrienols, tocopherols and carotenes among other antioxidants that are useful mediators in the prevention of several diseases especially heart maladies. It is important to monitor the processes for the production of the red palm especially the finished products prior to storage. Rancidification of oil and fat containing foods due to oxidation is a common problem. It affects food quality. To prevent this, synthetic or phenolic antioxidants are added [5]. Butylatedhydroxytoluene (BHT), Butylatedhydroxyanisole (BHA), Propyl Gallate (PG) and 2-ethylhexyl 4-methoxycinnamate (EEMC) are lipophilic (fat-soluble) organic compounds which are derivatives of the compound phenol. They are useful for their antioxidant properties and are used as standard for antioxidant assay [6]. BHT, BHA, PG and EEMC are commonly used antioxidants found in several foods which include cheese, butter, chewing gums, baked foods, processed foods and pharmaceuticals. They are used to preserve odour, colour and flavour of foods. Synthetic phenolic antioxidants find their use as food additives used to prevent rancidification, due to their suitability cheapness and availability. However, numerous studies by various researchers have shown that the use of phenolic antioxidants above the requirement limit can result to serious health risks [6]. A number of synthetic antioxidants, about thirty of them have been permitted to be used in foods as additives directly or indirectly [6]. Their use in food may be singly or in combination with other synthetic antioxidants. As a result, it is of paramount importance to determine qualitatively and the widespread use of permitted synthetic antioxidant. A number of analytical instruments and methods have been documented for the qualitative and quantitative determination of synthetic antioxidants in foods and edible oils. These methods include UV–visible photometry [6], [7], paper and thin-layer chromatography [8], gas chromatography [9]-[11], and liquid chromatography [12]. The sample extraction steps of most of the methods mentioned are time consuming and cumbersome. Phenolic antioxidants are oxidized preferentially in fats or oils and protect the foods from spoilage. There are some reports about the possible carcinogenic effect of antioxidants like BHA, BHT, PG and EEMC in high doses and long-term use may have

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potential health risks [7]. Conversely, phenolic antioxidants are advocated as dietary supplements, which have antiviral properties and are useful against herpes and some family viruses [7]. In lower doses, BHT is not carcinogenic. The reason for BHT non-toxic property to be used in foodstuffs is that the tert-butyl groups surround the hydroxyl group. The alkyl groups being so bulky, the hydroxyl group is screened off from possibilities of chemical activity and above all from the possibility of hydrogen bonding [9]. Due to this controversial reports the detection and estimation of BHT in food products is considered important.

Phenolic compounds such as BHA and BHT, PG and EEMC are added singly or in combination to prevent oxidative rancidity in personal care products [3]. This makes it possible for the unstable peroxide radicals [4], [5] to inhibit reactions promoted by oxygen, thus avoiding the oxidation and are intended to prevent the appearance of ketones and aldehydes that can give a product a disagreeable smell and rancidity [5]. To prevent edible oils from peroxide radicals we must use antioxidant compounds, which have the ability to neutralize those radicals through the transfer of hydrogen to this radical, stabilizing the radicals. Analysis is performed to determine the amount of BHT, BHA, PG and EEMC in virgin palm oil. A method was proposed to achieve high throughput, low flow rate using minimal mobile phase solvent. Reversed Phase High Performance Liquid Chromatography method with UV/Vis detector (RP-HPLC-UV/Vis) is an important analytical technique with strong chromophores that absorb light in the wavelength region from 200nm to 800nm [12]. The UV/Vis detector is a suitable detector for the determination of BHA, BHT, PG and EEMC as a phenolic antioxidant added singly or in combination in red palm oil. To our knowledge, no method has been proposed or developed for the determination of the phenolic/synthetic antioxidants in virgin palm oil. There is need to develop a RP-HPLC method, which could be employed for the routine analysis of virgin palm oil either singly or in combination using simple mobile phase composition. The present study was undertaken to develop, optimize, and validate a RP-HPLC method for analysis of BHA, BHT, PG and EEMC.

Therefore, the aim is to determine the optimum analysis condition and validate the method for a simultaneous detection, identification and quantification of synthetic/phenolic antioxidants with the objectives of developing an analytical method for the evaluation and quality control of phenolic/synthetic compounds in virgin palm oil using Reverse Phase High Performance Liquid Chromatography method (RP-HPLC).

II. METHODOLOGY

A. Sampling Sites, Sample Collection and Preparation

Red palm oil was sampled from the Headquarters of Nigeria Institute for Oil Palm Research (NIFOR) in Benin and from its substation. One of the oils from a substation in Apoje oil palm plantation was used to validate the analytical method. The oil palm plantation sampling point was partitioned into four points and samples were collected from each point. The sampling locations were also based on factors such as population density, farm settlements, and areas receiving significant pollution characteristics. Storage containers for sampling were sterilized 1-liter plastic containers. The samples were preserved in an ice chest at 4 ℃. The samples were transferred to the laboratory and stored in the refrigerator at 4 ℃ prior to analysis. Glassware were washed with detergent and rinsed with distilled water. They were further soaked overnight in 10% analytical grade nitric acid, and then rinsed in high purity deionized water.

B. Chemicals

Butylhydroxytoluene (BHT) (≥99% pure), Propyl Gallate (PG) 99.8% pure, (HPLC grade) butylatedhydroxyanisole (BHA), 2-ethylhexyl 4-methoxycinnamate (EEMC), n-hexane, methanol (HPLC) grade, acetonitrile, ethanol, acetic acid, Petroleum ether. All chemicals were purchased from Sigma Aldrich USA and were of analar/chromatography grade.

C. Preparation of Standard Solution

Individual stock solution of BHA, BHT, PG and EHMC (20mg/L in methanol) were prepared accurately. The mixture was shaken until a homogenous and clear solution was formed and a serial dilution of each was prepared to obtain a linear calibration curve. The solution was covered with aluminum foil and stored in a freezer (4 ℃); (this was away from heat and sunlight for a maximum of one month). Before analysis was performed, standard working solutions were prepared by diluting appropriate amounts of the stock solutions in methanol.

D. Extraction Procedure

About 0.5g of the virgin palm oil was weighed into a centrifuge tube using an electronic weighing balance. Acetonitrile (3mL) which was saturated with hexane was added to the mixture in the centrifuge tube. The mixture was homogenized for 5 minutes at 1800rpm using a shaker followed by centrifugation using a centrifuge for 5 minutes at 4000rpm.

The non polar phase which is the acetonitrile phase was collected and the oily layer was re-extracted thrice using similar extraction procedures. All the acetonitrile phases were recombined into a 10 mL capacity volumetric flask and diluted to the mark with acetonitrile. The solution was set aside to be analysed by RP- HPLC.

E. Instrumentation

This is a modified procedure of Dr. Ancos *et al.* An ODS C_{18} Chromatographic column was used (4.6cm×250cm). The ODS C_{18} Chromatographic column used for the analysis had a particle size of 5μL. An Isocratic system with mobile phases of 75:25 v/v methanol: water mobile phase was employed. The Agilent Technologies (1200 series) RP-HPLC Instrument used for the analysis was equipped with UV/Vis detector and quaternary pumps. The maximum wavelength chosen was 280nm and 1mL/min flow rate.

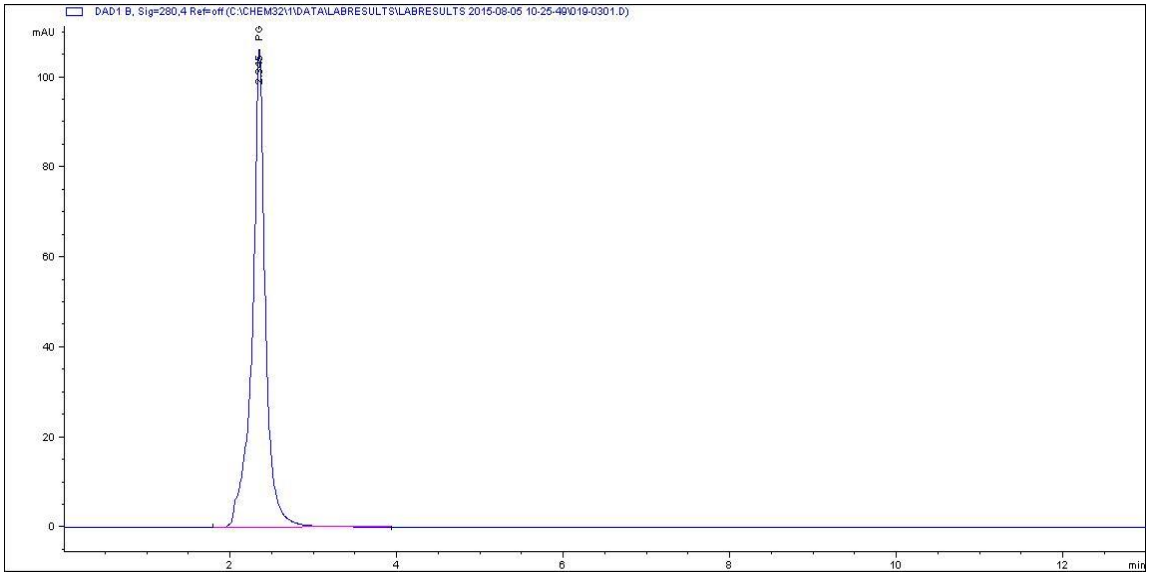

Figure 1. The chromatogram of the standard calibration curve for PG.

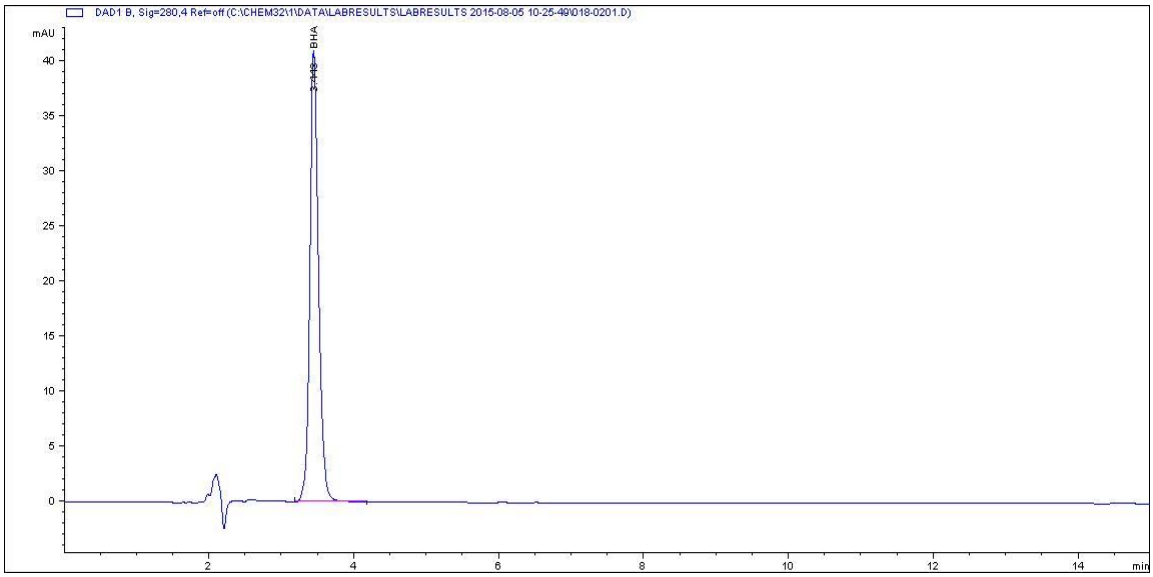

Figure 2. The chromatogram of the standard calibration curve for BHA

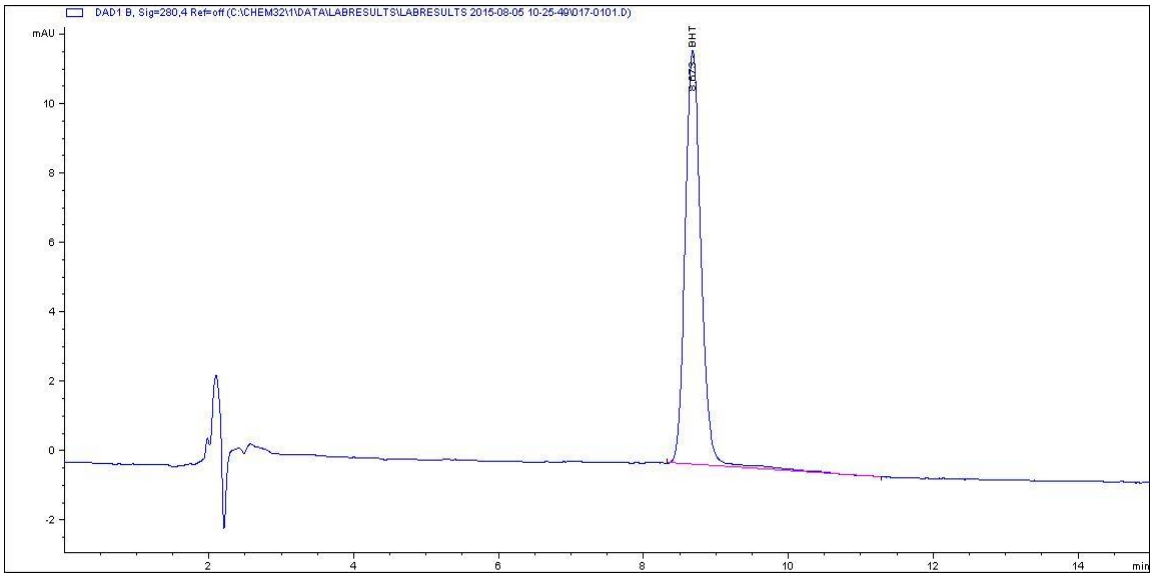

Figure 3. The chromatogram of the standard calibration curve for BHT

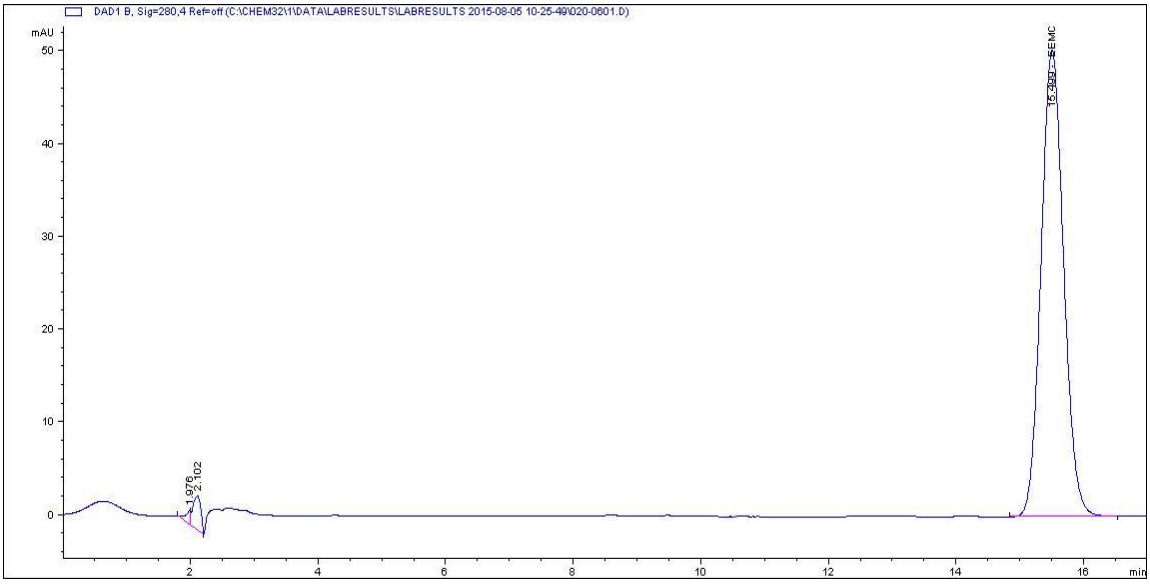

Figure 4. The chromatogram for the standard calibration curve for EEMC

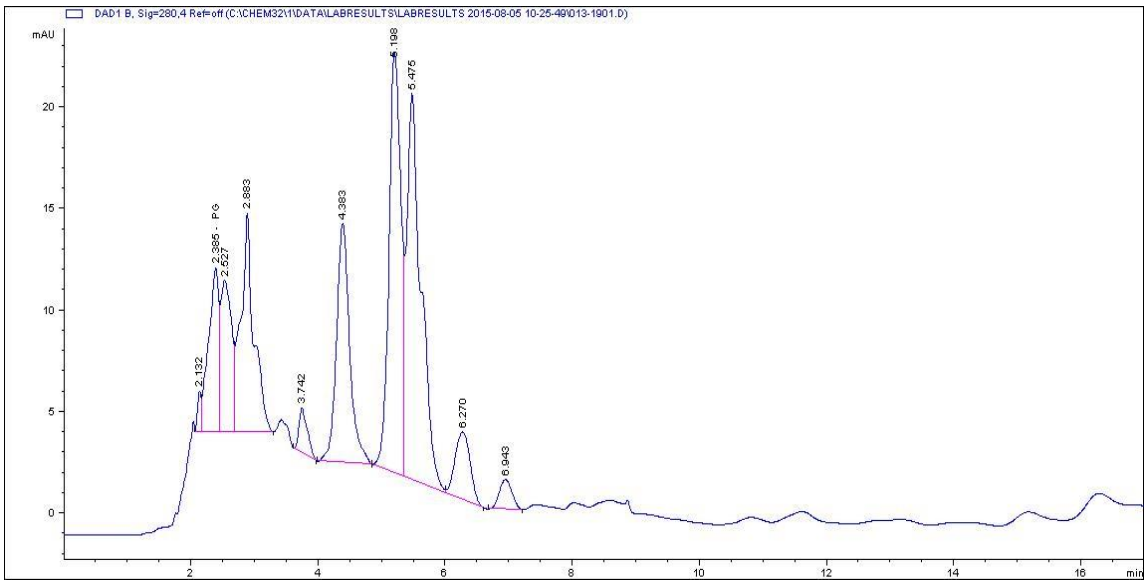

Figure 5. The chromatogram of the result of the analysis for Apoje sample

III. RESULTS AND DISCUSSSION

A. Results

Fig. 1 shows the chromatograph of the standard calibration curve for PG. The retention time of PG was within 2 minutes at a flow rate of 1mL/min and 50 µL injection volume.

Fig. 2 shows the chromatograph of the standard calibration curve for BHA. The retention time of BHA was within 3-4 minutes at a flow rate of 1mL/min and 50 µL injection volume.

Fig. 3 presents the retention time for BHT which was within 8 minutes at a flow rate of 1mL/min and 50 µL injection volume.

Fig. 4 shows the retention time for EEMC which was within 15-16 minutes at a flow rate of 1mL/min and 50 µL injection volume.

B. Analysis of Real Samples

Real samples of red palm oil were analysed. In this study, optimization of the extraction procedures for red palm oil sampled on Apoje plantation was evaluated to obtain the best analysis time was evaluated. The result is presented in Fig. 5. A peak was observed at the retention time of 2 minutes which could be PG. The concentration of the peak was poorly resolved. From the calibration curve represented in the graph in Fig. 6, the peak area was measured and RF determined. The concentration of the calibration curve was greater than the concentration of the PG as shown in Fig. 5. PG was resolved within 2 minutes. The calibration curve shows that the analyte gave a linear curve and this determined the linear dynamic range. PG was detected in the sample but it was below the linear dynamic range. Based on the result obtained from the initial extraction procedure which involved the use of acetonitrile and hexane, it can be

deduced that the synthetic phenolic antioxidants are absent in the sample of virgin palm oil or it can be deduced that the extraction procedure is inaccurate. A new extraction technique was proposed to validate the extraction procedure. The analysis is in progress.

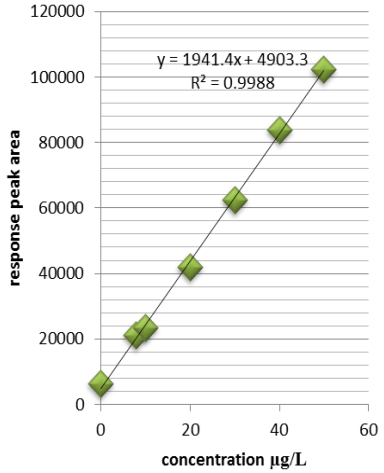

Figure 6. The linear calibration curves for the antioxidants

C. Validation of the Analytical Procedure

The linearity of the method was determined by linear calibration curves for each of the antioxidants. Table I shows the regression data obtained from the calibration curve.

TABLE I. THE REGRESSION DATA OBTAINED FROM THE CALIBRATION CURVE

Antioxidant	Equations	R^2	LOD	LOQ
PG	*y=1941x+4903*	0.998	0.001	0.01

D. Recovery Efficiency and Method Performance

The accuracy of the analytical method was determined using external standardization addition methods for four spiked samples at 1, 5, 10 and 25mg/L and comparing with a standard chromatogram of similar concentration.

IV. CONCLUSION

Virgin Palm oil was investigated to determine the levels of synthetic phenolic antioxidants. Virgin palm oil contains important dietary nutrients such as antioxidants which are mediators of cellular functions and cures for several ailments including cancer and cardio vascular diseases. The rancidity of virgin palm oil during storage causes the oil to have an offensive odour, colour and taste. Synthetic phenolic antioxidants may be added to offset these changes in the oil during storage. The study was aimed to know quantitatively and qualitatively the type and amount of these synthetic phenolic antioxidants. This is of paramount importance because the levels of the synthetic phenolic antioxidants above the Recommended Dietary allowance have been investigated to be a major contributor of some forms of cancer. Other health effects include hyperactivity, irritability, and migraines. The modified RP-HPLC procedure by Dr. Ancos *et al.* with an ODS C18 Chromatographic column was employed using 75:25 v/v methanol: water mobile phase. PG was detected below the detection limit of the machine using the procedure. A new extraction procedure was proposed and the analysis is in progress.

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