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CURRENT STATE OF RESEARCH ON THE STEROL MOLECULES CONATINED IN NUTS

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ABSTRACT:

The current review mainly focuses on the Extraction of Sterols and the analysis of different types of sterols in the Hazelnut, pecan nuts and wall nuts. The types of sterols found in different nuts are free esterified sterols, β- sitosterol, campesterol, D-5-avenasterol, 4-desmethylsterol, 4-monomethylsterol and 4,4-dimethylsterol.

The Composition of different type of sterols in Hazelnuts and wall nuts are discussed, in Pecan nuts the percentage of 4-desmethylsterol, 4-monomethylsterol and 4, 4-dimethylsterol found in the nut in the different stages of growth of the pecan nut is studied. β- sitosterol is the primary sterol found in walnuts and hazelnuts. Thephytosterol composition of whole pecan kernel was quantified by Gas Chromatography–Flame Ionization Detection (GC–FID) and identified by Gas Chromatography–Mass Spectrometry (GC–MS).

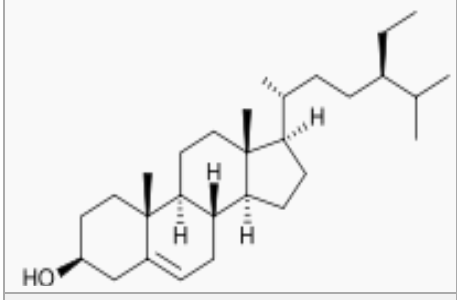
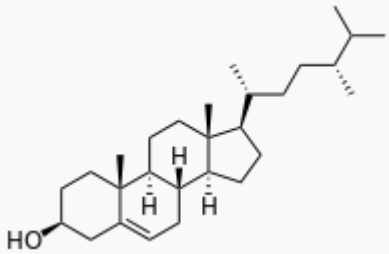
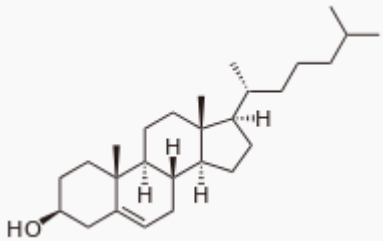
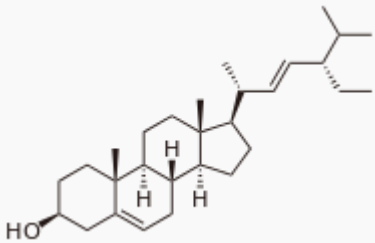
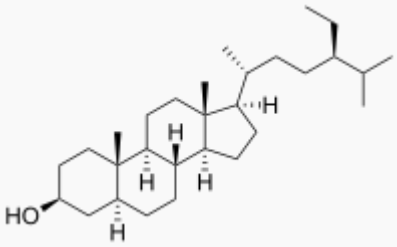
Sterols have many health benefits and therapeutic effects, The Sterols found in the pecan nuts have Health Benefits like lowering LDL-cholesterol and preventing heart disease. The Effect of sterols on the enlarged prostate gland and sterols anti-cancer and anti-inflammatory activities are also discussed here.

INTRODUCTION

Sterols, also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. Sterols may be found either as free sterols, acylated, alkylated, sulfated or linked to a glycoside moiety which can be itself acylated (acylated sterol glycosides). Phytosterols are a group of steroids produced by plants. They are

structurally and functionally related to cholesterol and comprise a major component of the human diet [1, 2]. A small fraction of cholesterol can be found in the membranes of some plant cells [3, 4]. However, for the most part, the sterol content of plant cell membranes is made up by other sterols, e.g. campesterol, β-sitosterol and stigmasterol [4].

These sterols in plant cell membranes differ structurally from cholesterol only with regard to their side chain. Campesterol has a methyl group at carbon 24, while β-sitosterol and stigmasterol have an ethyl group in this position. Stigmasterol furthermore contains an additional trans double bond between carbons 22 and 23. Carbon 24 in campesterol and β-sitosterol has R chirality, while the equivalent carbon in stigmasterol has S chirality due to the trans configuration of the side chain double bond [2] and [5].The structures of different type of plant sterols are given below.

β-sitosterol	campesterol	cholesterol
		
stigmasterol	**Stigmastanol**	
		

Plant sterols, plant stanols and conjugates of these induce a decrease in the serum total and low-density lipoprotein cholesterol level, which in turn may cause suppression of atherogenesis [6]. The mechanisms for the decrease in cholesterol concentration related to plant sterols is based on the plant sterols and stanols reducing the rate of intestinal cholesterol absorption [7]. Plant sterols are absorbed from the intestine to a lower extent in comparison to cholesterol [8]. Nevertheless, when absorbed, plant sterols are transported in lipoproteins and may get incorporated

in cellular membranes [9, 10]. A change in sterol content could be critical for the membrane functions of erythrocytes, which lack the ability to adjust the sterol content of their plasma membrane [11].

There are several studies that have explored the effects of plant sterols in phospholipid membranes, such as effects on phospholipid condensation [12], membrane permeability to small solutes [13], phospholipid order [14, 15], membrane interfacial qualities, the thermotropic properties of phospholipid bilayers [16], and on membrane domains [17]. Researchers are trying to better understand the properties of sterol-rich domains in plant sterol-containing phosphatidylcholine and sphingomyelin systems.

β-sitosterol (24-ethylcholesterol) is one of the most abundant dietary phytosterols present in many beans, nuts, and seeds [2]. It is also an important constituent of saw palmetto, devil's claw, stinging nettle and several other natural remedies. [18, 19] β-Sitosterol consumption decreases blood cholesterol levels by preventing its intestinal absorption [7]. It also has been shown to have anti-inflammatory and analgesic properties in various animal models [20]. Additionally, in both animal models and human clinical trials, β-sitosterol has demonstrated a significant effect on reducing the symptoms of benign prostatic, hyperplasia [21]. β-sitosterol intake may also be partially responsible for the decreased incidence of prostate, colon and breast cancers among vegetarians and men and women in Asian countries who consume much larger amounts of β-sitosterol than most Westerners [22]. It is also used for enhancing sexual activity. Marathon runners use β-sitosterol to reduce pain and swelling after a run. β-sitosterol is applied to the skin for treating wounds and burns. High levels of β-sitosterol concentrations in blood have been correlated with increased severity of heart disease in men having previously suffered from heart attacks. Gene–diet interactions seem to play a role modulating cholesterol/plant sterols absorption and secretion. These influences may interfere with the response to drugs [23].

In the current review we talk about the extraction of sterols from some of the nuts like hazel nut, wall nut and pecan nut. A brief explanation of the methods of sterol extraction along with their analysis and therapeutic uses is given.

METHODS

(1) Extraction and Analysis of sterols from various Nuts:

(i) Extraction and Analysis of Sterols from Hazel Nuts [24]:

(a) Extraction:

Hazel nuts varieties of Katalon´ski and Webba Cenny commonly cultivated in Polandare collected and they are manually cracked and shelled before chopping and the fibrous skin was removed by hand and the pellicle is sent for

analysis. The hazelnut oil was extracted using the procedure described in[25]. Oil sample was homogenized with Chloroform/methanol solution as solvent. After 3 min of homogenization, the filtrate was mixed thoroughly with 1 M KCl solution and left overnight at 4 ºC in order to phase separation, and this extract is called as total lipid extract. To determine the free + esterified phytosterols, dihydrocholesterol was added to fat from Total lipid extract and saponification was conducted at room temperature. After saponification, the organic fraction was washed and unsaponificable matter was extracted in sequence using diethyl ether/water and 0.5 N aqueous KOH. The diethyl ether solvent was removed under vacuum and the unsaponificable matter was used for the free + esterified sterol determination.

(b) Analysis:

To determine the total sterols fat from Hot Saponification extract was used for analysis. The unsaponificable matter from TL and HS extracts were silylated and they were analysed using a GC/MS in the chromatographic Phytosterols identification was achieved by comparing peak mass spectra with peaks of standard mixture and with GC–MS data.

Seven sterols and three stanols were identified. Table 1presentstheir free + esterified, bound and total form content. Total content of phytosterols found in Katalon´skiand Webba Cenny samples was 1522.2 and1303.2 mg/kg d.w. nut, respectively. No statistical differences were reported in free + esterified phytosterols content in both samples total free + esterified sterolcompounds were the 62.0% and 75.7% of total sterols in Katalon´skiand Webba Cenny samples, respectively ($p < 0.05$).Free + esterified sterols found were β-Sitosterol, campesterol, Δ5-avenasterol along with major saturated sterols, namely sitostanol and campestanol. Other minor free + esterified sterol compounds that were determined were Δ7-avenasterol, stigmasterol, chlerosterol, fucosterol and cholesterol.

Bound sterols in Katalon´skiand WebbaCennysamples were the38% and 24% of total sterols, respectively. As reported for the freesterols, sitosterol, campesterol and Δ5-avenasterol were the first, second and third bound sterols in all samples. The sum of sitosterol, campesterol and Δ5-avenasterol was 92% and 85.5% of total bound sterols in Katalon´skiand WebbaCenny, respectively. The most abundant phytosterols, namely, sitosterol, campesterol, and Δ5-avenasterol found in Katalon´skiand Webba Cennyoils accounted for 1894.5 and1689.7 mg/kg, 128.9 and 101.5 mg/kg, 88.4 and 80.2 mg/kg, respectively. Obtained sterol results for both cultivars are in the wide agreement with those presented in literature [29].

Table1.1 Sterols content in two hazelnut varieties cultivated in Poland (mg/kg d.w. nut) [24]

Sterols		Variety	
		Kataloński	*Webba Cenny*
Cholesterol	Free + esterified	2.4 ± 0.2 a	1.6 ± 0.6 a
	Bound	6.7 ± 0.4 b	1.8 ± 0.2 a
	Total	9.1 ± 0.5 b	3.4 ± 0.3 a
Campesterol	Free + esterified	53.5 ± 1.0 b	50.0 ± 0.6 a
	Bound	35.8 ± 2.1 b	17.3 ± 1.5 b
	Total	89.3 ± 1.3 b	67.3 ± 1.0 a
Campestanol	Free + esterified	11.0 ± 1.0 b	8.0 ± 0.7 a
	Bound	2.7 ± 0.6 a	6.1 ± 1.5 b
	Total	13.7 ± 0.8 a	14.1 ± 1.0 a
Stigmasterol	Free + esterified	6.1 ± 0.5 a	6.0 ± 0.1 a
	Bound	3.2 ± 0.3 b	1.2 ± 0.0 a
	Total	9.3 ± 0.4 b	7.2 ± 0.5 a
Stigmastanol	Free + esterified	1.2 ± 0.3 a	0.9 ± 0.1 a
	Bound	2.3 ± 0.5 b	1.2 ± 0.1 a
	Total	3.5 ± 0.3 b	2.1 ± 0.1 a
Chlerosterol	Free + esterified	5.7 ± 0.3 b	4.3 ± 0.7 a
	Bound	7.1 ± 1.2 b	2.8 ± 0.6 a
	Total	12.8 ± 0.5 b	7.1 ± 0.3 a
Sitosterol	Free + esterified	789.6 ± 31.2 a	832.7 ± 36.2 a
	Bound	472.1 ± 23.4 b	242.1 ± 18.4 a
	Total	1261.7 ± 48.5 b	1074.8 ± 29.2 a
Sitostanol	Free + esterified	28.2 ± 1.7 a	32.7 ± 0.2 b
	Bound	15.9 ± 0.8 a	29.1 ± 0.9 b
	Total	44.1 ± 0.9 a	61.8 ± 1.2 b
Δ^5-Avenasterol	Free + esterified	36.7 ± 2.6 a	39.5 ± 1.7 a
	Bound	24.7 ± 2.5 b	11.3 ± 2.0 a
	Total	61.4 ± 2.7 b	50.8 ± 2.5 a
Fucosterol	Free + esterified	3.4 ± 0.5 a	4.2 ± 0.6 a
	Bound	1.1 ± 0.1	1.3 ± 0.3 a
	Total	4.5 ± 0.4 a	5.5 ± 0.5 a
Δ^7-Avenasterol	Free + esterified	6.1 ± 0.1 a	6.8 ± 0.4 a
	Bound	6.7 ± 0.3 b	3.8 ± 0.5 a
	Total	12.8 ± 0.6 b	9.0 ± 0.4 a
Total	Free + esterified	943.9 ± 47.0 a	986.7 ± 31.6 a
	Bound	578.3 ± 33.4 b	316.5 ± 30.8 a
	Total	1522.2 ± 41.6 b	1303.2 ± 36.3 a

(ii)Extraction and Analysis of Sterols from walnuts [26]:

(a) Extraction:

Walnut kernels were ground into a fine powder and the oil was extracted usinga Soxhlet extractor with petroleum ether as a solvent. After 6 h of extraction, samples were evaporated under vacuum, weighted and their oil yield was determined. Unsaponifiable lipids were determined by saponifying lipid extract with ethanolic KOH using a-cholestanol) and 1-eicosanol solution, as internal standard. Unsaponifiable matter was extracted four times with petroleum ether. The ether extracted was dried over anhydrous Na_2SO4 and evaporated. The dry residues were dissolved in chloroform for TLC analysis.

The unsaponifiable matter was separated into sub-fractions on preparative silica gel thin layer plates using 1-dimensional TLC with hexane/diethyl ether as the developing solvent. The band corresponding to sterols and alcohols was scraped off, extracted with chloroform/diethyl ether, filtered to remove the residual silica, dried in a rotary evaporator and stored at -10 ºC. Sterols and alcoholic fractions were evaluated by GLC-FID using a HP5890 gas chromatograph, equipped with a FID, HP-5MS capillary column. Quantification was made by the addition of internal standard (a-cholestanol and 1-eicosanol, for sterols and alcoholic fractions, respectively). Apparent β-sitosterol was calculated as the sum of β-sitosterol, Δ5, 23-stigmasterol, clerosterol, sitostanol, and Δ5,24-stigmastadienol.

(b) Analysis:

All extractions and determinations were conducted in triplicate. The data were analysed using the analysis of variance (Anova). Comparisons of means were achieved using the Statistical Analysis System XLSTAT. Differences between varieties were assessed using Duncan test. Differences at $p < 0.05$ were considered.

Sterols constitute the major fraction of the unsaponifiable matter in many oils. They are of interest due to their antioxidant activity and beneficial impact on human health [30].Among the identified phytosterols, β-sitosterolwas the major form (69.42–89.26%), followed by campesterol (0.33–5.24%) and Δ5-avenasterol (0.1–7.34%). The results [26 Table 2]are in agreement with those from [30] who reported that the predominant sterol was β-sitosterol followed by other common 4-desmethylsterols such as campesterol, stigmasterol and Δ5-avenasterol.

There were two saturated plant sterols (sitostanol, campestanol) were identified in small amounts in all studied varieties. The Local gd variety has the highest amount of stanols. Despite their minority, phytostanols are more effective than phytosterols in lowering cholesterol levels in mammal [6]. Statistical analysis showed that the phytosterols and stanol composition and contents were significantly different between the six varieties ($p < 0.05$). In this study, the composition and the levels found in the different varieties grown under similar conditions vary considerably. This could be explained by the fact that ecological factors do not solely affect the composition of cultivar; genetic factors might also be responsible for this variability.

(iii) Extraction and Analysis of Sterols from Pecan nuts [27]:

(a) Extraction:

The Pecan nuts were collected and frozen at -20 C until the extraction process begins. Oil was extracted from dry milled pecankernels using a Soxhlet apparatus with light petroleum ether for 6 h at 42 ºC. The solvent was evaporated using a rotary evaporator at 50 ºC then the oil was weighed and stored in dark glass bottles at 4 C until analysis. Unsaponifiable fraction was determined by saponifying pecan nut oil, with methanolic potassium hydroxide (1 N) solution and dihydrocholesterol solution was used as an internal standard in a cappedflask. The mixture was allowed to react over night under shaking at room temperature. After adding distilled water and dichloromethane (DCM), the mixture was vortexed vigorously and centrifuged at 3000 rpm for 3 min. The organic fraction was transferred in a second capped flask. The unsaponifiable matter was extracted twice with dichloromethane. The combined dichloromethane extract was washed with distilled water until the neutrality. The solvent was then evaporated to dryness under a stream of N_2 at 40 ºC. Analyses were performed in triplicate.

The sterol TMS derivatives were prepared according [28]. After removal of DCM, the residue was mixed with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (with 1%TMCS) and pyridine for derivatization. The 4-desmethylsterols, triterpene alcohols, 4-monomethylsterols and stanols were quantified by Gas Chromatography–Flame Ionization Detection.

(b) Analysis of Sterols in different stages of Ripening of Pecan nut[27]:

The total ion chromatogram of the unsaponifiable fraction of pecan nut oil showed 15 phytosterols: nine 4-desmethylsterols,two 4a-monomethylsterols and four 4,4-dimethylsterols (Fig. 1).During the ripening of C. illinoinensis, the highest levels of phytosterols were detected at an early stage of maturity in the three varieties (Fig. 2A). Indeed, the early stage of fruit ripening is a period of excessive cell division, which necessitates the biosynthesis of compounds essential for controlling membrane fluidity, such as phytosterols [5]. There is a significant decrease in the phytosterols during complete maturity. This decline in total phytosterols can be explained by the fact that in last ripening stages the enzymatic activity of sterol biosynthesis was stopped which yielded to the end of their biosynthesis, and the existed phytosterolswere converted to other sterol forms or to stanols [32].

Analysis revealed that in pecan nut oils, the main constituents of the phytosterols fraction were the 4-desmethylsterols, ranging from 83% to 87% of the total phytosterols content and 4α-amonomethylsterolsand 4,4-

dimethylsterols at lower amounts (5–6% and 7–12% of total sterols, respectively). These proportions were quantitatively close to those of hazelnut oil [24].

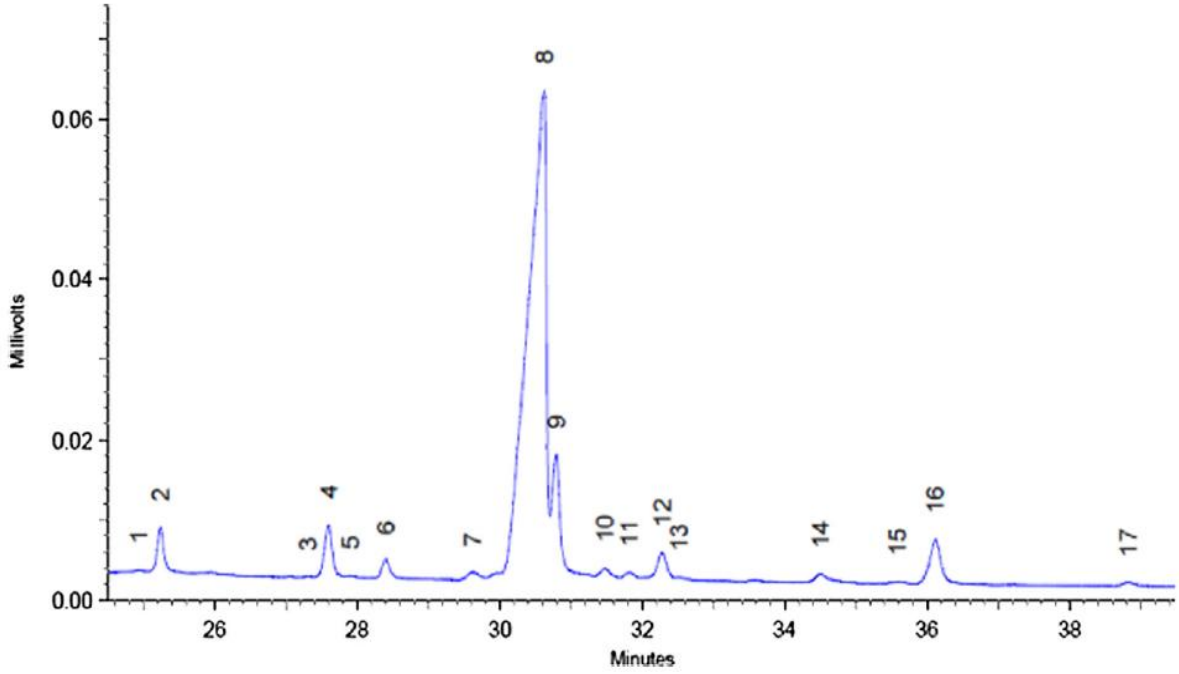
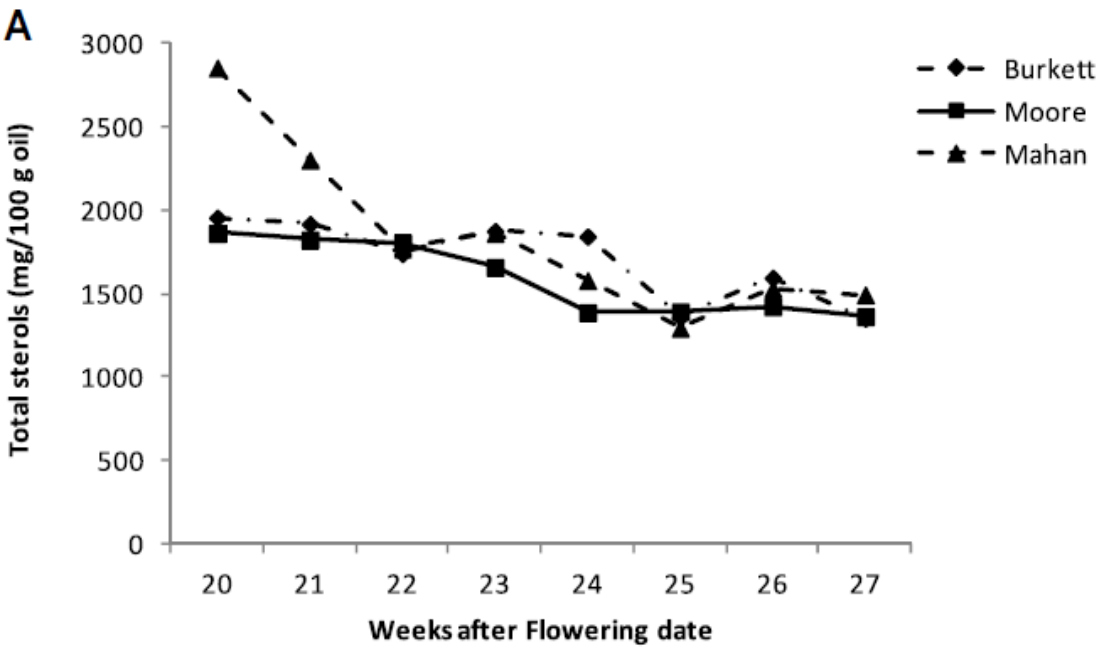
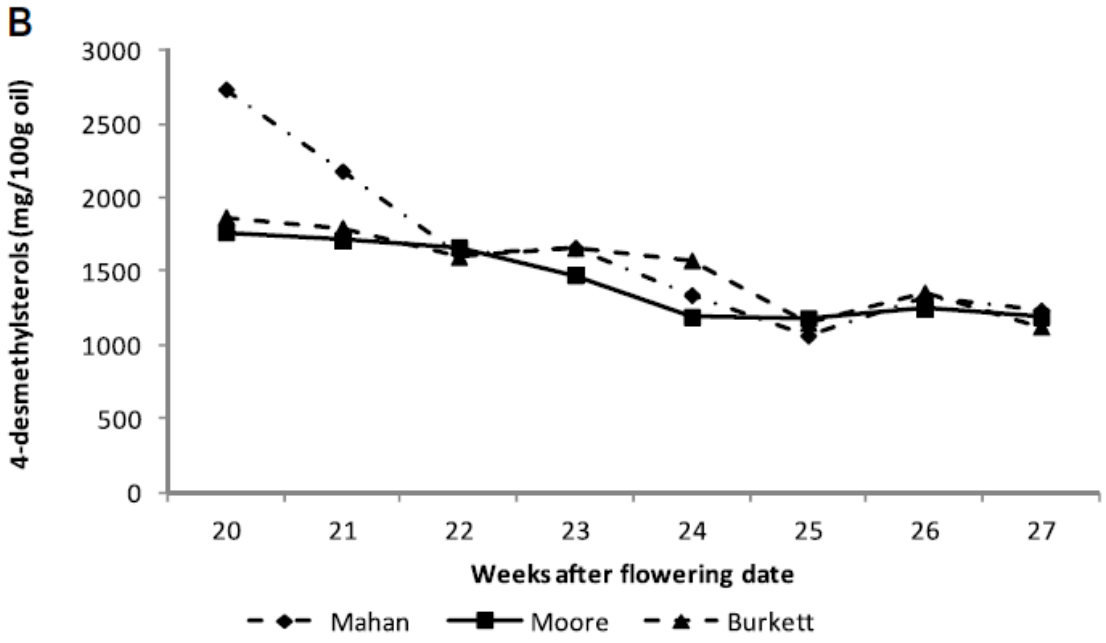


Fig.1.GC chromatogram of the unsaponifiable fraction of pecan nut oil. 1: cholesterol; 2: dihydrocholesterol; 3: 24-methylene cholesterol; 4:campesterol; 5: campestanol; 6: stigmasterol; 7: clerosterol; 8: b-sitosterol; 9: Δ5-avenasterol; 10: fucosterol; 11: gramisterol; 12: cycloartenol; 13: Δ7-avenasterol; 14: 24-methylene cycloartenol; 15: lupeol; 16: citostadienol; 17: betulin

The Δ5-avenasterol is considered the second most abundantsterolic compound in pecan nut oil after the β-sitosterol. The third sterolic compound of pecan nut oil is the campesterol which decreased during the maturity process of peanuts. These major 4-desmethylsterols cover approximately 94% of the total 4-desmethylsterols. Other minor components were detected fucosterol, stigmasterol, and clerosterol with small amounts of Δ7-avenasterol, cholesterol and 24-methylene cholesterol. This difference in sterol composition may be attributed to genetic and geographic factors. It may also be related to storage conditions, soil type and climatic differences where pecan nut had been grown. The evolution of these major compounds during Mahanpecans maturation is presented in Fig. 2C4-desmethylsterols showed that these compounds exhibited the same accumulation patterns during the development of the different varieties (Fig. 2B). During the ripening of C. illinoinensis, the highest levels of phytosterols were detected at an early stage of maturity in the three varieties (Fig. 2A). These amounts were of 2852.5, 1861.9 and 1955.2 mg/100 g of oil for Mahan, Moore and Burkett, respectively.


A
Total sterols (mg/100 g oil)
3000
2500
2000
1500
1000
500
0
20
21
22
23
24
25
26
27
Weeks after Flowering date
Burkett
Moore
Mahan


B
4-desmethylsterols (mg/100g oil)
3000
2500
2000
1500
1000
500
0
20
21
22
23
24
25
26
27
Weeks after flowering date
Mahan
Moore
Burkett

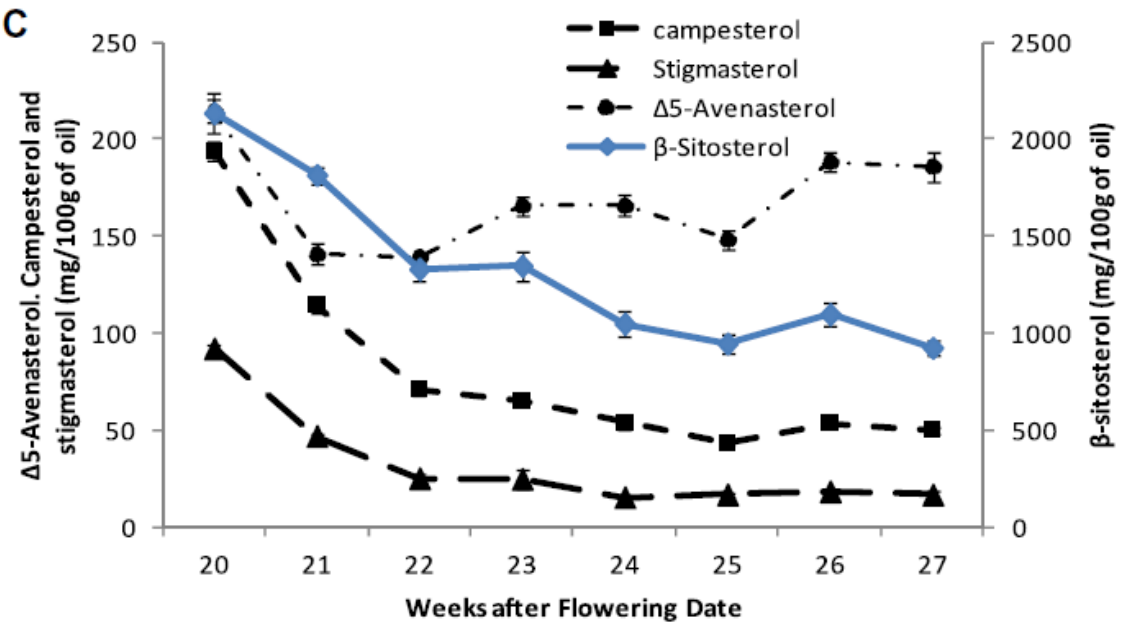


Fig.2. (A) Changes in total phytosterols content (expressed in mg/100 g oil) during ripening of three varieties. (B) Changes in total 4-desmethylsterols content (expressed in mg/100 g oil) during ripening of three varieties. (C) Changes in the content of the major 4-desmethylsterol compounds (expressed in mg/100 g oil): b-sitosterol, Δ5-avanesterol, campesterol and stigmasterol during ripening of Mahan pecan nuts. Each value is a mean of a triplicate analysis performed on different samples. [27]

These studies this study indicate that variety and degree of ripening are among the most important factors affecting the phytosterols content in pecan nuts but not the phytosterols composition which naturally accumulate these high-value constituents in pecan nut oil, offering improvements in oil quality based on the health benefits provided by phytosterols.

(2) Sterols Uses:

Sterols shows anti-inflammatory activity, anti- cancer activity and it acts on enlarged prostate gland also. β-sitosterol has been reported to inhibit the growth, migration, and invasion of prostate cancer cells and is used to treat enlarged prostate [19, 33].Almost all human tissues possess detectable 17β-HSDactivity, and 17β-HSD4 is thought to be an important housekeeping enzyme responsible for inactivating the most potent estrogen,17-estradiol, in all tissues. [34]Loss of 17β-HSD4 activity also leads to a severe d-bifunctional protein deficiency that is usually lethal bythe age of one. β-Sitosterol may therefore derive its activity against prostate cancer through modulation of 17β-HSD4 activity.[35]

The identification of two new binding proteins for β-sitosterol that has been shown to be effective against enlarged prostate in human clinical trials. β-Sitosterol also has anti-cancer and anti-inflammatory activities in cell culture including prostate, breast, colon, leukemia, T-cells, and macrophages[19].

Enzyme (5a-reductase) was known to be inhibited by β-sitosterol. It remains unclear if inhibition of5a-reductase is responsible for the effects of β-sitosterol on benignprostatic hyperplasia and prostate cancer, and modulation of additional targets may be essential. Moreover, inhibition of5a-reductase is unlikely to play a role in the anti-inflammatory activity of β-sitosterol or in its effects on other cancers. [33, 35]

CONCLUSION

The most common method used in the extraction of Sterols in nuts is Saponification and, in some cases, total lipid extract can also be used to analyse the Sterol content in nuts. The Sterol content in Nuts is different in different nuts, even in a Particular nut the sterol content varies with species. From the Pecan nut studies it is evident that in pecan nut the content of sterols in the nut's changes with stage of development of nut. The content of Campesterol decreases and content of 4-desmethyl Sterols increases as the Pecan nuts ripens. Wallnut studies show that β-Sitosterol has the highest percentage among different type of sterols followed by campesterols. In Hazelnuts the method used for the extraction is Saponification; HPLC is used for the analysis of the Extract of Saponification. The data were analysed using the analysis of variance (ANOVA) Comparisons of means were achieved using the Statistical Analysis.

Among the different plant sterols identified and quantified in pecan nut oil, β-sitosterol, campesterol, stigmasterol and Δ5-avenasterol exerted antioxidant effects against lipid peroxidation. The decline in total phytosterols is since in last ripening stages the enzymatic activity of sterol biosynthesis was stopped which yielded to the end of their biosynthesis, and the existed phytosterols were converted to other sterol forms.

In wall nuts β-sitosterol was the major form, followed by campesterol and Δ5-avenasterol. In hazelnut β-Sitosterol was the first free sterol in the two samples tested. The other free + esterified sterols, in decreasing order of abundance, were campesterol, and Δ5-avenasterol. Other major compounds were the saturated sterols, namely sitostanol and campestanol. In hazelnuts and walnuts it is found that β-sitosterol is the major sterol, the content of β-sitosterol is more compared to all other sterols, β-sitosterol has also lot of uses and it is a therapeutically active compound it is used to lower the cholesterol, treat the inflammation in prostrate, high levels of β-sitosterol in patients suffered with heart attacks leads to severe heart problems.

All the Extractions are generally made by Saponification; total lipid extract is also used. Nuts of different type from various geographical regions are collected and the analysis on the content of sterols in those nuts is the major research going on. These sterols have lot of pharmacological/Therapeutical effects hence the phytosterols that can be taken as a part of diet has gained importance.

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