Dietary Phospholipids and Phytosterols: A Review on Some Nigerian Vegetable Oils

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Abstract: Dietary phospholipids and phytosterols have proven to be potential sources of bioactive lipids with widespread effects on pathways related to inflammation, cholesterol metabolism, and high-density lipoprotein function. Due to their biological and physicochemical properties, they are important in human nutrition. The efficient separation and accurate quantification of phospholipids and phytosterols can be achieved with high-performance liquid chromatography–evaporative light scattering detection (HPLC-ELSD) and gas chromatography (GC) often combined with mass spectrometry respectively. The phospholipid and phytosterol compositions of some Nigerian vegetable oils were reviewed. From the literature, the phospholipid concentrations (mg/100g) of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, lysophosphatidyl choline, phosphatidyl inositol and phosphatic acid are in the range of 2.60 – 1168.00, 1.13 – 558.00, 0.10 – 336.00, 0.72 – 596.00, 1.01 – 611.00 and 9.24 – 94.06, respectively. Total phospholipids range from 14.30 mg/100g in Ethiopian pepper to 2040.00 mg/100g in cooked groundnut. The values of cholesterol, cholestane, ergosterol, campesterol, stigmasterol, 5 – avenasterol and sitosterol range between 1.6e³ – 9.28, 4.80e⁶ – 2.28, 4.59e⁴ – 30.20, 9.39e³ – 103.00, 1.24 – 45.60, 5.62e³ – 53.50 and 17.21 – 351.00, respectively for the vegetable oils under review. Malaba spinach records the highest total phytosterols content (442.91 mg/100g), followed by big alligator pepper (369.34 mg/100g) while Ethiopian pepper contains the least total phytosterols of 23.82 mg/100g. The results of this reviewed work indicate that Nigerian vegetable oils have potentials in finding application either in food industry as emulsifiers, emulsion stabilizers or industrial purposes such as biomedical applications, cosmetics and even drug delivery.

Keywords: Phospholipids, phytosterols, vegetables, oils, Nigeria

Introduction

In developing countries like Nigeria, sub-optimal diet is the major leading risk factor for disability and death. Hunger and malnutrition cause enormous suffering among the disadvantaged populations in all nations especially developing countries. Consequently, diet-related cardiometabolic diseases such as coronary heart disease (CHD), stroke, type 2 diabetes mellitus, and obesity produce even larger global health burdens. Other vascular conditions including peripheral arterial disease, chronic kidney disease, cognitive decline, heart failure, and atrial fibrillation are also influenced by diet-related risk factors. Worldwide, chronic diseases will cause $17.3 trillion of cumulative economic loss between 2011 and 2030 from healthcare expenditures, reduced productivity, and lost capital [1]. Considering these health and economic burdens, diet-related illnesses are among the leading priorities of our time.

Atherosclerosis is a key contributor to cardiovascular disease (CVD) (coronary heart disease (CHD)) and is characterized by the hardening and thickening of the artery wall caused by accumulation of fatty plaque. Atherosclerosis is an insidious and progressive chronic inflammatory disease that takes decades to develop in humans [2]. Atherosclerosis is not only an inflammatory disease characterized by infiltration of immune cells, but also a lipid disorder; subendothelial accumulation of lipids derived from plasma lipoproteins is a key initiator of plaque development [3]. Lipoprotein metabolism is therefore critical to the development of atherosclerosis [4]. Lipoproteins have evolved to facilitate the extracellular transport of water-insoluble lipids in multicellular organisms [5]. Apolipoprotein B-containing lipoproteins that originate from the liver, such as very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), contribute to the CVD process.

In contrast, high-density lipoprotein (HDL) improves CVD through its ability to remove excess lipid from the artery and transport it back to the liver for...
excretion from the body, a pathway termed “reverse cholesterol transport” (RCT) [6]. The atheroprotective effect of HDL is mainly attributed to its role in RCT, with plasma HDL-cholesterol (HDL-C) considered to be a surrogate metric for this pathway [7]. The relationship between blood cholesterol and CVD is well-established, with the lowering of LDL-cholesterol (LDL-C) levels being the primary target of preventive therapy [8]. There has also been considerable interest in studying the relationship between dietary cholesterol intake and CVD risk [9]. Dietary phospholipids and phytosterols have emerged as a potential source of bioactive lipids that may have widespread effects on pathways related to inflammation, cholesterol metabolism, and HDL function.

Phospholipids (PLs) are complex lipids which contain one or more phosphate groups. Phospholipids are amphipathic in nature that is each molecule consists of a hydrophilic portion and a hydrophobic portion thus tending to form lipid bilayers. In fact, they are the major structural constituents of all biological membranes, although they may be also involved in other functions such as signal transduction. There are two classes of phospholipids, those that have a glycerol backbone and those that contain sphingosine. Phospholipids that contain glycerol backbone are called as glycerophospholipids, which are the most abundant class found in nature. The most abundant types of naturally occurring glycerol phospholipids are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI), phosphatidyl glycerol (PG) and cardiolipin (CL). The structural diversity within each type of phosphoglyceride is due to the variability of the head group, variability of the chain length and degree of saturation of the fatty acid ester groups.

Vegetable oils (such as cotton seed, soybean, peanut, palm, corn, sunflower and rapeseed) and animal tissues (such as bovine brain and egg yolk) are the main sources of phospholipids. But in terms of production, soybean and egg yolk are the most important sources for phospholipids [10]. As important components of the cell membranes of living species that perform multiple roles in cell processes and life activities, the main function of phospholipids is defining the permeability barrier of cells and organelles by forming a phospholipid bilayer. This bilayer serves as the matrix and support for a vast array of proteins involved in important functions of the cell such as energy transduction, signal transduction, solute transport, DNA replication, protein targeting and trafficking, cell-cell recognition, secretion, cardiovascular health, nerve health, liver function, and digestion. During the digestive process, phospholipids form clusters to help move vitamins, nutrients and fat-containing molecules through the body. Phospholipids are active participants that influence the properties of the proteins associated with the membrane and serve as precursors to important cellular components. Since nutritional adequacy of dietary components depends on both their amount and bioavailability in the diet, then insufficient dietary intake or excessive loss of macro- and micronutrients will strongly affect the phospholipids of cellular membranes in all living organisms, leading to pathophysiological situations of clinical importance in human health [11, 12, 13].

Phytosterols (plant sterols and stanols) are naturally occurring compounds found in all food of plants origin that include sitosterol and campesterol, and their saturated counterparts sitostanol and campestanol. They resemble cholesterol both in structure and biological functions. The most biologically relevant phytosterols are sitosterol, campesterol, stigmasterol and brassicasterol. Sitostanol and campestanol, the major plant stanols, are 5, 6-saturated analogues of sitosterol and campesterol [14].

Phytosterols are found in the lipid-rich and fibre-rich fractions of all plant foods. In particular, vegetable oils and products made from oils like spreads and margarine are good sources of plant sterols [15]. Other foods which contribute to the daily intake of plant sterols are cereal grains, cereal based products, nuts, legumes, vegetables and fruits [16 – 17]. Plant stanols are also found in some foods, but at much lower concentrations. They are found in some cereals grains like rye, corn and wheat and in non-hydrogenated vegetable oils [15]. Plant stanols are also found in plant material from coniferous trees such as pine and spruce. Dietary intake of plant sterols ranges from 150 to 400 mg/day with 65% of intake as b-sitosterol, 30% as campesterol and 5% as stigmasterol [18 – 19]. The daily intake of plant stanols is in the magnitude of about 25 mg/day [20]. Phytosterols have been used for the last half-century because of their cholesterol-lowering properties. They have been shown in a vast number of human studies to be safe and effective in lowering plasma total and LDL-cholesterol concentrations. The underlying mechanisms of the cholesterol-lowering action of phytosterols relate to the inhibition of intestinal cholesterol absorption [14]. In addition to their well-established cholesterol-lowering effect, other potential health benefits of phytosterols include antioxidant and anti-inflammatory actions, benefits on the immune system and anticancer properties.

Regrettably, there exists a paucity of data presently on the concentrations or quantities of phospholipids and phytosterols in the Nigerian plant foods.
Therefore, the aim of this review work is to provide useful information on the potentiality of some Nigerian plant foods in providing phospholipids and phytosterols to serving as components of a healthy lifestyle, to reduce plasma low-density lipoprotein-cholesterol (LDL-C) levels, and thereby lower cardiovascular risk.

Methodology

**Determination of Phospholipids in Food Samples**

One of the problems in lipid analysis is the lack of a satisfactory detection method that enables the quantification of all of the major classes of lipids present in food matrices. On the other hand, the demand for fast and reliable quantitative screening methods of complex food matrix extracts has led to the development of more widely applicable detection strategies. With regard to this, the analysis of phospholipids has been performed by several different methods, including thin-layer chromatography (TLC) [21, 22, 23], high-performance liquid chromatography (HPLC), [24, 25, 26, 27] and solid-phase extraction (SPE) [28, 29, 30]. In earlier articles, the lipid quantification had been performed exclusively by TLC, but the method has several disadvantages; namely, the separation of individual classes is very difficult and time consuming, and the technique is not always accurate. Over the course of the past few decades, HPLC has become the preferred method for determining PLs, because a quantitative and qualitative analyses can readily be obtained at a relatively low cost.

In recent years, HPLC coupled to an evaporative light-scattering detector (ELSD) represented a useful alternative. The use of an ELSD approach for spectrophotometric derivatization (i.e., insertion of chromophoric groups) is feasible and therefore the drawbacks of derivatization (dependence on experimental parameters, incompleteness of derivatization reaction, use of salt-laden mobile phases, prolonged analysis time, additional cost for derivatization system and reagents) can be eliminated. In particular, the use of an ELSD for lipid analysis has become essential, because it is a universal detector that is compatible with a broad range of solvents and gradient elution (unlike the refractive index detector) and the signal is independent of the degree of saturation and chain length of an acyl chain (unlike the UV detector) [31, 32, 33, 34].

**Determination of Phytosterols in Food Samples**

Gas chromatography (GC) is the most common method for analyzing phytosterol composition in food matrices [35]. There are HPLC methods available for separating and quantifying the various forms of phytosterols, such as free sterols, steryl fatty acid esters, steryl glycosides, acylated steryl glucosides, and hydroxycinnamic acid esters of sterols [36, 37, 38]. However, with over 200 different structures, GC, often combined with mass spectrometry, is the best and most widely used tool for the chromatographic separation, identification, and quantification of phytosterols. There are several standardized methods for phytosterol analysis that are approved by organizations such as the Association of Official Analytical Chemists (AOAC) [39], American Oil Chemists’ Society (AOCS) [40], and the International Organization for Standardization (ISO) [41]. Most standardized methods are somewhat tedious in terms of the amount of glassware, sample, and solvent required and in the number of steps in the procedure, which make it difficult to analyze multiple samples at the same time. These procedures have been modified by users to require smaller sample sizes, less solvent, and to eliminate some of the time-consuming cleanup steps [42]. However, for the most part, the latest techniques employ the same basic steps that the traditional methods use: (1) Sample preparation (sample weighing, lipid extraction (optional), addition of internal standard, acid and/or alkaline hydrolysis, extraction of unsaponifiables, optional sample cleanup/further purification (2) Derivatization and (3) GC analysis.

**Overview of the Phospholipids Composition of Nigerian Vegetable Oils**

Recent researches have shown that phospholipids can have a positive nutritional effect on human health, such as reducing the risk of cardiovascular disease [43, 44, 45], reducing blood cholesterol levels [46], and enhancing brain function [47]. Moreover, their antioxidative properties [48], bacteriostatic properties [49], and the inhibitory effect of sphingolipids on colon cancer have been intensively studied [50].

The phospholipids composition of some Nigerian vegetable oils are presented in Table 1. The phosphatidylcholine (PC) value range between 2.60 in Ethiopian peppers to 1168.00 mg/100 g in cooked groundnut. PC is the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL [51]. PC’s role in the maintenance of cell-membrane integrity is vital to all of the basic biological processes which include: information flow that occurs within cells from DNA to RNA to proteins; the formation of cellular energy and intracellular communication or signal transduction. PC rich in polyunsaturated fatty acids has a marked fluidizing effect on cellular membranes [52, 53]. When membrane damages are present, PC is in high demand to help form, proliferate, restore, and regenerate cell membranes and to enhance membrane-dependent metabolism.
Phosphatidylethanolamine (PE) is a major phospholipid in nervous tissue such as the white matter of brain, neural tissue, nerves and in spinal cord. PE is the most abundant lipid on the cytoplasmic layer of cellular membranes, with significant roles in cellular processes such as membrane fusion [54], cell cycle [55], autophagy [56], and apoptosis [57]. The phosphatidylethanolamine ranges from 1.13 in small alligator peppers to 558.00 mg/100 g in malaba spinach. The phosphatidylserine (PS) of the reviewed plant foods is found to be in the range of 0.10 in African wild mango to 336.00 mg/100 g in malaba spinach. PS is the major acidic phospholipid class that accounts for 13–15 % of the phospholipids in the human cerebral cortex [58]. In the plasma membrane, PS is localized exclusively in the cytoplasmic leaflet where it forms part of protein docking sites necessary for the activation of several key signaling pathways. These include the Akt, protein kinase C (PKC) and Raf-1 signaling that is known to stimulate neuronal survival, neurite growth and synaptogenesis [59, 60, 61]. PS has been demonstrated to speed up recovery, prevent muscle soreness, improve well-being, and might possess ergogenic properties in athletes involved in cycling, weight training and endurance running. The US Food and Drug Administration (USFDA) had stated that consumption of PS may reduce the risk of dementia and cognitive dysfunction in elderly persons [62].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PE (mg/100 g)</th>
<th>PC (mg/100 g)</th>
<th>PS (mg/100 g)</th>
<th>LPC (mg/100 g)</th>
<th>PI (mg/100 g)</th>
<th>PA (mg/100 g)</th>
<th>Total (mg/100 g)</th>
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<td>214.53</td>
<td>94.06</td>
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<td>148.16</td>
<td>0.10</td>
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<td>10.61</td>
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<td>725.00</td>
<td>262.00</td>
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<td>75.70</td>
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<td>1168.00</td>
<td>275.00</td>
<td>235.00</td>
<td>110.00</td>
<td>-</td>
<td>2040.00</td>
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<td>5.13</td>
<td>32.82</td>
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<td>6.01</td>
<td>35.07</td>
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<td>31.17</td>
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<td>3.83</td>
<td>107.32</td>
<td>-</td>
<td>530.94</td>
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<td>195.26</td>
<td>18.18</td>
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<td>-</td>
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<td>Big Alligator pepper</td>
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<td>18.30</td>
<td>1.82</td>
<td>1.16</td>
<td>1.01</td>
<td>-</td>
<td>23.59</td>
<td>[68]</td>
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<td>Small Alligator pepper</td>
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<td>17.90</td>
<td>4.52</td>
<td>1.05</td>
<td>9.18</td>
<td>-</td>
<td>33.78</td>
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<td>Ethiopian peppers</td>
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<td>2.60</td>
<td>1.45</td>
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<td>7.99</td>
<td>-</td>
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<td>Ginger</td>
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<td>27.40</td>
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<td>1.04</td>
<td>-</td>
<td>36.68</td>
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<td>49.50</td>
<td>99.90</td>
<td>3.52</td>
<td>18.50</td>
<td>-</td>
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</tr>
<tr>
<td>Wonderful kola</td>
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<td>195.03</td>
<td>204.75</td>
<td>8.72</td>
<td>59.87</td>
<td>27.51</td>
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<tr>
<td>Steeped wheat</td>
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<td>79.90</td>
<td>321.00</td>
<td>1.45</td>
<td>611.00</td>
<td>-</td>
<td>1382.35</td>
<td>[71]</td>
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<td>Sprouted wheat</td>
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<td>65.10</td>
<td>302.00</td>
<td>1.89</td>
<td>604.00</td>
<td>-</td>
<td>1292.99</td>
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<td>Bridelia ferruginea</td>
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<td>79.80</td>
<td>0.91</td>
<td>5.19</td>
<td>6.93</td>
<td>-</td>
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<td>43.60</td>
<td>13.20</td>
<td>5.19</td>
<td>177.00</td>
<td>-</td>
<td>263.39</td>
<td>[69]</td>
</tr>
<tr>
<td>Luffa cylindrica</td>
<td>51.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[72]</td>
</tr>
<tr>
<td>Adenopus breviflorus</td>
<td>14.40</td>
<td>60.10</td>
<td>-</td>
<td>-</td>
<td>25.20</td>
<td>-</td>
<td>99.70</td>
<td>[72]</td>
</tr>
</tbody>
</table>

PE = Phosphatidylethanolamine, PC = Phosphatidycholine, PS = Phosphatidylserine, LPC = Lysophosphatidylcholine, PI = Phosphatidylinositol, PA = Phosphatic acid, - = Not Available

Lyso phosphatidylcholine (LPC) is present in cell membranes, oxidized lipoproteins, and atherosclerotic tissues. It has the ability to alter endothelial functions and is regarded as a causal agent in atherogenesis [73]. The lysophosphatidylcholine of the reviewed vegetable oils ranges from 0.72 in Indian spinach to 596.00 mg/100 g in raw groundnut. LPC is believed to play
an important role in atherosclerosis and inflammatory diseases by altering various functions in a number of cell-types, including endothelial cells, smooth muscle cells, monocytes, macrophages, and T-cells. It activates several second messengers and impairs the endothelium-dependent relaxations mediated by endothelium-derived relaxing factors and directly modulates contractile responses in vascular smooth muscle [74]. Phosphatidylinositol (PI) plays a key role in the membrane recruitment and/or activation of proteins [75]. In this way, PI provides a means for the temporal and spatial regulation of complex cellular processes [76]. From Table 1, the PI values range from 1.01 in big alligator peppers to 611.00 mg/100 g in raw wheat. PI is the primary precursor of the endocannabinoid 2-arachidonoylglycerol in neurons, and it is also an essential cofactor for phospholipase D and so affects the cellular production of phosphatidic acid with its specific signalling functions [77]. The phosphatidic acid (PA) value is in the range of 9.24 to 94.06 mg/100 g for wonderful kola and water melon, respectively. PA has emerged as a new class of lipid mediators involved in diverse cellular functions in plants, animals, and microorganisms. It mediates cellular functions through different modes of action, such as membrane tethering, modulation of enzymatic activities, and/or structural effects on cell membranes. The regulatory processes in which PA has been implicated include signaling pathways in cell growth, proliferation, reproduction, and responses to hormones and biotic and abiotic stresses [78].

The intake of phytosterols enriched foods or supplements have been shown to effectively lower total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations [85, 86]. Based on recent meta-analyses, a phytosterols intake of 2 g/d lowers LDL-cholesterol by on average 0.31 - 0.34 mmol/L, or 8 - 10% [87 – 89]. High total cholesterol, especially LDL-cholesterol, is an established risk factor for cardiovascular disease (CVD) and reducing cholesterol by dietary or drug interventions is known to reduce the risk of CVD [90, 91]. Hence, the cholesterol-lowering properties of PS have been acknowledged by health associations such as the National Cholesterol Education Program Adult Treatment Panel III [92], the American Heart Association [93], the European Society of Cardiology and the European Atherosclerosis Society [94]. PS lower plasma cholesterol by partly inhibiting cholesterol absorption in the gut, mainly through competition with cholesterol for micellar incorporation [95].

Table 2 presents the phytosterols content of some Nigerian vegetable oils. The values of cholesterol, cholestanol, ergosterol, campesterol, stigmasterol, 5α-avenasterol and sitosterol from the reviewed samples are $1.6e^5 – 9.28$, $4.80e^6 – 2.28$, $4.59e^4 – 30.20$, $9.39e^3 – 103.00$, $1.24 – 45.60$, $5.62e^3 – 53.50$ and $17.21 – 351.00$. Malaba spinach records the highest total phytosterols content (442.91 mg/100 g), followed by big alligator peppers (369.34 mg/100 g) while Ethiopian pepper contains the least total PS of 23.82 mg/100 g.

Cholesterol which is predominately of animal origin, is being synthesized in the human liver, and has an essential role in the human body, either for the cell walls or as a building block for steroid hormones, such as testosterone and estrogen. It is carried from the liver to the cells by the low density lipoproteins (LDL), through the blood, and these may originate fat deposits in the arteries, increasing the risk of coronary heart disease (CHD), and leading ultimately to heart attack or stroke [96]. On the contrary, the high density lipoproteins (HDL) exert a protective effect to the heart, since they carry the excess of bad cholesterol back to the liver, where it is eliminated. Sitosterol is the most abundant phytosterols but campesterol and stigmasterol are also present in important quantities [37].
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**Table 2: Phytosterols Composition (mg/100 g) of Some Nigerian Vegetable Oils**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CLR</th>
<th>CLN</th>
<th>EGR</th>
<th>CPR</th>
<th>SMR</th>
<th>5-ANR</th>
<th>STR</th>
<th>Total</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Tea bush</td>
<td>0.00</td>
<td>1.04e-3</td>
<td>1.84e-3</td>
<td>8.14</td>
<td>4.42</td>
<td>3.89</td>
<td>26.30</td>
<td>42.75</td>
<td>[63]</td>
</tr>
<tr>
<td>Indian spinach</td>
<td>6.33e-4</td>
<td>4.08e-4</td>
<td>1.91e-3</td>
<td>67.70</td>
<td>8.23</td>
<td>52.60</td>
<td>196.00</td>
<td>324.53</td>
<td>[65]</td>
</tr>
<tr>
<td>Malaba spinach</td>
<td>3.09e-3</td>
<td>4.10e-4</td>
<td>2.70e-3</td>
<td>103.00</td>
<td>11.40</td>
<td>53.50</td>
<td>275.00</td>
<td>442.91</td>
<td>[65]</td>
</tr>
<tr>
<td>Raw Groundnut</td>
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<td>0.00</td>
<td>8.47</td>
<td>7.85</td>
<td>9.05</td>
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</table>

CLR = Cholesterol, CLN = Cholestanol, EGR = Ergosterol, CPR = Campesterol, SMR = Stigmasterol, 5-ANR = 5-Avenasterol, STR = Sitosterol, - = Not Available, ND = Not Detected

Besides their cholesterol lowering effect, phytosterols also have other positive activities, such as anti-inflammatory [100 – 101], antioxidant [102 – 103] and anti-atherosclerosis. Phytosterols were also found to be protective to ulcers [104] and to have anti-fungal activity [105]. The intake of phytosterols is beneficial to prevent or treat many different types of cancer, including breast [106], prostate [107 – 108], lung [109], esophagus [110], stomach [111], endometrial [112], and ovary [113].

**Conclusion**

Phospholipids (PLs) and phytosterols (PS) are important components of the cell membranes of all living species that contribute to the physicochemical properties of the membrane and thus influence the conformation and function of membrane-bound proteins, such as receptors, ion channels, and transporters. They influence cell function by serving as precursors for prostaglandins and other signaling molecules and modulating gene expression through the transcription activation. They have been shown also, in a vast number of human studies to be safe and effective in lowering plasma total and LDL-cholesterol concentrations. In view of the findings of this reviewed work, Nigerian vegetable oils can be said to be potential source of phospholipids and phytosterols. But due to the paucity of data on the concentrations of phospholipids and phytosterols in the Nigerian plant–based oils as well as their importance in food, cosmetics, pharmaceuticals and biomedical industries, more research needs to be done on the vast variety of both utilized and under-utilize plant–based oils in order to examine their PL and PS compositions.

**Acknowledgement**

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**References**


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92. NCEP ATP III (2002). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III).


