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Investigation into Level of Retinol and Other Antioxidants Present in Vegetable Oil Consumed in Ekiti/Ondo State Region of Nigeria

Ayorinde D. Olowookere[1*], Olamide O. Ojo[1], Sunday A. Malomo[2]

[1]Department of Nutrition and Dietetics, College of Health Sciences and Technology, Ijero Ekiti, Nigeria.
[2]Department of Nutrition and Dietetics, Federal University Technology, Akure, Nigeria.

*Corresponding author: rindezon@gmail.com; +2348068294305
ORCID No: https://orcid.org/0000-0002-1323-459X

Abstract

Twenty-two different brands of vegetable oils were collected in different markets in Ekiti/Ondo State of Western Nigeria and tagged as samples A to V, respectively. The oils were estimated for retinol and various antioxidants like; phenol, tocopherol, ascorbic acid and iron reducers using standard methods. The study showed no significant ($p>0.05$) difference in the concentrations of the retinal, tocopherol and ascorbic acid present in the different oil brands but with significance ($p<0.05$) differences in the concentration of tannic acid and reducing properties of the oils. For instance, the Sample B, F and N had the highest (857.8 USP/g) while sample I had the lowest (816.6 USP/g) concentrations of retinol, respectively. However, the level of added retinols and other antioxidants were within the limits approved by the laws and controls of the Regulatory bodies (NAFDAC and SON) for the imported but edible vegetable oils. The report of this present analytic survey showed the degree of compliance stipulated by the regulatory bodies by the importers and local producers, (in the areas of the added retinol and antioxidant concentrations) in these imported vegetable oils ranges.

Keywords: *Antioxidants, retinol, tocopherol, ascorbic acid, carotene*

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1. Introduction

Edible oil is vegetable oil derived purely from plants sources, which may be high, low or moderate in saturated, mono-unsaturated and polyunsaturated fatty acids and therefore may be liquid or congeal at temperature of <26 °C. However, these characteristics depend on the type of oils and processing technique being employed during their productions (Feskanish *et al.*, 2002). Fats and oils are lipids of nutritional importance and serve as a source of essential fatty acids. Essential fatty acids are necessary for growth and normal skin condition. Fat and oils aids in the transportation of the fat-soluble vitamins A, D, B, and K, and help the body to absorb them. They improved the palatable and flavor of food and baked products (Champe, 1986). The demand for edible vegetable oils has globally increased due to population growth, rising standards of living as well as consumer preference, arising partly from health consideration (WHO, 2022). Palm oil is a natural food being consumed for more than five thousand years. It is produced from the fruit of the oil palm *Elais guineensis*, which originated in the tropical rain forest region of West Africa. Malaysia is the largest producer and exporter of palm oil in the world (Champe and Harvey, 1994). Palm oil's contribution to the world's edible oil supply is likely to continue to increase because it is relatively cheap to produce and the yield per hectare far exceeds that of any other perennial or annual oil producing plant (Bergerk, 1996). Palm oil plays a critical role in producing nutrition worldwide, it is used as a cooking oil, shortening for making margarine, ice cream and also incorporated as a component into numerous fat blends and wide variety of food product. They are also used in the manufacture of grasses, soap, candle, lubricants chemical, cleaning products and detergents (Kirk and Sawywer, 1999).

The groundnut or peanut, Arachis hypogea belongs to the family Leguminosae. It is grown as an annual crop in tropical and subtropical regions and the warmer areas of temperate regions of the world, principally for its edible oil and protein rich kernels or seeds. Groundnut oil is pressed and extracted from the seeds of the plant. Groundnut oil is used as cooking oil for production of margarine, shortening in pastries and bread. It is valuable as a lubricant and emulsion for insecticides. It is used as an excipient for the delivery of fat-

soluble drugs in medicine and pharmaceuticals. It can be used as a message oil and ointment base in hydrogenated form (Feskanish *et al.*, 2002). Another edible oil is also obtained from *Guna* melon (*Colocynthis citrullus*) from the family *curcurbitacae*. It is one of the many varieties and the smallest melon commonly called *egusi* in the southem and northern part of Nigeria. It is sold all over the country. Melon seed oil constitutes a potential source of nutrient especially essential fatty acids in human nutrition. Vegetable oils are inherently in fatty acid composition. They are devoid of the fibre and other components of the seeds or kernels from which they are derived (Badifu and Abbah, 1991). In the literature, there is limited information on the lipid composition of some of these edible vegetable oil produced in Nigeria. Since palm oil, ground nut, soya oil and melon seed oils are widely and commonly consumed edible vegetable oils among Nigerian populace, it is imperative to ascertain the lipid composition of these edible oils produced are consumed in Nigeria and the information obtained from the study will be useful to both the producer and consumers of these edible vegetable oils.

This research work thus aimed to determine the levels of retinol in various vegetable oils consumed in Ekiti State and its environment, estimate the level of added antioxidants in these vegetable oils and ascertain whether the processor comply with regulation prescribe by regulatory bodies.

2. Materials and Methods

2.1 Materials

Twenty-two different brands of vegetable oils were bought from different market stalls in Ado Ekiti, Nigeria as shown in Table 1.

2.2 Determination of retinol (vitamin A) in oil samples

The vitamin A is extracted from the oil sample and then purified by saponification and then dissolved in optically pure isopropanol, hexane or cyclopean the method is based on the measurement of light absorption of the vitamin solution. The light absorption is proportional to the concentration of the vitamin at a wavelength of 325 nm where maximum absorption occurred (Mahindru, 2000). **The Carr-price** method was used as described by Mahindru (2000). The oil sample to be assayed was saponified with ethanol KOH and vitamin A was extracted with petroleum pet ether. The extracted vitamin was reacted with antimony trichloride (SbCl3), this resulted in a blue-coloured solution which was then measured in a colorimeter at 6l0-620 nm and the calculation of vitamin A content was carried out with reference to a calibration curve. The sample was made peroxide free by adding wet zinc foil approximately 80cm^2/litre, cut in strips long enough to reach at least half way up the container. In which the zinc strips have been previously immersed in diluted. CuS04 solution for one minute and subsequently washed with water. **T**he chloroform is washed thrice with fresh 10% aqueous solution of $Na_2S_20_3$ in a separating funnel and this was dried with $Cacl_2$ then filtered and distillate Over anhydrous $Na_2S_20_3$ in all glass apparatus with a fractionating Column of 70°m- only the middle fraction of the distillate was collected while as well as the last 10% of the distillate was discarded. Antimony trichloride (sbcl3) solution: this is prepared by dissolving 113.4g into 350ml trichloro-methane. $CHCI_3$.

Saponification (using 5 ml of the sample) was accurately weighed and then transferred quantitatively into saponification - flask (150 ml). 30 ml of ethanol (95%, v/v) and 5 ml of KOH (50%, w/v) was added and the mixture was kept in dark corner. A stream of nitrogen gas was passed through solution with a jet tube for 5 min to replace air. The content was saponified under reflux over an electrically heated water bath for 35 min. After saponification the contents were cooled, then 30 ml water was added and this was transferred qualitatively to a conical, 250 ml capacity separating funnel. The saponified solution was extracted thrice with 50, 30 and 20 ml of pet ether, respectively. These pet ether extracts were combined in another separating funnel and washed with 50 ml of ice cold water containing a little $NaHSO_4$ which just enough to neutralize the alkalinity of the extract, these washings were repeated with only ice cold water, till the washings were neutral to litmus The pet ether extract was transferred to a stopper measuring cylinder (l00 ml), then the volume was made up to 100 ml with more pet ether while 15 g of Na_2SO_4 granular in form was added, the contents mildly shaken and then left to settle in dark in a cool place.

A little quantity of the saponified oil was accurately weighed i.e. the residue obtained after evaporating the pet ether under moderate heat and reduced pressure. The residue was dissolved in a definite volume of chloroform that is, 2 ml of then chloroform solution with a fixed volume of the $SbCl_3$ solution would give an absorbance of about 0.5 to 0.2. The Jenway UV-Visible spectrophotometer (Jenway Technologies Inc., London, UK) was set at 100% transmittance with 2 ml $CHCI_3$ with fixed volume of $SbcI_3$ solution used as blank. The colorimetric reading was recorded. Vitamin A content was determined from the standard from the standard curve and units of vitamin A/ 100 g of the sample were calculated.

C = (g/100ml) of hexane (Amount of assay sample); L = Length of light path in absorption cell; W = Weight of the sample in grams

$$\text{Vitamin A (USP units/g)} = \frac{Abs\ x\ dilution\ factor\ x\ 18.30}{L\ x\ W}$$

Preparation of Calibration Curve

A suitable aliquot of the pet ether solution of the unsaponifiable extract was evaporated to about 5ml and the remaining Pet ether was evaporated off at low heat under reduced pressure. The residue was shaken up in sufficient trichloro methane so that after addition of antimony chloride ($SbCI_3$) solution, an absorbance of about 0.8 in photoelectric colorimeter was obtained from this stock solution of the standard, a series of dilutions in trichloromethane ($CHCI_3$) were made to give absorbance valves of 80, 60, 40 and 20% of the original absorbance. Absorbance of the blue colour formed when 1 ml aliquot of each of these five solutions plus l ml of $CHC1_3$ with a drop of acetic anhydride was treated with the volume of antimony chloride $CHCI_3$ solution. The blank was adjusted to 100% transmittance using a tube containing 2 ml of $CHC1_3$ and a fixed volume of the $SbC1_3$ solution. Using a rectangular coordinate paper, the five absorbents obtained

against known quantities of vitamin A are plotted and the best smooth curve from the origin through those points is drawn.

2.3 Determination of total phenolic content

Folin-ciocalteu reagent was used as described by (Hatch, 1995). Briefly, the **a**liquots of the samples (200 ml) was oxidized with 1.0 ml of folin coicalteu reagent and then neutralized by 0.8 ml of sodium carbonate (7.5%). The solution was then incubated at 300 ºC for 90 min. Then the sample was place in spectrometer and measured at 765nm.

2.4 Determination of Ascorbic Acid (Vitamin C)

Ascorbic acid reduces the 2,6 diclorophenol dye to a colourless leno base and the ascorbic acid in the oil sample get oxidized to dehydroascorbic acid (AOAC, 2000). Oxalic acid 4% (4 g oxalic acid dissolve in distilled water and make up to l00 ml). Dye solution: weighing 42 mg of $NaHCO_3$ into a small volume of distilled water, then 52 mg of 2.6 dichlorophenol was dissolved in it and make it up to 200 ml. 100 ml of the sample was dissolved in 100 ml of 4% oxalic solution in a standard flask (1 ml/ml). 25 ml of the sample was pipetted into 100 ml conical flask. 10 ml of 4% oxalic acid was added and then titrated against the dye Solution. The appearance of pink colour which persisted for a few minutes signalled the end-point. The amount of the dye consumed is equivalent to the amount of ascorbic acid.

2.5 Determination of reducing properties

The reducing properties of the samples were determined by assessing the ability of the sample to reduce iron (ii) chloride solution as described by (AOAC, 2000). 2.5 ml of the sample was mixed with 2.5 ml sodium phosphate buffer (pH 6.6) and 2 m1 of 1% potassium ferro cyanide. The mixture was incubated at 50 °C for 20 min, after incubation 2.5 ml, 10% trichloroacetic acid and then centrifuge at 650 *x g* rpm for 10 min. Then 5 ml of the supernatant was mixed with equal volume of water and l ml of 0.1% ferric chloride and the absorbance measured at 700 nm. A higher absorbance indicates a higher reducing power.

2.6 Determination of tocopherol in oil sample

Vitamin E activity in food is due to various tocopherols and its determination was done using the previously described method of (AOAC, 2000). 5 ml of the oil sample was transferred into 100 ml flask fitted with a reflux condenser. 10 ml absolute alcohol and 20 ml alcoholic sulphuric acid was added. The condenser and the flask were wrapped in aluminium foil. This was refluxed for 45 min and then cooled 50 ml water was added and the mixture transferred into a separating funnel covered with aluminium foil. 50 ml extra distilled water was added and the unsaponifiable matter was extracted using 30 ml diethyl pet ether. Then the combined pet ether was washed with distilled water in order to make it free from acid and then dried over anlydrous sodium sulphate. The extract was evaporated at a low temperature. The flask containing the extract was placed on a water bath 90 °C for exactly 3 min. The absorbance of 5l5 nm was measured against a blank containing 5 ml absolute alcohol and 1 ml concentrated nitric acid.

2.7 Statistical Analysis

All determinations were carried out in triplicates. Data were subjected to analysis of variance (ANOVA) using SPSS (version 21, USA), while means was separated using New Duncan Multiple Range Test (NDMRT) at 5% level of significance ($p<0.05$).

3. Results and Discussion

The result of the various retinol and antioxidant are shown in Tables 1 to 4. From the result obtained it was revealed that, sample B and N has 857.8 v.s p/g at 325 nm concentration of vitamin A, which were shown through their highest concentration of retinol while sample I has the lowest concentration of retinol with 816.6 USP (Table 1). Most of the oils partially but not fully complied with the regulations from regulatory body on fortification. The fortification rule stated that oil should not be manufactured, packaged, imported, exported, advertised, distributed or sold as specified in the first schedule of the regulation unless it is fortified with Vitamin A to a level not below 2000 I.U/kg (NAFDAC, 2019).

The determination of tocophenol at 515 nm revealed that the sample E has the highest concentration of vitamin E with 4.76 mg/100 g while B, F, K, P, S and V had the lowest concentrations (3.57 mg/100 g) as shown in the Table 1.
The ascorbic acid determined revealed that sample E has the highest concentration with 3.90 mg/100 g while sample F, G and I had the lowest concentrations with 2.75 mg/100 g as shown in Table 2, respectively.

Moreso, various antioxidants were estimated and the result obtained showed that sample K, L and P had the highest concentration of phenols with 0.512 mg/ml while sample E has the lowest concentration of 0.3 mg/ml at 700 nm as shown in Table 3.

Interestingly, the results presented in Table 4 revealed that the samples A, B, K, L, M, P and Q all had the same Fe^{2+} reducing properties (0.09%), respectively. Finally, the analysis of the variance further showed the concentrations of retinol, tocopherol, ascorbic acid, total phenol and iron reducing properties varied from one oil product to the other after adding these vitamins and antioxidants.

4. Conclusion

The retinol and antioxidant concentrations of some of these oil samples were within the stipulated limits recommended by the Codex Alimentarus and other international and national regulatory bodies. It can then be concluded that these oil samples showed some degrees of compliance to the regulatory bodies' laws and regulations, stability quality and resistance to oxidation. Therefore, the consumption of some of these oils posed no threat to health or as carcinogenic agents nor heart problem. However, people should be aware of the nutritional contents of the oils they consume in order to avoid high loads of cholesterol, which could cause high blood pressure and invariably leading to hypertension, stroke and death.

Table 1: Fat-soluble vitamins contents

Sample	Tocopherol (mg/100 g)	Retinol (U.SP/G)
A	4.16[a]	834.90[a]
B	3.57 [b]	857.90[a]
C	3.86[b]	830.90 [a]
D	4.46[a]	834.90 [a]
E	4.76 [a]	846.40 [a]
F	3.57 [b]	857.90 [a]
G	4.17 [a]	834.90 [a]
H	3.86 [b]	829.23 [a]
I	4.46 [a]	816.60 [a]
J	4.46 [a]	840.53 [a]
K	3.57 [b]	830.43 [a]
L	4.17 [a]	840.53 [a]
M	3.86 [b]	830.43 [a]
N	3.86 [b]	857.80 [a]
O	4.17 [a]	835.00 [a]
P	3.57 [b]	829.73 [a]
Q	3.57 [b]	815.23 [a]
R	4.17 [a]	834.90 [a]
S	3.57 [b]	930.43 [a]
T	3.86 [b]	829.46 [a]
U	4.17 [a]	852.63 [a]
V	3.57 [b]	828.53 [a]

Values followed by different superscript along the same row are significantly different (P<0.05)

Table 2: Ascorbic acid or Vitamin C contents

Sample	Vitamin C (mg/100 g)
A	3.50[a]
B	3.70 [a]
C	3.30 [a]
D	3.31 [a]
E	3.90 [a]
F	2.75 [b]
G	2.75 [b]
H	3.21 [a]
I	2.75 [b]
J	3.44 [a]
K	3.67 [a]
L	3.50 [a]
M	3.31 [a]
N	2.65 [bc]
O	3.24 [ab]
P	2.25 [bc]
Q	3.44 [a]
R	3.44 [a]
S	3.57 [a]
T	3.60 [a]
U	3.25 [ab]
V	3.31 [a]

Values followed by different superscript along the same row are significantly different (P<0.05)

Table 3: Total phenol contents

Sample	Total Phenol (mg/ml)
A	0.51 [a]
B	0.48 [a]
C	0.46 [a]
D	0.49 [a]
E	0.39 [b]
F	0.47 [ab]
G	0.46 [ab]
H	0.47 [ab]
I	0.46[ab]
J	0.47 [ab]
K	0.51[a]
L	0.51[a]
M	0.49[a]
N	0.50 [a]
O	0.51 [a]
P	0.51 [a]
Q	0.49 [a]
R	0.49 [a]
S	0.48 [a]
T	0.51 [a]
U	0.46 [a]
V	0.47 [a]

Values followed by different superscript along the same row are significantly different (P<0.05)

Table 4: Reducing properties

Sample	Fe^{2+} Reducing Properties (%)
A	0.09 [e]
B	0.09 [e]
C	0.17 [e]
D	0.10 [e]
E	0.24 [b]
F	0.17 [cd]
G	0.20[bc]
H	0.17 [cd]
I	0.21[b]
J	0.15[c]
K	0.09 [d]
L	0.09 [d]
M	0.09 [d]
N	0.89 [a]
O	0.89 [a]
P	0.09 [d]
Q	0.09 [d]
R	0.10 [d]
S	0.15 [c]
T	0.10 [d]
U	0.20 [b]
V	0.16 [c]

Values followed by different superscript along the same row are significantly different (P<0.05)

Declaration

Author Contributions

Author ADO performed the experiment, collected and analyzed the data, prepared the draft of the manuscript as well as serving as the Corresponding Author. Author OOO performed the experiment and analyzed the data. Author SAM supervised the study and edited the manuscript.

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Data Availability Statement
Data are available upon request by contacting the authors.

Conflicts of Interest
The authors declare that they do not have any conflict of interest.

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