

Physicochemical Characteristics and HPLC Determination of Alpha-Tocopherol in Eighteen Edible Vegetable Oils Marketed in Nigeria

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ABSTRACT: Eighteen brands of vegetable oils available in the local market were extracted with *n*-hexane before analysis for alpha-tocopherol by RP-HPLC method. The chromatographic separation occurred isocratically with methanol-water [96:4% v/v] at 0.9 ml/min flow rate. Tocopheryl acetate was the internal standard and alpha-tocopherol was eluted at 7.87 min. Free fatty acids value [FFAVs], peroxide value [PV], iodine value [IV] and saponification values [SV] were determined as quality parameters. Calibration curve was linear [r^2 0.9969] and the method was precise with relative standard deviation of 0.35% and mean recovery, 87.39%. Alpha-tocopherol concentration ranged from 0-9.22 mg/100g with the highest in Tropical sunflower oil [9.22 mg/100g] and the lowest [1.16 mg/100g] in Laziz oil. Alpha-tocopherol was not detected in unbranded, local palm oil. The calculated percentage daily value [% DV] of vitamin E ranged from 0-8.60%. Significant difference [$p < 0.05$] between % DV and recommended dietary allowance [RDA] of vitamin E was observed. FFAs and PV ranged from 0.11-0.74% and 0.99-11.55 meq/kg while IV and SV ranged from 26.71-37.03 g/100g and 4.14-43.68 mg KOH/g, respectively. Seventeen samples [94%] were found to be within the acceptable limits while one [6%], failed for both quality parameters and α -tocopherol test. Strict regulatory control is advocated for these oils to safeguard the public health.

Key words: Vegetable oils, physicochemical characteristics, quality assessment, α -tocopherol, RP-HPLC

INTRODUCTION

Vitamins are organic compounds that promote and regulate essential biochemical reactions within the body, which itself is unable to synthesize these compounds hence are required in the diet in trace amounts.¹ Vitamin E, a fat-soluble vitamin, which can be classified into tocopherols and tocotrienols (Figure 1) is a naturally occurring antioxidant present in some foods. Each class consists of alpha, beta, gamma and delta forms² differing only in their chemical structures. Tocopherols differ from each

other by the number and position of methyl group in the phenolic part of the chromane ring.^{3,4} They have saturated phytyl tail while the tocotrienols have shorter unsaturated farnesyl tail with three isolated double bonds.⁵ Biological activities and anticancer effects for tocopherols have been reported.^{6,7} Alpha-tocopherol is an important antioxidant and essential micronutrient that protects the body from degenerative diseases. Vitamin E has been reported to protect cell wall fatty parts; prevent oxidation of polyunsaturated fatty acids, prevent nerves and muscle degeneration aside reducing oxygen requirement of muscles.¹

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Chromatographic conditions. The HPLC system was an Agilent 1100 HPLC series, USA equipped with a main controlling unit, quaternary pump, online degasser, waters x-bridge C18 column (100 x 4.6 mm ID, 5 μ m particle size), with 20 μ l

injector loop and UV-Vis chem-station software detector. Mobile phase was made up of HPLC grade methanol and water (96:4) and ran at a flow rate of 0.9 mL/min with temperature kept at 35°C. The detector wavelength was set at 292 nm.

Table 1. Details of vegetable oil samples.

Sample number	Name of brand	Ingredient Source	Date of manufacture	Expiry date
1.0	Devon Kings vegetable oil	Palm olein [fraction of palm oil]	12/07/16	12/07/17
2.0	Sunola soya oil	Seeds of Glycine max [soy bean]	09/2015	09/2017
3.0	Golden Penny pure soya oil	Glycine max [soy bean]	28/08/15	28/08/16
4.0	Adan soybean oil	Glycine max [soy bean]	28/05/15	27/05/17
5.0	Mamador pure vegetable oil	Palm olein [fraction of palm oil]	21/10/15	21/10/16
6.0	Power oil	Palm olein [fraction of palm oil]	21/03/16	21/03/17
7.0	Lesieur vegetable oil	Palm olein [fraction of palm oil]	05/2015	05/17
8.0	Laziz vegetable oil	Palm olein [fraction of palm oil]	09/11/15	09/11/16
9.0	Mazola corn oil	Embryo of Zea mays	25/12/15	25/12/16
10.0	Zok cotton seed oil	Seeds of <i>Gossypium</i> spp.	27/07/15	26/07/17
11.0	Tropical sunflower oil,	Seeds of <i>Helianthus annus</i>	-	09/2017
12.0	Borges extra virgin olive oil	Fruits of <i>Olea europaea</i>	-	10/2017
13.0	Classic olive oil	Fruits of <i>Olea europaea</i>	-	10/2017
14.0	Goya Spanish olive oil [plastic]	Fruits of <i>Olea europaea</i>	-	-
15.0	Goya extra virgin olive oil [bottle]	Fruits of <i>Olea europaea</i>	-	10/2017
16.0	Groundnut oil Kuli-kuli [local]	<i>Arachis hypogaea</i>	-	-
17.0	Palm oil [unbranded]	<i>Elaeis-guineensis</i> [fruit of oil palm]	-	-
18.0	Rodi palm oil	<i>Elaeis-guineensis</i> [fruit of palm oil]	25/09/15	25/09/16

Preparation of standards. DL- α -tocopherol (10 mg) was dissolved with ethanol (96%) and made up to 100 mL in a volumetric flask to form the stock solution of 0.1 mg/ml. It was protected from light by wrapping with aluminum foil. Different concentrations (1.0 μ g/ml, 2.5 μ g/ml, 5 μ g/ml, 10 μ g/ml & 20 μ g/ml) were then prepared through various dilutions. Each standard solution was filtered before being injected into the HPLC (Agilent 1100 HPLC series, USA). Tocopheryl acetate solution (300 μ g/ml) was used as internal standard. Each run lasted 12 minutes. Data was collected by Agilent chromatograph automated chem. station software. Peak area was plotted against concentration to generate the calibration curve. Linearity was determined from the correlation coefficient (r) of the standard plot.

Oil extraction and determination of α -tocopherol content. Each oil sample [1 g] was weighed into a 10-mL volumetric flask and made up to volume with hexane. 200 μ L was taken and mixed with 940 μ L of methanol and 60 μ L of internal standard (300 μ g/ml stock solution) in an ultracentrifuge bottle. It was centrifuged at 4000 rpm for 5 minutes. Tocopherol analysis was carried out by RP-HPLC with an Agilent 1100 HPLC series, USA using the method of Gimeno *et al.*²² with major modifications to column temperature, flow rate and dilution factor. 20 μ L of each prepared sample was injected into the HPLC previously described using the conditions specified under chromatographic procedures.

Physicochemical analysis. Physicochemical parameters used in evaluating the quality of vegetable oils such as Acid value (AV), Peroxide value (PV),

Iodine value (IV) and Saponification value (SV) were determined by established methods.^{34,37, 38,46}

Relationship between recommended daily allowance (RDA) and percentage daily value

$$mg/serving = \frac{conc \left(\frac{mg}{100g} \text{ of oil} \right) \times servingsize}{100}$$

$$\%DV = \frac{mg/serving \times 100}{RDA}$$

(%DV). Serving size of oils analysed according to labeled claim was 14 g/serving size. RDA and % DV were calculated using the formula⁴⁶ shown below:

RESULTS AND DISCUSSION

Physicochemical evaluation. Table 2 shows the results for the physicochemical characteristics: peroxide, free fatty acid, iodine, and saponification values of the analyzed vegetable oils.

Peroxide value. The peroxide value is used to determine the extent of rancidity of oils during storage, therefore, it can be used as an indicator in determining stability of fats and oils.³⁶ It is primarily used to determine the oxidation of lipids.³⁷ The peroxide results ranged from 0.99-11.55 mEq/kg as

presented in Table 2. However, the acceptable limit of peroxide value for edible oils is 10 mEq/kg.^{32,38} Sample number 17 had the highest value of 11.55 mEq/kg which was not within the official limit. Sample number 16 was also high in peroxide value, though it was within the acceptable limit. These high values indicate the extent of rancidity due to lipid oxidation. This could have been due to storage or “age”, since there were no labels on the oil, to indicate the date of manufacture/preparation.

Table 2. Saponification, Free fatty acid, peroxide, Iodine, and tocopherol values of different oils.

Sample number	Peroxide value [mEq/Kg]	Free fatty acid value [%]	Iodine value [g/100 g]	Saponification value [mgKOH/g]	Alpha tocopherol (mg/100g)
1.0	2.76	0.17	33.85	17.94	5.42
2.0	2.27	0.14	37.00	11.10	2.06
3.0	0.99	0.19	36.66	15.03	2.89
4.0	1.00	0.16	36.34	4.71	2.03
5.0	2.24	0.17	30.61	19.72	3.04
6.0	1.74	0.16	35.59	19.32	2.70
7.0	3.26	0.14	35.15	15.78	4.73
8.0	1.75	0.11	34.03	20.01	1.16
9.0	1.49	0.14	34.47	32.60	4.67
10.0	1.76	0.11	36.78	15.80	3.46
11.0	2.25	0.17	37.03	4.14	9.22
12.0	1.99	0.11	35.83	14.57	3.78
13.0	2.01	0.11	35.52	34.00	3.49
14.0	1.74	0.13	34.84	28.44	3.18
15.0	1.49	0.14	35.14	24.72	4.97
16.0	8.03	0.33	34.18	37.40	3.34
17.0	11.55	0.74	26.71	43.68	0
18.0	3.66	0.27	28.75	39.92	2.77

Free fatty acid value (FFAV). Free fatty acid is due to hydrolysis of fats and oils which may be promoted by oil's reaction with moisture.³⁹ Values obtained for free fatty acid ranged from 0.11-0.74% as presented in Table 2. The acceptable limit for FFA according to AOAC is 0.6%³³ while other samples were found to be within the acceptable limits. Sample number 17, the unbranded palm oil had an elevated FFA value of 0.74%, which is above the acceptable limit for edible oils.^{32,38} This indicates that hydrolytic rancidity and oxidative degradation might have occurred in sample 17, which could have been due to exposure to atmospheric moisture. This result also correlated with the peroxide value [10 mEq/kg] obtained for the same sample that was above recommended limit.

Iodine value. Iodine value is a measure of the degree of unsaturation of oils and fats.³⁴ Results for iodine value are presented in Table 2 and it ranged from 26.71-37.03 g/100 g. High iodine values indicates high level of unsaturation in the oils. Sample 11 (Tropical Sunflower oil) had the highest iodine value of 37.03 g/100g, an indication that it contains the most unsaturated fatty acids.

Saponification value. Saponification value is an index of average molecular mass of free fatty acid.⁴⁰ The saponification values ranged from 4.14-43.68 mg

KOH/g (Table 2). These values are below the official limit of 186-205 mgKOH/g specified by SON.³⁸ Low saponification value is an indication that the oil samples will not be suitable for soap making.

Quantification of α -tocopherol in oil samples

Standard calibration. The calibration curve of the standard is presented in Figure 2. The standard DL- α -tocopherol eluted at a retention time of 7.8 min and showed an acceptable linearity with a correlation coefficient of 0.997 in the concentration range studied. Tocopheryl acetate, the internal standard eluted at 10.1 min.

HPLC determination of α -tocopherol. The chromatogram of DL- α -tocopherol and tocopherol acetate is presented in Figure 3

The elution time of dl- α -tocopherol was different from the elution time of tocopherol acetate; hence they were well separated without interference. The elution time was used in determining the peak of the α -tocopherol in the vegetable oils studied, while the peak area was used in calculating the concentration of the α -tocopherol in the various oil samples. Modifications made to the method of Gimeno *et al.*²² allowed for better peak separation

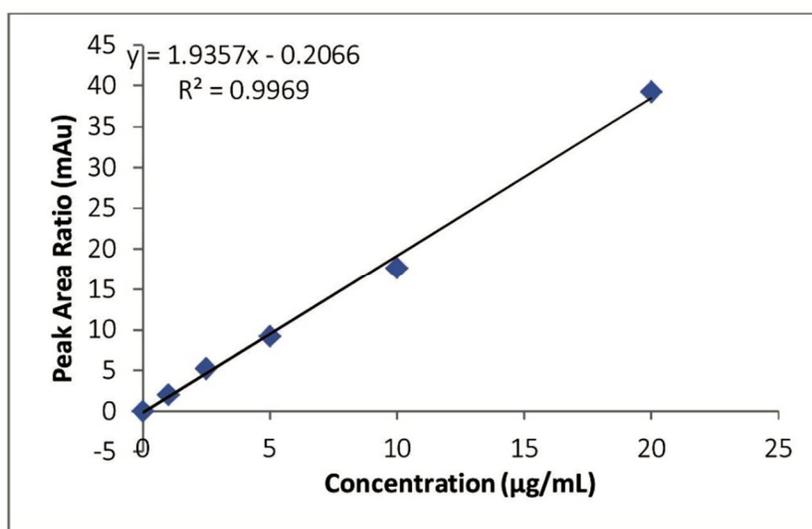


Figure 2. HPLC calibration curve for DL-alpha tocopherol

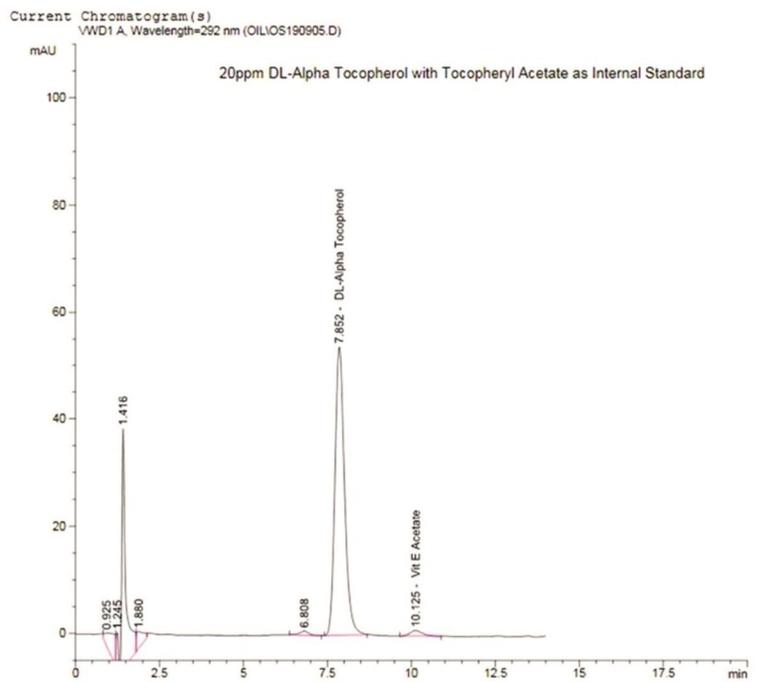


Figure 3. The chromatogram of DL- α -tocopherol and tocopherol acetate.

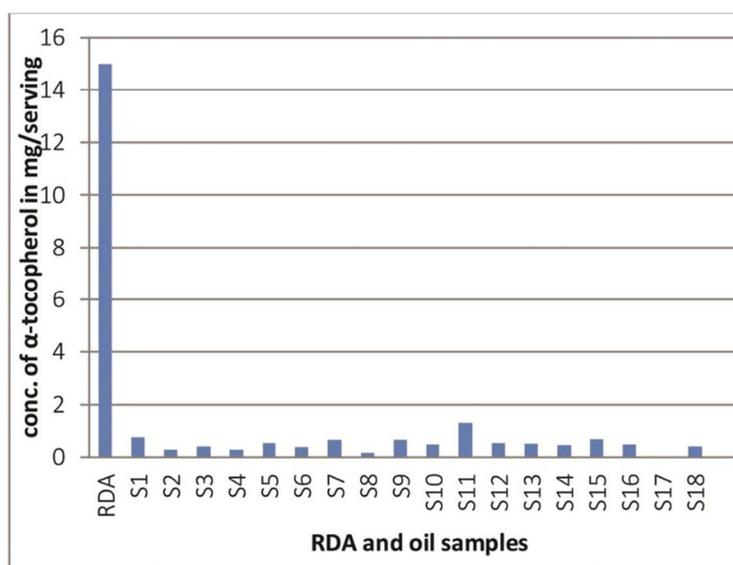


Figure 4. Relationship between daily requirement of vitamin E and RDA

and longer elution time a result of lower column temperature and flow rate. The results of the concentrations are presented in Table 2. Alpha-tocopherol values obtained were in the range of 0-9.22 mg/100 g. Tocopherols are phenolic potent

natural antioxidants that prevent rancidity of oils during storage and thus increase the shelf-life of edible oils.⁴¹ Tropical sunflower oil was the richest in alpha-tocopherol with a concentration of 9.22 mg/100g while the lowest [1.16 mg/100g] was found

in Laziz vegetable oil. This result was consistent with the previous study which reported that sunflower oil was the richest in alpha-tocopherol.⁴² Sample 17, the unbranded palm oil had no detectable α -tocopherol. This could be responsible for the high presence of free fatty acids and peroxide value obtained for sample 17. The presence and importance of vitamin E in these oil samples cannot be overemphasized consequent of the effect of its absence and oxygenated decomposition products on human health

including membrane damage, cardiac diseases, ageing, cancer and other degenerative diseases. Thus, oil quality and its stability are very important for both consumers and application industries alike.⁴³ Results from this study suggests that most [94%] of the sampled vegetable oils in the south-west Nigerian market meet the recommended standards of quality test for edible oils and this is in agreement with some previous studies.⁴⁴

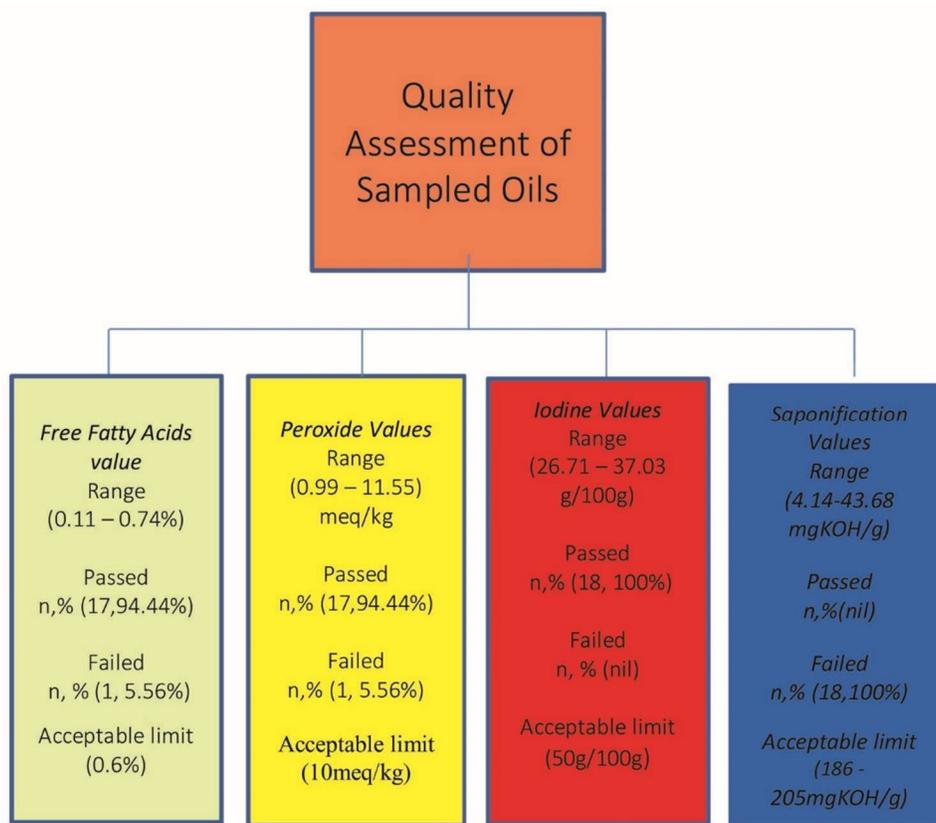


Figure 5. Summary of physicochemical parameters for oil samples used in the study

The recommended daily allowance for vitamin E for healthy individuals of aged 14 years and above is 15 mg of α -tocopherol.⁴⁵ A food is considered a source of vitamin E if the percentage daily value [%DV] is greater than 5% of Recommended Daily Allowance [RDA].⁴⁶ Figure 4 shows the relationship between daily requirement of vitamin E and RDA which ranged from 0-1.29 mg/serving while Figure 5 outlines a summary of the physicochemical

characteristics of the oil samples. Percentage daily value [%DV] ranged from 0-8.60%.

Tropical sunflower oil (sample 11) and Devon Kings vegetable oil (sample 1) had % DV of 8.60% and 5.07%, respectively. Therefore, samples 11 and 1 could be considered food sources of vitamin E. Other samples could not be judged as food sources of vitamin E because of the low % DV observed. Factors such as fruit or seeds quality, extraction

systems and refining procedures²⁶ could have influenced the concentration of alpha-tocopherol in these oils aside improper storage in transparent bottles and direct exposure to sunlight in all commercial markets.¹³

CONCLUSIONS

The reversed-phase high performance liquid chromatography used was a good method and linear in the calibration range studied for the quantitative assessment of α -tocopherol in the vegetable oils. Seventeen oil samples [94%] fell within acceptable limits for edible oils while one [6%], failed both quality and α -tocopherol test; hence may be unsuitable for human consumption. Strict regulatory control and quality assessment is advocated for these oils to safeguard public health.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest and received no funding.

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