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# β- SITOSTEROL: ANALYTICAL PROFILE AND COMPREHENSIVE REVIEW

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**ABSTRACT:** Phytosterols, a subclass of steroids, have gained considerable attention in scientific research and nutrition due to their potential health benefits. This article presents an overview of phytosterols, encompassing their sources, chemistry, extraction methods, analytical techniques, and biological activities. Phytosterols, structurally resembling cholesterol, are found abundantly in plants, animals, and fungi. Their physiological roles include influencing membrane fluidity and acting as second messengers in signal transduction pathways. Incorporating phytosterol-rich foods into the diet has been linked to various health benefits, particularly in reducing cholesterol levels, as recognized by regulatory bodies like the European Food Safety Authority and the FDA. The article discusses various extraction methods for phytosterols, emphasizing the need for environmentally friendly approaches to minimize the use of organic solvents. Traditional techniques such as Soxhlet extraction are effective but pose health and environmental concerns, driving the exploration of alternative extraction technologies. Phytosterol chemistry, characterized by specific carbon atoms and side chains, is explored, highlighting their steroid structure and different forms in nature. Analytical methods such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS) are discussed for identifying and quantifying phytosterols, with HPLC-MS being particularly sensitive and selective. The article concludes with an examination of the diverse biological activities of phytosterols, including anti-inflammatory, antimicrobial, and anti-cancer effects. The potential therapeutic applications of prominent phytosterols like β-sitosterol are also explored. Overall, this article provides a comprehensive overview of phytosterols, emphasizing their importance in nutrition and health, and the need for sustainable extraction methods to maximize their benefits.

**INTRODUCTION:** The term "phytochemicals" (phytochemicals) was introduced to the world in 1994 and has become a distinction and boundary for scientists and researchers where phytosterols, a subclass of steroids, are important working classes of bioorganic molecules.

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Phytosterols are common in plants, animals, and fungi and have a structure similar to cholesterol. Phytosterols play an important role in the physiology of eukaryotes.

For example, cholesterol is an essential part of animal cell membranes, affecting membrane fluidity and acting as a second messenger for signal development. The most important benefit of these metabolites is the inclusion of natural foods containing them in health promotion. The European Food Safety Authority (EFSA) recommends a daily intake of approximately 1.5 - 2.4 grams of plant sterols and/or stanols to lower cholesterol.

In addition, the FDA has approved foods containing phytosterol esters in foods low in saturated fat and cholesterol to reduce heart disease, especially when consuming at least 1.3 grams of sterols twice daily [1].

Natural foods and foods containing high phytosterols have been continuously marketed in many countries for many years. Vegetable oils and products made from vegetable oils, nuts, grain products, vegetables, fruits, and nuts are listed as the richest or richest in phytosterols. The three phytosterols, β-sitosterol, campesterol, and stigmasterol, are the main sterols in the human diet, accounting for 65%, 30%, and 3% of the food, respectively. Phytosterols have a long history in the food or pharmaceutical field and are generally considered safe (GRAS) with no side effects. An exception is their sterols to the intestines and brain [2].

Phytosterols, often called phytosterols, are naturally occurring bioactive compounds that represent various triterpenes. Phytosterols are found in all plant cell membranes and are particularly found in vegetable oils and fats, cereals and cereal products, vegetables, fruits, and berries. In addition to herbaceous plants and oilseeds, wastes from the soft and hardwood industry (high bituminous and sulphate soaps) are also important for phytosterols. These phytosterols contain 10% to 15% phytosterols. New and virtually untapped natural resources Phytosterols are now receiving a lot of attention, for example, in microalgae. Phytosterols are important components of lipid bilayers in cell membranes. They have a main structure of 28 or 29 carbon atoms and are similar to cholesterol in structure (tetracyclic steroid core, 3β-hydroxyl group, and often 5,6-double bonds) and function (stabilization of phospholipid bilayers in cell membranes). Most phytosterols have a side chain of nine to ten carbon atoms, while cholesterol has a side chain of eight carbon atoms. More than 200 phytosterols have been found in various plants.

Of these, β-sitosterol, campesterol and stigmasterol are abundant in nature. Other phytosterols such as brassicasterol, Δ5-avenasterol, sitosterol and campesterol are found in very small amounts in almost all plants. In nature, phytosterols exist as free sterols or conjugated fatty acid esters, glycosides, and acetylated glycosides. Phytosterols, natural components of lipids, have received great interest in reducing blood cholesterol and heart disease risk in humans. In addition, phytosterols have anti-inflammatory, antibacterial, antiulcer, and antitumor properties [3].

Plaza *et al.* (Year) reported that foods containing phytosterols improve the health of consumers and prevent different diseases. Many chromatographic techniques allow rapid analysis of biological compounds. However, its results may depend on the extraction method, including the extraction method, the nature of the target, and the plant matrix. The quality and variety of bioactive compounds in plant materials depend only on the selection of appropriate extraction methods.

Until now, the extraction process has been investigated to restore the beneficial effects of the plant for the economy. Since, phytosterols are lipids in nature, various extraction methods can be used to extract non-polar components. Currently, many traditional and controversial techniques are used to extract relevant elements from different plant matrices. Among the traditional extraction methods, Soxhlet extraction is the best method and is still considered to be used for new designs. However, the organic solvents used in the extraction process are always harmful to human health and the environment [4, 5].

Due to the large number of organic solvents used in conventional extraction, there is always a greater need for new technologies to reduce the use of organic solvents and provide some advantages over traditional extraction methods. Extraction methods focusing on the removal of bioactive substances other than phytosterols from plant matrices have been reviewed in many areas. Then, the purpose of this review is to explain the beneficial effects of phytosterols and their extraction and analysis methods and to offer some suggestions for the commercial use of environmentally friendly strategies [2, 6, 7].

**Chemistry of Phytosterols:** The phytosterols are chemical steroids, made of cyclopentanoperhydrophentherene ring. Phytosterols (PS) are fatty compounds of plant origin (steroids) that represent the largest

proportion of unsaponifiable plant lipids [4], whereas cholesterol is found in animals. They have a steroid skeleton characterized by the presence of a combination of C-5 and C-6 of the sterol moiety. They have an aliphatic side chain attached to the C-16 atom and a hydroxyl group attached to the C-3 atom. Cholesterol and phytosterols were first discovered as non-esterified and esterified forms or glycosides of cinnamic acid/fatty acid (FA). The bound form is hydrolyzed mainly in the intestine by pancreatic enzymes. It is estimated that up to 50% of free cholesterol is absorbed in the human intestine due to changes in PS structure and molecular weight [3]. The above-mentioned structures are frequently found in medicinal plants, a few of them are listed in below **Table 1.**

**TABLE 2: BIOLOGICAL SOURCE AND USES**

| Sr. no. | Herbs | Biological Sources | |
|---|---|---|---|
| 1. | Saw Palmetto | dwarf palm tree of *Serenoa repens* Family*: Arecaceae* | Saw palmetto is a rich source of phytosterols. It contains beta-sitosterol, campesterol, stigmasterol, and other phytosterols. |
| 2. | Nettle Leaf | It contains the leaf *Urtica dioica L.* Family: Urticaceae | It is also a good source of phytosterols, including beta-sitosterol |
| 3. | Pygeum Bark | It contains the bark of *Pygeum africanum* Family*: Rosaceae* | It is also a good source of beta-sitosterol. |
| 4. | Fenugreek | It contains seeds of *Trigonella foenum graceum.* Family: *Leguminosae* | It is also a good source of phytosterols, including stigmasterol and beta-sitosterol. |
| 5. | Ginseng | It contains the dried root of *Aralia quinquefolia* Family: *Araliacaea* | It is also a good source of phytosterols, including beta-sitosterol |
| 6. | Milk Thistle | It contains the leaves of *Silybum marianum* Family*: Asteraceae* | It is also a good source of phytosterols, including beta-sitosterol. |
| 7. | Black Seed | It contains the seed of Nigella *sativa* Family: Ranunculaceae | It is also a good source of phytosterols, including beta-sitosterol. |

**Extraction Techniques for Phytosterol:** Extraction techniques allow the separation of plant-soluble metabolites in selective solvents. The cleavage process of phytosterols depends on the nature of the matrix and the form of the phytosterols (free, esterified, and glycosylated). Each extraction process should use different conditions to get the best results from botanicals.

The quality of the extract usually depends on the plant material, weight, extraction process, etc. affected by many factors such as During the extraction process, all parameters must be optimized to obtain a high-quality extract with a large number of desired products.

As mentioned above, there are traditional and ineffective extraction methods. The main objectives of these techniques are:

**a)** Extraction of relevant bioactive compounds from plant matrices,

**b)** Improvement of bioassay sensitivity by increasing the concentration of target compounds,

**c)** Development of analytical methods

**d)** Conversion transformations into more suitable materials for the detection and extraction of biologically active compounds.

**e)** Providing robust and reproducible methods independent of sample matrix variations.

The methods involved in the extraction and analysis of phytosterols are introduced (Roini *et al.*). The effects of various phytosterol extraction methods such as Soxhlet extraction, ultrasonic, supercritical carbon dioxide, and supercritical carbon dioxide with cocoa butter co-solvent were investigated.

The authors reported that the highest phytosterol content was achieved using supercritical carbon dioxide and co-solvents. Abbas et al. developed a method to extract sterols from corn fiber using ethanol.

They claim that the extracted phytosterols are selected from the alpha, beta, and gamma forms of sitosterol, sitosterol, stigmasterol, stigmasterol, campesterol, campesterol, spinasterol, phytosterol esters, phytosterol esters, and compounds [8, 9].

**TABLE 2: EXTRACTION OF PHYTOSTEROLS USING CONVENTIONAL TECHNIQUES**

| Plant matrices | Extraction technique | Used solvent | Detection method | Internal standard | Main Analysed phytosterol | Reference |
|---|---|---|---|---|---|---|
| Grape seed | SFE | SC-$CO^2$ | GC-FID | Dihydrocholestrol | β-sitosterol, campesterol stigmasterol | Orozco-Solano *et al* |
| Leaves of piper gaudichaudianum Kunth | Soxhlet | Petroleum ether or ethanol | GC-MS | Perylene | Stigmasterol, β-sitosterol, | Péres *et a* |
| Anise, coriander, caraway, white mustard, nutmeg seeds | Soxhlet | Hexane | GC | 5α-cholestane | Sterols | Kozłowska *et al* |
| Ripe pulp of mango | Soxhlet | Dichloromethane | GC-MS | Tetracosane | Fucosterol β-sitosterol, campesterol stigmasterol | Vilela *et al*. |
| Sea buckthorn Seed | Cold Pressing | | GC FID GC-MS | 5α-cholestane | Sitosterol Lanosterol Campesterol, Clerosterol | Li *et al.* |
| C. nutans | Soxhlet | Ethanol | HPLC | | Total phytosterols, β-sitosterol | Mustapa *et al* |
| Pumpkin seed | Cold pressing | | GC-MS | Cholesterol | β-sitosterol and spinasterol | Rabrenović *et al* |
| Corn, sesame, oat and peanut | Hexane Extraction | Hexane | UPLC-APCIMS | 6 ketocholestao | β-sitosterol, Ergosterol,camp esterol stigmasterol | Lu *et al* |

**Analysis of Phytosterols:** As mentioned earlier, phytosterols occur in nature as free sterols, sterol esters, sterile glycosides, and acylated sterile glycosides. For the determination of all phytosterols, sample preparation must include all sterol conjugates. Because plant lipids contain phytosterols with other non-saponifiable components, reliable analytical techniques are required to analyze them. Many analytical methods have been developed since the discovery of phytosterols. The first-generation method widely used for the determination of phytosterols is the digitonin precipitation method.

Later, a sensitive colorimetric method involving enzymatic oxidation was introduced for the determination of phytosterols. However, this method has become automated due to tedious procedures and expensive reagents. The products from the various methods were analyzed by different chromatographic methods, including gas chromatography (GC), column chromatography (CC), high-performance liquid chromatography (HPLC), and capillary electrochromatography (CEC) to identify and characterize sterol. Preliminary measurements were made by thin-layer chromatography (TLC). Today, GC and HPLC are widely used in phytosterol determination. HPLC can operate at pressures above 40 MPa. Instead, ultra-performance liquid chromatography (UPLC), a new technique capable of operating at pressures of up to 100 MPa, was used to determine phytosterols in rice. Phytosterols can be detected by flame ionization detection (FID), nuclear magnetic resonance (NMR), infrared, ultraviolet (UV) detection, evaporative light scattering detection (ELSD), and mass spectrometry (MS). GC-FID and GC-MS are widely used for the analysis of phytosterols.

However, phytosterols can be better identified and quantified by gas chromatography combined with an electron beam or chemical ionization mass spectrometry. A comprehensive review of analytical methods for phytosterols in food products is provided by Abidi and Lagarda *et al.*[10] A combination of flash liquid chromatography and silver ion liquid chromatography was developed by Francavilla *et al*. All sterol fractions are isolated and the most abundant phytosterols are purified.

Using both methods together, they claim a purity of 97.87% and a recovery of 98%. And demonstrate the analysis of phytosterols using GC and HPLC [11]. Individual phytosterols were analyzed through several sequential steps such as lipid extraction, saponification and/or acid hydrolysis, and finally purification and quantification. Many chromatographic methods require the saponification of extracts before phytosterol analysis [8].

**Gas Chromatography:** GC is the most common and widely used method for sterol determination. Compared to filled lines, capillary lines have advantages such as shorter analysis time, better concentration, and high thermal stability. Most phytosterols were determined by nonpolar stationary phase gas chromatography. The stationary phase usually consists of cross-linked polysiloxane. A less polar stationary phase containing 5% biphenyl and 95% dimethylpolysiloxane shows good thermal stability and improves the peak solubility of individual phytosterols [12].

For GC analysis of sterols, the non-extracted material is derivatized as trimethylsilyl (TMS). Without derivatization, it is difficult to improve the quality and solubility of individual phytosterols and phytosterols. N-methyl-N-(trimethylsilyl) trifluoro-acetamide and bis(trimethylsilyl)-trifluoro-acetamide containing 1% trimethylchlorosilane in anhydrous pyridine are frequently used as derivatizing agents. Dry pyridine trimethyl-chlorosilane and hexamethyldisilane can also be used for derivatization. Phytosterols are usually analyzed by GC-FID based on retention time. Instead, GC-MS was used to confirm the peak identity and its phytosterol amount [13, 14].

**High-performance Liquid Chromatography (HPLC):** Liquid chromatography, especially HPLC, has many advantages over GC. HPLC can operate at low concentrations and, thanks to non-destructive detection, phytosterols can be identified and collected in purified form. HPLC was used for the identification and quantitative preparation of phytosterols. HPLC is suitable for the analysis of sensitive phytosterols. If the extracted lipids are in simple form, the phytosterols can be analyzed directly by HPLC without losing the sample [15].

Normal phase HPLC was used to separate and quantify the five major lipid levels of phytosterols (sterol esters, free sterols, sterol glycosides, acylated sterol glycosides, and sterol ferules). In reverse phase HPLC, the addition of a low volatility polar organic solvent to water leads to rapid equilibration of the bound silica stationary phase and mobile phase solvent compared to normal phase HPLC [16]. Octadecyl silica (ODS C18) columns are widely used for the analysis of phytosterols. Many organic solvents such as acetonitrile, methanol, isopropanol, tetrahydrofuran, and acetic acid are used for the separation of phytosterols in high-performance liquid chromatography. Sterols were detected using UV spectroscopy, ELSD, diffraction detection, and MS in the 200 to 210 nm range [17, 18].

Phytosterols in Alpinia cane were analyzed by HPLC equipped with a C8 column, PDA, and UV detector. Isocratic elution with acetonitrile and water (95:5 v/v) produced better results and solubility for β-sitosterol and stigmasterol and reduced base and unexpected differences. Liu and Ruan reported a new HPLC-based method for the quantification of phytosterols using benzoyl chromophore as the derivatizing agent. The incorporation of benzoyl groups into phytosterols through simple derivatization greatly improves the UV response at 254 nm [17, 19].

**HPLC-MS Analysis:** HPLC-MS is also used for phytosterol analysis. More importantly, atmospheric pressure chemical ionization (APCI) combined with liquid chromatography-mass spectrometry (LC-MS) was used for the analysis of phytosterols from natural sources. Due to the high lipophilicity of polar functional groups in phytosterols, it is difficult to ionize sterols by the conventional electrospray method. Electron ionization (EI) and atmospheric pressure photoionization (APPI) techniques are also good alternatives for lipophilic compounds [23].

A method for the analysis of phytosterols by HPLC based on positive ion APCI tandem mass spectrometry (LC-MS/MS) is presented. Dietary concentrations of phytosterols and triterpene alcohols in edible oils, including brassicasterol, campesterol, cyclosterol, β-sitosterol, stigmasterol, and lupeol, were measured using APCI LC-

MS/MS. This new method provides a good combination of speed, selectivity, and sensitivity for phytosterol analysis [20, 21].

**Capillary Liquid Chromatography (CLC) Analysis:** The use of capillary liquid chromatography (CLC) and nano-liquid chromatography (nano-LC) in analysis is increasing. Phytosterols in extra virgin olive oil were analyzed by UV spectrophotometric and nano-LC coupled with MS. Analysis of phytosterols was also done by supercritical liquid chromatography (SFC) using SC-CO2 as the mobile phase. Combining SFE with SFC has the advantage of a single process, including extraction, processing, proportioning, and quantification of phytosterols [25].

**IR Spectral Analysis:** Infrared spectrum analysis shows broad peaks at 3549.99 $cm^{-1}$ for the OH group, 2935.73 $cm^{-1}$ for the $CH_2$ group, 2867.38 $cm^{-1}$ for the CH group, 1637.63 $cm^{-1}$ for the C=C group and 1063 $cm^{-1}$. The C-O group is 34 $cm^{-1}$. The molecular weight determination shows that its molecular formula is C29H50O. Similar results were observed where IR peaks were obtained at 3426.89, 2924.52, 2855.1, 1738.51, and 1057.31 $cm^{-1}$ [24, 31].

**NMR Spectral Analysis:** The 1H NMR spectrum shows an olefinic signal (δ H 5.08) indicating the presence of a >C=C< system in the ring. A proton-wide multiplet at δ H 4.44 shows the crossover peak between the C-2 proton and the C-4 proton in HETCOR, and the signal is assigned to the C-3 methine proton. Differences were observed in the δH1 range.

1-2.14 is documented in the presence of different methylene and methine protons in the steroidal structure. Another proton resonance was assigned to glucopyranocytes. 13 C NMR [34]. also provided additional evidence showing a resonance at the 35 carbon atom.

The C3 carbon resonates at 71.73 ppm. The anomeric and oxymethylene carbons of the sugar occur at 100 ppm and 61 ppm, respectively. Therefore, the structure of this compound is defined as stigmast-5-en-3-O-β-D-glucopyranoside (β-sitosterol glucoside) according to spectroscopic data.

Another study showed that the 1H NMR spectrum of BS **Table 1** (400 MHz, CDCl3) showed a triplet state with a proton corresponding to the proton attached to the C-3 hydroxyl group as twice the δ3. 53 is indicative of the steroid core, its location, and many of its differences. The correct olefinic H-6 of the steroid skeleton manifests as an δ 5.36 (J = 6.4) triplet that can convert to a proton. The spectrum also spawns two singlets at δ 0 [33, 35, 37].

Three proton energies of 68 and 1.01 ppm are assigned to two tertiary methyl groups at C-18 and C-19, respectively. Three proton energies are assigned to the two tertiary methyl groups at C-18 and C-19, respectively. The NMR spectrum also shows two peaks at 8 0 (J = 6.4). 83 and 0.81 are attributed to two methyl groups at C-26 and C-27. The binary at 8 0.93 (J = 6.5) is attributed to the methyl group at C-21.

On the other hand, the triplet of three proton energies δ 0.84 (J = 7.2) is assigned to the first methyl group attached to C-29 [32, 34].

**GC-MS:** Gas chromatography coupled to mass spectrometry (GC-MS) for the simultaneous detection of BS in other sterols by various selective ion detectors. The process is based on alkaline hydrolysis of sterol esters, and extraction and derivatization of free sterols. Recovery for all sterols ranged from 76% to 101%. Except for the method recently described by Srividya *et al.*, the process is often labour-intensive and time-consuming [9].

Simultaneous detection of GC-FID and GC-MS after alkaline hydrolysis and liquid-liquid extraction is considered ideal for phytosterol bioanalysis. So, there is a lot of room to improve performance as search and quantity limit [34].

**Thin Layer Chromatography (TLC):** Using a glass plate coated with silicon G. Each extract was individually co-chromatographically analyzed using the correct sterols as markers. This plate was formed in a gas-pressure chromatography chamber saturated with a solvent mixture (hexane: acetone: 8:2) [15]. Other solvents such as benzene and ethyl acetate (85:15) [16]. Benzene: ethyl acetate (3:1) was also used, but hexane: acetone (8:2) gave a better separation.

The plates are air-dried and examined under ultraviolet (UV) light and marked with fluorescent dots corresponding to the markings. The plates were sprayed with 50% sulfuric acid and anisaldehyde reagents, respectively, and heated at 110°C for 10 minutes [38].

**Biological Activity of β-Sitosterol:**

**β-Sitosterol as Anti-Cancer agent:** β-sitosterol has anti-cancer properties for breast cancer, prostate cancer, breast cancer, lung cancer, breast cancer, stomach cancer, ovarian cancer and leukaemia. Studies have shown that BS interacts with many cell signalling pathways, including cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation. Most studies are inconclusive, in part due to the low power of BS. But nearly all research groups ignored the fact that, unlike all existing cancer treatments, it's generally non-toxic. To prevent the effectiveness of BS, it is possible to create BS children for "specific cancer therapy" therapy.

Delivery of BS via liposomes is a demonstration method and has proven very successful. However, further studies were not successful in delivering BS drugs or enhancing BS-mediated anticancer activities, thus making BS a good food [39].

**Antioxidant Activity:** Many studies have shown that BS has antioxidant effects [7]. It has also been shown to modulate antioxidant enzymes and human estrogen receptors. According to the study, BS reduced free oxygen radical and hydrogen peroxide levels in RAW 264.7 cells stimulated with phorbol myristate acetate (PMA), but did not act as a free radical scavenger.

**Antimicrobial Activity:** BS obtained from different plants has been proven to have antibacterial and antifungal properties and no toxicity in the brine shrimp lethality test. BS containing formulations or plant extracts showed mosquito larvicide activity and anti-trypanosomal activity. BS has been reported to have anti-inflammatory properties with a zone of inhibition comparable to other anti-inflammatory drugs.

**Anti-Inflammatory Activity:** BS contains antibodies against human aortic cells as well as mice. Various animal studies have shown that besides oedema, BS reduces the release of pro-inflammatory cytokines and increases the production of anti-inflammatory cytokines [40].

**Immune Modulation and Anti-HIV Effect:** BS has been shown to be a potent immunomodulator. BS exhibits immunomodulatory activity in HIV patients. It has also been reported that BS specifically targets T helper (Th) lymphocytes, increases Th1 activity, and increases T lymphocyte and natural killer (NK) cell activity. In other studies, BS has been shown to stabilize CD4 cell numbers in AIDS1 and reduce apoptosis of CD4 lymphocytes, thereby slowing the spread of HIV. In the same study, IL-6 levels were lowered, indicating that the viral load in infected cells slowed and reduced the number of infections.

**Anti-Pulmonary Tuberculosis Effect:** BS has been shown to be effective in weight loss due to tuberculosis. The same study showed that patients treated with BS showed significant differences in some haematological parameters such as lymphocyte, eosinophil, and monocyte counts. The details of this effect have not been investigated. The efficacy of BS as an immunomodulator in the treatment of multidrug-resistant tuberculosis should be further investigated. Studies have reported that plant extracts containing BS have significant anti-inflammatory activities [26].

**Anti-Arthritic Activity:** BS reduced the activation of the NF-κ-B transcription factor in PMA-stimulated macrophages. However, more research is needed on the ability of BS to treat arthritis [28].

**Antidiabetic Effects:** Many studies have identified PS as a regulator of glucose metabolism, leading to AMP-activated kinase (AMPK) activation or peroxisome proliferator-activated receptor (PPAR) transcriptional 5Słda, regulatory pathway. Huang et al. β-sitosterol (20 μM) treatment of L6 myocytes has been shown to increase translocation of GLUT4 to the plasma membrane, thereby increasing glucose uptake [19, 22, 25]. Zucker diabetic rats supplemented with 5-campestenone (0.6%) had increased blood glucose levels and improved insulin secretion after oral administration of high glucose [48]. It has been reported that after eight weeks of feeding with 0.3% 5-campestenone in db./db. Mice, blood sugar decreases and urinary glucose excretion is prevented [37].

**Effects on Lipoprotein Metabolism:** According to various studies, PS administration reduces total cholesterol (TC) and LDL-C levels by preventing dietary cholesterol absorption and also affecting liver/intestinal biotransformation [24]. When male C57BL/6 J mice were supplemented with a high-fat diet and given 3.1% PS for three weeks, low-density lipoprotein (VLDL) secretion was reduced with no difference in chylomicron secretion [22].

Epidemiological and experimental data have confirmed that a high-cholesterol diet is detrimental to intelligence in animal models. Rui *et al.* It was found that mice fed a high-cholesterol diet containing 2 g/100 g PS for 6 months maintained the same body weight, decreased blood lipids, improved cognition, increased pyramidal cell counts, and decreased astrocytes counts [30].

Various studies have shown the role of PS in the regulation of plasma and high triglyceride (TG) levels [49]. Rideout *et al.* The effect of a diet supplemented with 2% w/w PS was evaluated in Syrian golden hamsters fed a high-fat diet for 6 weeks. Surprisingly, the decrease in serum TG in the PS group was consistent with ezetimibe [39].

Similar results have been found in other animal studies, including factors such as diet type, PS dose, test duration, and circadian regulation of lipid levels, which may differ in TG levels and related processes [31, 32]. Therefore, based on the main results of animal experiments, it has been shown that PS can reduce TG levels by decreasing TG and FA uptake, increasing fecal FA excretion, decreasing duodenal bile acid excretion, and modulating hepatic FA and TG metabolism.

Several *in-vivo* studies on different animal models have yielded conflicting results, as have data from Brufau et al. Researchers investigating the effects of 2.45% g/100 g PS supplementation in guinea pigs fed a high-fat diet for 4 weeks [33, 37].

The authors did not account for changes in medium-chain FA excretion, but decreased medium/short-chain FA excretion and increased long-chain FA excretion [43]. Also, while fecal excretion of all saturated fatty acids is unchanged, increased excretion of long-chain and more hydrophobic saturated fatty acids indicates a reduced fat-soluble capacity in the gut, and the study time chosen for this test also affects the results [40, 41].

**Anti-atherosclerotic Effects:** The anti-atherogenic properties of phytosterols have been well-documented in various animal models and are directly related to their cholesterol-lowering effects [28]. Shariq *et al.* (2015) studied the antiatherogenic effect of coconut oil, which was reported to increase PS levels in male Wistar rats. Studies have shown the importance of high-density lipoprotein (HDL) associated with a reduction in body weight, TC, TG, LDL and VLDL, and atherogenic effects [42].

Various studies have also described the modulation of PS on atherosclerosis due to its anti-inflammatory effects. PS has been shown to reduce the production of proinflammatory cytokines, namely IL-6 and tumour necrosis factor (TNF)-α, and increase the Th1/Th2 ratio and the level of the Treg cytokine IL-10 in splenocytes of ApoE mice [43, 47].

Increased IL-10 levels have also been associated with a reduction in atherosclerotic lesion size. The reduction in the size of atherosclerotic lesions is also associated with a reduction in other characteristics such as the number of foam cells and cholesterol clefts, the amount of extracellular matrix, and the apparent degree of proliferation of muscle tissue [44, 45].

Dietary supplementation of ApoE-deficient mice with 2% PS for 14 weeks resulted in the transcription of 132 genes, specifically genes involved in the regulation of sterol biotransformation [46]. All these changes allow us to better understand how PS properties exert their cardioprotective effects.

**CONCLUSION:** In conclusion, the introduction provides an overview of phytosterols, their importance in health promotion, and the need for their extraction from various plant sources. The chemistry of phytosterols is explained, including their structure and occurrence in different herbs. Additionally, the extraction techniques for phytosterols are discussed, emphasizing the need for environmentally friendly methods. The data also delves into the analysis of phytosterols, discussing various analytical methods such as gas

chromatography (GC), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectroscopy. It provides insights into how these methods are applied to identify and quantify phytosterols in different plant sources. Furthermore, the biological activities of β-sitosterol are explored, highlighting its potential as an anti-cancer agent, antioxidant, antimicrobial agent, anti-inflammatory agent, immune modulator, and more. The review emphasizes the diverse range of health benefits associated with β-sitosterol, making it an intriguing compound for further research. Overall, the data provides a comprehensive overview of phytosterols, from their chemistry to extraction and analysis techniques, as well as their potential health benefits. This information is crucial for researchers and scientists working in the fields of nutrition, pharmacology, and health sciences, as it highlights the significance of phytosterols in promoting human well-being.

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