

A Comparative Study of α -Tocopherol as an Antioxidant Factor Extracted from some common Nigerian Seed Oil

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ABSTRACT

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols which are fat soluble vitamins with antioxidants properties. This study evaluated the concentration of tocopherol in onion oil, benne seed oil and palm kernel oil. Oil from the seed samples were extracted using soxhlet apparatus with n- hexane and ethanol as solvents used. The oils were further analyzed using U.V spectrometer. All the oils analyzed contained various levels of tocopherol, with benne seed oil having the highest with concentration of 9.60 μ g/ml, palm kernel oil with concentration of 8.83 μ g/ml and onion oil with concentration of 6.41 μ g/ml. Since all the oils analyzed contained substantial amount of α tocopherol, they should be consumed more and conclusively, consuming the oil will help in protecting against free radicals which has been the underlying mechanism of threatening human diseases.

INTRODUCTION

Therapeutic use of antioxidants has gained considerable interest during the last decade. Vitamin E has received considerable attention in the treatment and/or prevention of human diseases, such as, atherosclerosis, preeclampsia or hypertension (Rodrigo, Guichard, & Charles, (2007)). The most active component of Vitamin E is α

tocopherol. This organic substance is one of the most powerful antioxidants in the lipid phase of the human body (Wang & Quinn, 2000). It protects against free radical damage which has been the underlying mechanism of injury in a number of life threatening human diseases, such as atherosclerosis, diabetes, cancer and aging (Bonney, Draai & Kostka, 2002; Huang *et al.*, 2006;

Flora, 2007; Munteanu & Zingg, 2007). Antioxidants are compounds which destroy the free radicals in the body, thereby preventing against harmful oxidation-reduction reactions. The best sources are fruits and vegetables which provide a variety of antioxidants like Vit. A, C, E & carotenoids. Currently available data are compatible with the notion that these vitamins act as chemopreventives against some important cancers, e.g. carotenoids for lung cancer, ascorbic acid for salivary gland cancer, tocopherols for head and neck cancers etc. Thus, a greater consumption of fruits and vegetables should be encouraged as they are the natural sources of these chemopreventive antioxidants (Nimmi, Rajarshi, Deepak, Mamta, and Devika. 2013).

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α -tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form (Herrera & Barbas, 2001). It has been claimed that the α -tocopherol form is the most important lipid-soluble antioxidant and that it protects

membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α -tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol. This is in line with findings showing that α -tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase (GPX4)-deficient cells from cell death. GPX4 is the only known enzyme that efficiently reduces lipid-hydro peroxides within biological membranes (Herrera & Barbas 2001). Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamin C, E, A and other substances (Sies, 1997).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical

intermediates and inhibit other oxidation reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). The term lipid embraces a variety of naturally occurring compounds which have in common the property of being soluble in organic solvents, and sparingly soluble in water. All lipids yield monocarboxylic acids (saturated and unsaturated) on hydrolysis. Lipids are classified as Simple lipids, Compound lipids and Derived lipids. Simple lipids are made up of fats and oil. Fat is solid at room temperature while oil is liquid at room temperature. Fats are the most concentrated source of diet yielding 9.3kcal per gram as compared to 4.1kcal per gram of protein and carbohydrate. It plays a great role in safety value of food, that is, they give feeling of satisfaction and delay on the onset of hunger. It is because fats retard the rate at which food leaves the stomach. They belong to the group of esters which results from the reaction of alcohol (glycerol) that is three hydroxyl groups of the glycerol molecule and the resulting ester is called triglyceride. Oil is a water insoluble substance which consists of predominantly of long chain acid ester from simple alcohol glycerol. Oil is a mixture of the triglyceride that is liquid at room

temperature. Oils are classified according to their ability to absorb oxygen from the atmosphere. Oils are generally classified into three main groups, namely: Nondrying oils, semi-drying oils and drying oils. Nondrying oil remains liquid at room temperature and is incapable of forming elastic films even after long exposure to air as they do not interact with atmospheric oxygen. They are largely glycerides of saturated acids and oleic acid with little or no linoleic acids. Examples are groundnut, palm, olive, castor, and grape. Semi-drying oils are intermediate between drying and non-drying oils and are characteristics in having large amounts of linoleic and saturated acids but no linolenic acid. They absorb atmospheric oxygen slowly, producing only a soft film after prolonged exposures to air. Examples are cotton seed, sesame, corn, sunflowers. Drying oils are fairly rich in glycerides of the unsaturated fatty acids, particularly linoleic and linolenic and a few of oleic compounds. Such oils readily absorb oxygen on exposure to air and form a tough, elastic but resistant. Examples include soybean, safflower and hempseed.

Due to the current trend in the consumption of oils containing

non-essential fatty acids, there is an increase in the oxidation of low density lipoprotein LDL (bad cholesterol), which leads to atherosclerosis and finally leading to the formation of cardiovascular disease. This project aims at determining the concentration of α tocopherol in onion oil, benne seed oil and palm kernel oil.

Dietary Sources of Vitamin E

Vegetable oils are rich sources of Vitamin E; e.g wheat germ oil, sunflower oil, safflower oil, cotton seed oil. The actual vitamin E content of a food depends on harvesting, processing, storage and cooking because vitamin E is highly susceptible to destruction by oxygen metals, light and deep frying (Wardlaw & Kessel, 1996).

Recommended Daily Allowance

1. Males 10mg per day
2. Females 8mg per day
3. Pregnancy 10mg per day
4. Lactation 12mg per day
5. 15mg of Vitamin E is equivalent to 33 international units. The requirement increases with higher intake of PUFA. Pharmacological dose is 200-400 IU per day.

ANTIOXIDANTS

It involves the mechanism of action of antioxidants. Two

principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system, example lipid radicals. The second mechanism involves removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst.

Composition of Onion

Most onion cultivars are about 89% water, 4% sugar, 1% protein, 2% fibre, and 0.1% fat. Onions contain low amounts of essential nutrients, are low in fats, and have an energy value of 166 kJ (40 kcal) per 100 g (3.5 oz). They contribute their flavor to savory dishes without raising caloric content appreciably. Onions contain phytochemical compounds such as phenolics that are under basic research to determine their possible properties in humans (Yang, 2004). Considerable differences exist between onion varieties in polyphenol content, with shallots having the highest level, six times the amount found in Vidalia onions, the variety with the smallest amount. Yellow onions have the highest total flavonoid content, an amount 11

times higher than in white onions. Red onions have considerable content of anthocyanin pigments, with at least 25 different compounds identified representing 10% of total flavonoid content (Slimmorsen, Fossen & Vagan, 2007).

Benne Seed Oil

Sesame (*Sesamum indicum*) is a flowering plant in the genus *Sesamum*, also called *benne*. Numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods or "buns". The world harvested 4.2 million metric tonnes of sesame seeds in 2013, with India and China as the largest producers. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. Sesame has many species, most being wild and native to sub-Saharan Africa. *Sesame indicum*, the cultivated type, originated in India and is tolerant to drought-like conditions, growing where other crops fail.

Sesame has one of the highest oil contents of any seed. With a rich, nutty flavor, it is a common ingredient in cuisines across the

world. Like other nuts and foods, it can trigger allergic reactions in some people.

Composition of Benne Seed

For a 100-gram serving, dried whole sesame seeds are rich in calories (573 kcal) and are composed of 5% water, 23% carbohydrates, 12% dietary fiber, 50% fat and 18% protein. The flour that remains after oil extraction from sesame seeds is 35-50% protein and contains carbohydrates. This flour, also called sesame meal, is a high-protein feed for poultry and livestock (Ray, 2011).

Soybean Oil

Soybean is the dominant oilseed produced in the world, due to its favorable agronomic characteristics, its high-quality proteins, and its valuable edible oil. It contributes over a half of all oilseeds produced worldwide. The production of soybeans and soybeans oil is driven by the need for soy protein meal, which is used extensively in commercial feeds for poultry, swine and cattle. Soybean oil accounted for 80-90% of total edible oil consumption in the US (USDA-NASS) in 1998 because of its availability and its many desirable characteristics, including compositional and functional properties. Soybean oil

is the predominant vegetable oil produced in the world, with palm oil being the second. Mature soybeans are oval shaped and their sizes are variety-dependent. The seed consists of three major parts: Seed coat or hull, cotyledon, and germ or hypocotyls.

Palm Kernel Oil

Palm Kernel Oil (PKO) is the smaller of the two lauric oils among the 17 major oils and fats in world production, coconut oil (CNO) being bigger by about 20% (Oil World, 2001). The two oils have very similar fatty acid composition and both are derived from the fruit of palm trees, but they belong to different species. The coconut palm is *Cocosnucifera* while the oil palm, which gives both palm oil (PO) and PKO is *Elaeisguineensis* (Hartley, 1988). This tree is generally believed to have originated in the humid jungle forests of East Africa and there is some evidence that palm oil derived from the flesh of the fruit may have been consumed in Egypt at the time of the Pharaohs, some 5000 years ago.

The variety cultivated in nearly all the world's plantations is the hybrid *Tenera*, which is the cross between the varieties *Dura* and *Pisifera* and gives the highest yield of oil per hectare of any crop. The

economic efficiency of the oil palm is easily seen from the following simple calculation. The palm fruit is oval shaped, about 3cm long and looks like a small red plum. The outer fleshy mesocarp gives the PO while the kernel, which is inside a hard shell, gives the PKO. The two oils from the same fruit are entirely different in fatty acid composition. Unfortunately, these oils have often been confused and this has led to some grossly erroneous statements. The palm fruit is formed in large clusters of 15-25kg each, called fresh fruit bunches (FFB), which grow and mature on the tree progressively, so that harvesting is done throughout the year, every 10-14 days. The average yield from an FFB, is about 19% PO and 5.5% kernels (PK) (Malaysia 2000 average) and are called the oil extraction ratio (OER) and kernel extraction ratio (KER) respectively. They are very important parameters in plantation economics. In commercial crushing's, the ratio of PO/PKO obtained from the fruit is about 8:1-a useful fact for oil traders to remember when they try to estimate production from incomplete data.

MATERIALS AND METHOD

Sample Collection

Vegetable oil seeds; palm kernel, onion and benne seed were procured from Bukuru market of Jos South Local Government Area of Plateau State. The seeds were stored in plastic bags at 4°C on the day before use.

Solvent Extraction

When dealing with relatively low oil content materials, extraction with a solvent normally hexane is the most efficient for oil recovery. Oil produced by this technique is of high quality, because very little heat treatment is required. This extraction is performed over a long period of time in a soxhlet continues extraction apparatus.

Determination of α -Tocopherol

Preparation of Standard Curve

0.2% of α tocopherol in absolute ethanol was prepared and kept at 4°C. For working solution, 0.5ml of the stock solution of alpha tocopherol was diluted with purified ethanol to obtain a concentration of 20 μ g/ml. This working solution was used to

construct a standard curve ranging from (1 μ g/ml-4 μ g/ml).

The determination was carried out using a modification of the method by (Fabianek, Defillipi, Rickards & Herp, 1968). A standard curve was obtained by plotting absorbance vs. amount of tocopherol. The level of α tocopherol in the test (unknown) sample was obtained from this standard curve.

RESULTS AND DISCUSSION

A standard curve was prepared using the standard solution of α tocopherol ranging from (1 μ g/ml-4 μ g/ml). A typical dose response curve was obtained by plotting the amount of α tocopherol (in μ g/ml) vs. the absorbance at 536nm. The curve is linear and this procedure is sensible such that as low as 0.2 μ g of α tocopherol can be easily detected.

Table 1: Standard Concentration and Absorbance

Results of the measurement of absorbance at 536nm using microprocessor visible spectrophotometer AVI-722 are shown below.

S/No	Standard concentration in (μ g/ml)	Absorbance at 536nm
1.	1	0.436
2.	2	0.558
3.	3	0.768
4.	4	1.026

Table 2: Concentration of Tocopherol in the Samples and Absorbance

Measurements of sample concentration in $\mu\text{g/ml}$ are indicated below:

S/No	Sample(s)	Absorbance	Concentration in ($\mu\text{g/ml}$)
1.	Onion oil	1.70	6.41
2.	Benne seed oil	2.542	9.60
3.	Palm kernel oil	2.340	8.83

DISCUSSION

The assay is based on the principle that α tocopherol being a reducing agent can convert ferric iron into ferrous iron which can then be coupled with a chelating agent, such as 1, 10-phenanthroline to produce a pink ferrous 1, 10-phenanthroline colored complex. The absorbance of this colored complex at 536nm is proportional to the concentration of α tocopherol in the reaction. The assay is specific for α tocopherol. Addition of orthophosphoric acid in the assay reduces the interference of carotene (Fabianek *et al.*, 1968). From the linear curve plotted, the concentration of the test sample was obtained from the standard curve. Onion oil at 536nm, it mean absorbance was 1.70, the concentration was $6.41\mu\text{g/ml}$, benne seed oil at 536nm, it mean absorbance was 2.542, the concentration was $9.60\mu\text{g/ml}$ and palm kernel oil at 536nm, it mean absorbance was 2.340; the concentration was $8.83\mu\text{g/ml}$. From the results, it implies that benne seed oil has the

higher concentration of tocopherol. Benne seed is a reservoir of nutritional components with numerous beneficial effects along with health promotion in humans. The bioactive components present in the seed include vital minerals, vitamins, phytosterols, polyunsaturated fatty acids, tocopherols and unique class of lignans such as sesamin and sesamolin. The presence of phenylpropanoid compounds namely lignans along with tocopherols and phytosterols provide defense mechanism against reactive oxygen species and increases keeping quality of oil by preventing oxidative rancidity. In consequence, value addition efforts in sesame would enable development of genotypes with high antioxidant activity and subsequently prevention of free radical related diseases. Sesame seed contains 50-60% of high quality oil which is rich in polyunsaturated fatty acids (PUFA) and natural antioxidants, sesamin, sesamolin and tocopherol

homologues (Brar & Ahuja 1979). Recent studies on the antioxidant and anti-carcinogenic activities of sesame seed have greatly increased its applications in health food products that assert for liver and heart protection and tumor prevention (Cheng, Jinn, Hou & Tzen 2006). Tocopherols are a class of plant phenolics that have important antioxidant and nutritional properties (FASEB, 1999). All the analyzed oils contain α tocopherol being natural antioxidants, they inhibit oil oxidation. They act as biological kidnappers of free radicals and could prevent diseases, besides possessing an important nutritional function for human beings as a source of vitamin E (Brigelius and Traber, 1999).

CONCLUSION

This study showed that all the samples contained substantial amount of α tocopherol with benne seed oil containing the highest amount. This implies that oils are major source of vitamin E which is a natural source of antioxidant. Since the major function of vitamin E is to protect PUFA and other component of cell membrane and low density lipoprotein from oxidation by free radicals, therefore damage to the cell membrane is one of the major pathologies associated with

vitamin E deficiency (Vitamin and mineral requirement in human nutrition, 2004). These oils are rich sources of polyunsaturated fatty acid that possess health benefits such as regulating blood cholesterol levels and lowering elevated blood pressure (Eromosele *et al.*, 1997). All the vegetable oils have good health benefit that can protect individual's cardiovascular system.

RECOMMENDATION

The result showed that benne seed have the highest concentration of α tocopherol, but all the oils exhibit biological and antioxidant capability due to their composition of fatty acid, so they are all good for healthy consumption. I wish to recommend that people consume these oils.

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